

A WITHIN SUBJECTS COMPARISON OF TWO ANTEGRADE FLUSHING REGIMENS
IN CHILDREN

By

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To my husband William Aden Cover, Jr. and my son Adam Bennett Jarczyk

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	10
LIST OF FIGURES.....	11
LIST OF ABBREVIATIONS.....	13
ABSTRACT.....	14
CHAPTER	
1 INTRODUCTION.....	16
Epidemiology.....	16
Background.....	16
Theoretical Framework.....	19
Review of Pertinent Literature to Guide Theory Development.....	19
Physioanatomic Considerations.....	20
Linkages between bowel, bladder, and pelvic floor function.....	20
Nervous system associations.....	23
Animal studies.....	23
Human studies.....	24
Developmental Considerations.....	26
Pain and learning in children.....	27
Toilet training.....	28
Psychosocial Considerations.....	29
Psychological associations.....	29
Social associations.....	30
Anthropometric and Nutritional Considerations.....	31
Obesity.....	31
Dietary considerations.....	32
Genetic Considerations.....	32
Summary of Pertinent Literature to Guide Theory Development.....	33
Identification of a Metaparadigm to Guide Theory Development.....	34
Probabilistic Epigenesis.....	35
Historical Overview.....	35
Constructs and Relational Statements.....	36
The Applicability of Probabalistic Epigenesis to Pediatric Incontinence Research.....	38
The Integrative Model of Pediatric Incontinence and Dysfunctional Elimination.....	39
Proposed Program of Research.....	41
Problem.....	41

Purpose, Aims, and Hypothesis:.....	43
Null Hypotheses for Aim 1	43
Null Hypotheses for Aim 2	44
Null Hypothesis for Aim 3.....	44
2 LITERATURE REVIEW	47
Overview.....	47
Studies Addressing the Effectiveness of ACE Therapy in Promoting Continence	47
Studies Addressing Side Effects Associated with ACE Therapy	53
Studies Addressing Gut Microbiota	55
Significance and Innovation	58
3 METHODOLOGY	63
Design.....	63
Rationale for Utilization of Within Subjects Designs	64
Inclusion and Exclusion Criteria	68
Regulatory Considerations and Study Expenses	69
Subjects	70
Setting.....	70
Measures	72
Materials	75
High Volume Flush	76
Low Volume Flush	76
Procedures	77
High Volume Regimen	77
Low Volume Regimen	78
Optimal Dose Regimen	79
Regimen Comparative Phase.....	79
Specimen Collection.....	80
Statistical Analysis	81
Social Validity	85
Threats to Validity	87
4 FINDINGS.....	93
Subject Characteristics	93
Aims.....	93
Null Hypotheses for Aim 1	94
Frequency of Administration.....	94
Titration Time	94
Cost Burden	95
Null Hypotheses for Aim 2	96
Continence	96

Infusion Time.....	96
Procedural Time.....	97
Side Effects.....	97
Quality of Life.....	98
Electrolytes.....	99
Stool Calprotectin.....	99
Child's Flushing Preference.....	100
5 DISCUSSION AND CONCLUSIONS.....	132
Aimes Specific Discussion of Outcomes.....	132
Frequency of Administration.....	132
Flush Titration Time to Continence.....	133
Cost Burden.....	134
Fecal Continence.....	134
Infusion and Procedural Time.....	137
Side Effects.....	138
Electrolytes.....	138
Stool Calprotectin.....	139
Quality of Life.....	140
Study Limitations.....	140
Implications for Future Research and Practice.....	142
Probabilistic Epigenesis as a Framework for Pediatric Incontinence	
Research.....	142
Implications for future research.....	142
Implications for Clinical Practice.....	144
Conclusions.....	144
APPENDIX	
A THEORETICAL AND OPERATIONAL LINKAGES FOR RESEARCH	
ADDRESSING INCONTINENCE.....	146
B REQUIRED MATERIALS FOR FLUSHING PROCEDURE.....	147
C PROCEDURAL INSTRUCTIONS FOR COMPLETING SALINE FLUSH.....	148
D PROCEDURAL INSTRUCTIONS FOR COMPLETING USP GLYCERIN FLUSH.....	149
E MICROBIOME DNA EXTRACTION AND PROCESSING FOR DOWNSTREAM	
ANALYSIS.....	150
F THREATS TO STATISTICAL CONCLUSION VALIDITY.....	151
G THREATS TO INTERNAL VALIDITY.....	152

H	SOCIAL THREATS TO INTERNAL VALIDITY	153
I	THREATS POSED TO CONSTRUCT VALIDITY OF CAUSE AND EFFECT.....	154
J	NEMOURS CLINICAL RESEARCH COMMITTEE APPLICATION AND REVIEW.....	155
K	FDA IND APPLICATION.....	158
L	IAA AGREEMENT BETWEEN NEMOURS AND UNIVERSITY OF FLORIDA.....	184
M	MANAGEMENT OF RESEARCH PHARMACEUTICAL PRODUCTS	186
N	IRB SUBMISSION, APPROVAL AND APPLICATION FOR AND APPROVAL FOR AMMENDMENTS.....	192
O	KJ001 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND	242
P	KJ002 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND	252
Q	KJ003 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND	260
R	KJ004 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND	268
S	KJ005 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND	277
	REFERENCES.....	286
	BIOGRAPHICAL SKETCH.....	301

LIST OF TABLES

<u>Table</u>		<u>page</u>
2-1	Summary of Published Flush Solution, Dosing, Administration Frequency, and Procedural Time Outcomes	59
3-1	Itemized Estimate for Maximum Flushing Costs For 6 Subjects.....	88
3-2	Level of Soiling	88
3-3	Dependent Variables	89
3-4	Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Maintain Continence.....	90
3-5	Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Minimize Side Effects	91
4-1	Individual Subject Data and Descriptive Statistics	101
4-2	Cost Comparison Normal Saline vs USP Glycerin Flush.....	104

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
1-1 Gilbert Gottlieb's Model of Probabilistic Epigenesis. Depiction of the bidirectional coaction between levels of functioning over the course of development.....	45
1-2 Reduced Model of Probabilistic Epigenesis adapted to test pediatric continence.	45
1-3 Model depicting specific interactions between organ systems contained within the neural constitutive element specific to incontinence and dysfunctional elimination.	46
3-1 Procedural timeline.....	92
4-1 Frequency of flush administration.....	103
4-2 Titration time to achieve continence	103
4-3 KJ001 Absolute frequency of incontinence graph.....	105
4-4 KJ002 Absolute frequency of incontinence graph.....	106
4-5 KJ003 Absolute frequency of incontinence graph.....	107
4-6 KJ004 Absolute frequency of incontinence graph.....	108
4-7 KJ005 Absolute frequency of incontinence graph.....	109
4-8 KJ001 Frequency and severity of incontinence graph	110
4-9 KJ002 Frequency and severity of incontinence graph	111
4-10 KJ003 Frequency and severity of incontinence graph	112
4-11 KJ004 Frequency and severity of incontinence graph	113
4-12 KJ005 Frequency and severity of incontinence graph	114
4-13 KJ001 Procedural time graph	115
4-14 KJ002 Procedural time graph	116
4-15 KJ003 Procedural time graph	117
4-16 KJ004 Procedural time graph	118

4-17	KJ005 Procedural time graph	119
4-18	KJ001 Abdominal pain graph	120
4-19	KJ001 Cramping from flush graph	121
4-20	KJ002 Abdominal pain.....	122
4-21	KJ003 Abdominal pain.....	123
4-22	KJ003 Cramping with flush.....	124
4-23	KJ004 Cramping with flush.....	125
4-24	KJ005 Cramping with flush.....	126
4-25	KJ001 Vagal symptoms graph.....	127
4-26	Serum sodium levels	128
4-27	Serum potassium levels	128
4-28	Serum chloride levels	129
4-29	Serum carbon dioxide levels.....	129
4-30	Blood urea nitrogen levels	130
4-31	Serum creatinine levels (low levels normal in children)	130
4-32	Serum calcium levels.....	131
4-33	Stool calprotectin levels.....	131

LIST OF ABBREVIATIONS

ACE	Antegrade continence enema
BBD	Bowel and bladder dysfunction
FIC QOL	Fecal Incontinence Quality of Life Measure in Children with Spinal Bifida
GALT	Gut associated lymphoid tissue
IBD	Inflammatory bowel disease
IOA	Interobserver Agreement
Kg	Kilogram
L	Liter
LUTD	Lower urinary tract dysfunction
mL	Milliliter
mmol	Millimole
OAB	Overactive bladder
Peds QL	Pediatric quality of life
qd	Occurring or administered every day
qod	Occurring or administered every other day
QOL	Quality of life
STC	Slow transit constipation
WBFPRS	Wong-Baker Faces Pain Rating Scale
wk	Occurring or administered weekly

Abstract of Dissertation Presented to the Graduate School
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A WITHIN SUBJECTS COMPARISON OF TWO ANTEGRADE FLUSHING REGIMENS
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Fecal incontinence past the time of toilet training is devastating to affected children. Antegrade continence enema (ACE) therapy administered through a catheterizable stoma surgically placed in the cecum has helped children with intractable fecal incontinence attain continence for stool. Retrospective studies demonstrate favorable continence outcomes and prospective studies demonstrate improvement in quality of life on ACE therapy. There are no prospective trials comparing the effectiveness of different flushing regimens on continence. The purpose of this prospective study was to compare two distinct flushing regimens, saline and USP glycerin, in the immediate postoperative period in children requiring ACE therapy. The aims of this study were to (a) identify the minimal administration frequency, titration time to reaching effective dose, and cost of two flushing solutions; (b) compare which solution at an optimum dose was delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence and quality of life; and (c) process stool for down-stream identification of 16SrRNA microbiome gene. This prospective study utilized a repeated measures, single subjects alternating treatments

A-B-C-B'-C'-B₁' withdrawal design in which all subjects were tested under all conditions and each subject acted as his or her own control. A within subjects cross-over design was embedded in the dosing A-B-C dosing and maintenance B'-C'-B₁' treatment comparison phase of the study. Subjects were randomly assigned to one of two treatment sequences. Five subjects were recruited between 2015 and 2017. All subjects had fecal incontinence secondary to low level spinal cord lesions. Only one of five subjects (20%) gained continence on saline while four of five (80%) gained continence on USP glycerin. Moderate pain with flushing was consistently present in 1/5 subjects (20%) and 1/5 subjects (20%) was dropped from the study due to vagal symptoms. There was no electrolyte imbalance associated with either regimen. Saline was the more costly regimen. Findings did not reach statistical significance due in part to small sample size. Findings may have clinical relevance when applied to male children under the age of 8 with fecal incontinence due to low level spinal cord lesions.

CHAPTER 1 INTRODUCTION

Epidemiology

Incontinence and dysfunctional elimination impact a large percentage of children. Von Gontard, Heron, and Joinson (2011) reported a prevalence of 15.5% nocturnal enuresis and 7.8% diurnal enuresis in 7 ½ year olds. Reports detailing the prevalence of pediatric constipation world wide range from 0.7% to 29.6% with a median of 12% (Mugie, Benninga, & Di Lorenzo, 2011). Constipation accounts for 25% of referrals to pediatric gastroenterologists. Estimates of the prevalence of childhood fecal incontinence range from 1% to 3% (Culbert & Banez, 2007). The majority of children with incontinence, constipation, and lower urinary tract symptoms do not have an organic or structural etiology for their symptoms. The prevalence of incontinence due to organic or structural etiologies is difficult to determine. Bischoff, Levitt, and Pena (2009) estimate there are 200,000 infants with myelomeningocele and 8,500 infants with anorectal malformation born each year worldwide. Myelomeningocele is a part of a larger grouping of neurogenic etiologies including sacral agenesis, trauma, and tumor that often carry a poor functional prognosis for both urinary and fecal incontinence. Anorectal malformations are associated with a poor functional prognosis for fecal incontinence.

Background

A major developmental milestone in a child's life is the achievement of urinary and fecal continence. Incontinence in children past the expected time of toilet training is devastating and has been associated with substantial decrease in quality of life, increased abuse, bullying, anxiety, depression, greater social problems, and poorer

school performance (Kaugars et al., 2010; Youssef, Langseder, Verga, Mones, & Rosh, 2005). Incontinence is a subset within the broader category of dysfunctional elimination that includes chronic constipation, urinary frequency and urgency, and recurring urinary tract infection. Bower (2008) identified multiple domains including self-esteem, mental health, independence, family functioning, social interaction, and body image that were adversely affected by bladder and bowel dysfunction with or without incontinence.

Results from a multicenter prospective trial compared the impact of functional constipation to the impact of functional constipation plus fecal incontinence in four different age domains on quality of life using five instruments including Pediatric Quality of Life Inventory, Pediatric Quality of Life – Family Impact Module, Functional Disability Inventory - Parent Version, Pediatric Inventory for Parents, and Pediatric Symptom Checklist – Parent Report. The sample included 410 subjects ranging from 2 to 18 years of age. The four age domains were 2 to 4 years, 5 to 7 years, 8 to 12 years, and 13 to 18 years. Findings indicated children with combined constipation and fecal incontinence had worse family functioning that deteriorated as the child aged, significantly higher parental stress, and significantly lower emotional and behavioral well-being when compared to the children who suffered with functional constipation alone. Adolescents with constipation and fecal incontinence in particular were adversely impacted and had a substantially worse quality of life as measured by lower scores (0 minimum, 100 maximum). When the Pediatric Quality of Life scores in this study sample (57), were compared to reported norms in healthy adolescents (83), Inflammatory Bowel Disease (79), chronically ill children (77), and children with acute exacerbation of a chronic illness (79), the degree of devastation on the quality of life of

affected adolescents caused by fecal incontinence becomes explicit. When the Pediatric Quality of Life scores of children with constipation and fecal incontinence greater than 8 years of age were compared to published Pediatric Quality of Life scores in other studies, scores of affected children were worse than children with Type 1 Diabetes, cancer in remission, heart disease, and transplant recipients (Kovacic et al., 2015).

Bowel, bladder, and pelvic floor dynamics are closely interrelated. They share the same embryonic origins, are innervated from the same level of the spinal cord, and are all modulated by the central nervous system (Shafik, 1984). Because dynamics of the bowel, bladder, and pelvic floor are closely interrelated, organic or functional disruption in one of these systems can create a dysfunctional pattern of elimination affecting all of them. Incontinence and disordered elimination, whether it is primarily structural, organic, or functional in etiology, is further complicated by developmental, psychological, and social problems. Therefore, it is important to address the physical, developmental, psychological, and social issues comprehensively to achieve better outcomes. In the North American pediatric system of health care delivery, bowel, bladder, and psychiatric issues are managed within the subspecialties of gastroenterology, urology, and psychiatry/psychology respectively. Providers in these subspecialties do not share any forum in which they normally interact. They do not generally interface clinically and do not have journals, subspecialty societies, or national conferences in common. The care of these children is in effect “siloe” in the individual subspecialties with resulting fragmentation of services. Improved outcomes for this population of children will require a comprehensive and integrated understanding of the condition and a holistic approach to care.

Theoretical Framework

In an effort to find a unifying theory or framework to guide practice, an extensive review of the literature was conducted including searches in MEDLINE, Ovid MEDLINE, Cochrane Central Register of Controlled Trials, ERIC, EMBASE, CINAHL, and PsycINFO with the assistance of two librarians from different organizations using over 25 search terms. Review of the references at the back of journal articles was completed. A textbook on nursing theory (Glanz, Rimer, & Viswanath, 2008) and several nursing theory websites were reviewed. The search did not reveal a theoretical framework or model addressing incontinence and/or dysfunctional elimination in children.

Review of Pertinent Literature to Guide Theory Development

Pediatric dysfunctional elimination is a multifactorial, multidimensional, complex interplay of factors including physioanatomic, neurological, developmental, psychosocial, environmental, and genetic determinants. Theory is needed to integrate these factors into a coherent whole that would enable clinicians to provide comprehensive and effective care. The interrelationships between these factors have been demonstrated to varying degrees in the literature. The mechanisms involved in these interactions are poorly understood. A review of the literature was undertaken to gain an overview of pertinent concepts and their interrelationships to help frame the beginning of a theory for pediatric dysfunctional elimination and continence.

Physioanatomic Considerations

Linkages between bowel, bladder, and pelvic floor function.

There is a well-established association between bowel and bladder dysfunction. The symptom complex was initially termed dysfunctional elimination syndrome by Koff and colleagues in 1998 and was later renamed bowel and bladder dysfunction (BBD) by the International Children's Continence Society (Austin et al., 2014; Koff, Wagner, & Jayanthi, 1998). This disorder constitutes a symptom spectrum of lower urinary tract dysfunction (LUTD) that occurs concomitantly with constipation and/or fecal incontinence (Queiroz Machado, Monteiro, Pecanha, & Garcez da Fonseca, 2015; Santos, Lopes, & Koyle, 2017). Constipation is the presenting symptom in as many as 5% of visits to the pediatrician and 25% of visits to pediatric gastroenterologists (Loening-Baucke, 1996). Estimates of voiding dysfunction prevalence in the pediatric population range from 2-25%, with voiding dysfunction as the presenting symptom in as many as 40% of referrals to pediatric urologists (Feldman & Bauer, 2006; Vaz et al., 2006). It is estimated that 30% of children with constipation have lower urinary tract symptoms (Loening-Baucke, 1997). Constipation and occult megarectum have been linked to reversible urinary tract abnormalities including incomplete bladder emptying, urinary frequency, recurring urinary tract infection, daytime and nighttime urinary incontinence, and upper urinary tract dilation (Belman, 1998; Dohil, Roberts, Verrier Jones, & Jenkins, 1994; Hodges & Anthony, 2012; Neumann, DeDomenico, & Nogrady, 1973; O'Regan, Yazbeck, Hamburger, & Schick, 1986). Findings from a population-based study in Brazil demonstrated constipated children were 6.8 times more likely to have symptoms of lower urinary tract dysfunction, including incontinence, withholding,

infrequent voiding, urgency, and dysuria when compared to their non-constipated peers. Infrequent voiding and withholding behavior were independent predictors of constipation. In addition, an increase in constipation severity was associated with an increase in lower urinary tract symptom severity (Sampaio et al., 2016). Veiga et al. (2013) found that in addition to lower urinary tract dysfunction, 54.9% of children with overactive bladder (OAB) were constipated and approximately three times more likely to develop constipation than children without OAB. Of interest, although there is a well-documented correlation between LUTD and retentive constipation, two retrospective studies have demonstrated an association between OAB and non-retentive fecal soiling (Burgers et al., 2013; Combs, Van Batavia, Chan, & Glassberg, 2013). Dysfunctional elimination has been associated with both delayed resolution and post-operative recurrence of vesicoureteral reflux in children (Chen, Mao, Homayoon, & Steinhardt, 2004; Halachmi & Farhat, 2008; O'Regan, Schick, Hamburger, & Yazbeck, 1986; Koff, 1992; Koff, Wagner, & Jayanthi, 1998; Upadhyay et al., 2003). Rectal distention has been shown to heighten the sensation of bladder fullness at lower volumes (De Wachter & Wyndaele, 2003). Treatment of constipation has been shown to be beneficial in promoting the resolution of urinary tract symptoms, including recurring urinary tract infection, urinary frequency and urgency, dysuria, nocturnal enuresis, and daytime urinary incontinence (Chrzan, Klijn, Vijverberg, Sikkels, & De Jong, 2008; Loening-Baucke, 2005). Although counterintuitive, one study demonstrated treatment of OAB with anticholinergics resulted in amelioration of fecal incontinence with or without constipation in 75% of treated children (Coombs et al., 2013).

Although exact mechanisms have not been elucidated, shared embryological origins, close anatomic proximity, similar storage and emptying functions, cross-sensitization or 'cross-talk' via convergent neural pathways, and common innervation have been implicated in comorbid bowel and bladder dysfunction. The gastrointestinal system and urinary tract emerge from a shared cloaca dividing in the seventh week of gestation. An extensive nerve and vascular supply support the evolving tissue. Small changes in this differentiation process could lead to functional defects in both systems (Kaplan et al., 2013; Malykhina, Brodie, & Wilcox, 2016).

Shafik (1984) discussed the similarities in muscle composition, evacuation function, and continence mechanisms between the bladder neck and rectal sphincter, with the pelvic floor acting as a "common sphincter." Coordination of these individual intrahiatal outlets with the pelvic floor is necessary to maintain patterns of functional elimination and continence. Using this model, dysfunctional changes in elimination dynamics are thought to be caused by physical obstruction at the level of the pelvic floor. Support of the pelvic floor as a key contributor to elimination dynamics is supported by an extensive body of research demonstrating the effectiveness of pelvic floor biofeedback. Animated pelvic floor biofeedback has been shown to be particularly effective in treating dysfunctional elimination syndrome in children (Desantis, Leonard, Preston, Barrowman, & Guerra, 2011; Herndon, Decambre, & Mckenna, 2001; Kaye & Palmer, 2008; Mulders, Cobussen-Boekhorst, de Gier, Feitz & Kortmann, 2011). Neuromuscular involvement seen in cerebral palsy and degenerative neuromuscular disorders has the potential to negatively impact voiding and defecation dynamics

leading to dysfunctional elimination due to the inability to coordinate the muscles of elimination.

Nervous system associations

Although poorly understood, outcomes from human and animal studies suggest the presence of bladder-distal gut cross-sensitization termed “cross-talk” in which discreet afferent signals from the bowel and bladder are transmitted through convergent dorsal root ganglia and both superficial and deeper lumbosacral spine neurons for modulation of routine pelvic organ function. This cross-talk, or neural circuitry, also provides a pathway for the abnormal function of one pelvic organ to cause dysfunctional changes in another pelvic organ. Shared peripheral innervation and neuronal mechanisms in the presence of neural plasticity in early childhood allows physical insult or psychological perturbation to effect the restructuring and connectivity of neural circuitry affecting bowel and bladder function.

Animal studies

Penzzone, Liang, and Fraser (2005) measured bowel and bladder smooth and striated muscle reflexes during voiding and rectal distention in rats using external urethral and anal sphincter electromyography (EMG). They measured external anal EMG phasic firing during voiding synchronous with external urethra sphincter activity with phasic firing and tonic bursts independent of urethral sphincter activity. Colorectal distention in the absence of bladder irritation produced no change in abdominal wall EMG activity. Colorectal distention in the presence of acute bladder irritation resulted in abdominal wall EMG activity at much lower distention pressures, suggesting colonic afferent sensitization. Acute colonic irritation resulted in a 66% increase in bladder

contraction frequency, suggesting lower urinary tract afferent sensitization. Findings from this study provided evidence of a bidirectional cross-sensitization of the lower bowel and lower urinary tract. Researchers induced colonic inflammation in rats via enema administration of trinitrobenzenesulfonic acid and found significant attenuation in the amplitude of bladder contractions that returned to normal with resolution of the inflammatory stimulus (Noronha, Akbarali, Malykhina, Foreman, & Greenwood-Van Meervald, 2007).

Ustinova, Fraser, and Pezzone (2010) tested the effects of colonic irritation on the mechano-chemo-sensitive properties of bladder afferents in rats and found colonic irritation increased firing rates over resting rates by two-fold. In addition, a higher percentage of bladder afferents responded at lower distention pressures in the presence of colonic irritation. Wyndaele and colleagues (2013) evaluated the functional response of rat bladders to differing levels of colorectal distention and demonstrated reversible and pressure-dependent effects of colorectal distention on the bladder. Noxious levels of colorectal distention resulted in an initiation of an inhibitory rectovesical reflex demonstrated by an increased micturition volume threshold and a decreased bladder contraction, providing a possible explanation for increased bladder capacity and acontractility in the constipated child.

Human studies

A within subjects study evaluated urodynamic parameters completed in adult women with rectal balloon insufflation to 150 mL repeated in the same subjects without balloon insufflation (Panayi et al., 2011). They demonstrated statistically significant findings in the subjects during balloon insufflation, including a 46% decrease in volume

infused at first sensation, 33% decrease in volume infused at first desire, and 26% reduction in maximum bladder capacity. Of note, 13% of subjects who had been diagnosed with OAB were found to have detrusor over activity in the presence of balloon insufflation that was absent when the rectum was empty. Chang, Hsieh, and Yang (2012) compared post-void residuum urine volumes in children with constipation versus children without constipation and found constipation resulted in a statistically significant increase in absolute urine volume, a higher rate of residual >20 mLs, and a lower voiding efficiency. Investigators combined colonic motility and urodynamic studies in humans to explore the relationship of bowel and bladder dynamics between children with severe constipation and those with bladder dysfunction. Lucanto, Bauer, Hymen, Flores, & Di Lorenzo (2000) evaluated children with severe constipation and bladder dysfunction and found abnormal colonic motility suggestive of neuropathy in all of the children and abnormal urodynamics in 90.0% of the children studied. Burgers et al. (2010) investigated the effect of rectal distention on urodynamic parameters and found it significantly impacted bladder capacity, sensation, and instability in an unpredictable manner regardless of the presence of a history of constipation.

A study evaluating colonic transit time in children with refractory constipation and LUTD noted abnormal urodynamic features in 86.7% of affected subjects with 66.7% categorized as detrusor over activity and voiding dysfunction, 16.7% categorized as isolated detrusor activity, and 8.3% categorized as isolated voiding dysfunction. Colonic transit time was abnormal in 80% of constipated subjects, with 60% categorized as slow transit and 20% as outlet dysfunction. A high prevalence of slow transit constipation (STC) was found in children with refractory constipation. There was a significant

association between STC and OAB indicating the presence of a common neuropathy that affects both the colon and lower urinary tract (Queiroz Machado et al., 2015).

The bowel, bladder, and pelvic floor are innervated by the spinal cord at levels S1 through S4. Injury or disruption in spinal cord function at any of these levels has the potential to significantly impact bowel and bladder function. The concept of neuroplasticity may have significant relevance in dysfunctional elimination in the neurologically intact child. Afferent impulses generated from the bowel, bladder, or pelvic floor in response to aversive stimuli like pain may cause a significant change in efferent stimuli resulting in end organ change.

There is evidence to suggest the brain, not the bladder, is the primary site of lower urinary tract symptoms in children. Franco (2011) proposed a “neurocentric” model to explain the association between encopresis, urinary incontinence, and psychiatric comorbidities, postulating a central defect in the anterior cingulate gyrus or prefrontal cortex. Vortex based mapping analysis revealed a decrease in frontal grey matter in adults with a history of enuresis. Von Gontard & Hollman (2004) found children with comorbid functional urinary incontinence and encopresis had a significantly higher rate of abnormal electroencephalography.

Developmental Considerations

Central nervous system maturation is an essential consideration in issues related to pediatric continence and functional elimination. To successfully achieve independence in toileting, a child must have attained a certain level of cognitive and fine and gross motor development. Any significant cognitive or muscular disorder or delay

has the potential to prevent, substantially delay, or necessitate extraordinary means for achieving continence in the affected child.

Pain and learning in children

Conventional thought regarding the origins of dysfunctional elimination in children identifies painful elimination as the primary cause. Pain is an aversive stimulus. Young children learn by cause and effect and make the association between pain and elimination. Children will avoid things that cause pain, so they begin to actively withhold out of fear of pain. The function of the lower bowel is fluid reabsorption and storage of waste. When a child withholds stool, the stool becomes hard causing further discomfort during defecation, thereby reinforcing the withholding behavior. The rectum dilates to accommodate retained stool. The stool increases in caliber exacerbating the cycle. As the rectum accommodates to the increased stool burden, the urge to defecate is blunted and may vanish so the child can go for longer periods of time without passing a bowel motion. Fear and pain result in dyssynergic pelvic floor dynamics. Instead of increasing intra-abdominal pressure and relaxing the muscles of the pelvic floor, the child tightens the pelvic floor resulting in incomplete emptying of the bowel and bladder. Over time, loose stool from higher up in the colon leaks around the mass of hard stool in the rectal vault causing encopresis. The mass of stool in the rectal vault further compromises bladder dynamics compressing the bladder, decreasing its functional capacity, and causing an earlier sensation of bladder distention (Culbert & Banez, 2007; Halachmi & Farhat, 2008). The same basic dynamic is seen with children who have pain with urination. Dysuria leads to withholding behavior and dyssynergic voiding dynamics that is often the root cause for urinary incontinence and recurring urinary tract infection. It is

interesting to note that despite the accepted conventional wisdom proposing pain as a major precipitating factor in the development of dysfunctional elimination, there are no publications focused on aggressive treatment of constipation and dysuria as a preventive strategy.

Toilet training

Although achieving continence is a universal milestone for the normally developing child, there is limited and conflicting evidence regarding the ideal age for initiation of toilet training, required skills, and sequencing of those skills necessary for successful toilet training in the normal child (Joinson et al., 2009; Klassen et al., 2006; Schrum et al., 2002). Conventional wisdom has been to allow the child to determine when they are ready to initiate toilet training. The combination of improved diapers that prevent the child from experiencing a significant degree of discomfort when incontinent has resulted in children toilet training at a later age. Recent studies suggest earlier toilet training may be beneficial in preventing dysfunctional elimination. Joinson et al. (2009) found initiation of toilet training past 24 months of age was associated with increased odds of daytime urinary incontinence in school-age children.

Children with neuromuscular disorders, urologic and anorectal malformations, spinal cord anomalies, spinal cord trauma or tumor, nonneurogenic-neurogenic bladder, and megarectum are at high risk for urinary and/or fecal incontinence that is very difficult to manage using conservative measures (Tobias, Mason, Lutkenhoff, Stoops, & Ferguson, 2008). In a large portion of children with neurogenic bowel and bladder, extraordinary measures need to be employed to gain continence including clean intermittent catheterization and either retrograde enemas using continence apparatus or

antegrade continence enemas (ACE) administered through a catheterizable stoma using the appendix, a tubularized portion of the bowel, or a low profile button device from the abdominal wall into the cecum. Effectiveness of ACE therapy has been demonstrated in many retrospective studies with overall continence rates in children ranging from 55.7 % to 98% with a mean time to first relapse lasting as long as 121.9 +/- 29.7 months (Bani-Hani, Cain, King, & Rink, 2008; Basson et al., 2014; Curry, Osborne, & Malone, 1999; Dey et al. 2003; Driver et al., 1998; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Driver et al., 2001; Mousa et al., 2006; Ok & Kurzrock, 2011; Peeraully et al., 2014; Randall, Coyne, & Jaffray, 2014; Siddiqui, Fishman, Bauer, & Nurko, 2011; Sinha, Grewal, & Ward, 2008; Thomas et al., 2006; VanderBrink et al., 2012; Yardley et al., 2009). Prospective studies have demonstrated a significant improvement in somatic functioning, psychosocial functioning, and quality of life (QOL) following an ACE procedure (Aksnes et al., 2002; Mousa et al., 2006; Tiryaki, Ergun, Celik, Ulman, & Avanoğlu, 2010). There are a number of standard interventions such as dietary manipulation, timed emptying, suppository use, or retrograde enema therapy that are initially attempted prior to instituting more extraordinary measures. However, there is no research detailing the appropriate and most effective sequencing or combination of conservative therapies in children.

Psychosocial Considerations

Psychological associations

Recent studies have demonstrated a high rate of psychiatric comorbidities including attention deficit disorder in children suffering from urinary and fecal incontinence. The rate of psychiatric comorbidities in children with nocturnal enuresis is

as high as 30%, diurnal enuresis 40%, and fecal incontinence 50% (Von Gontard, Baeyens, Van Hoecke, Warzak, & Bachmann, 2011). Von Gontard and Hollmann (2004) found daytime urinary incontinence was associated with executive function, attention, separation anxiety, opposition, and conduct disorders, while fecal incontinence was associated with attention, activity, obsession, compulsion, and opposition disorders. Children with fecal incontinence were more likely to bully or be bullied and engage in antisocial behaviors than children who were continent (Joinson, Heron, Butler, & Von Gontard, 2006; Zavadenko, Kolobova, & Suvorinova, 2011). There is evidence to support comprehensive evaluation and treatment of underlying attention and psychiatric issues improve treatment outcomes in children with dysfunctional elimination and incontinence (Williamson, Gower, & Ulzen, 2011; Von Gontard, Baeyens, Van Hoecke, Warzak, & Bachmann, 2011).

Social associations

Maternal depression and anxiety were associated with daytime wetting and soiling in children (Joinson et al., 2008). Having a family member with a neuropsychiatric disorder placed the child without a neuropsychiatric disorder at higher risk for urinary tract symptoms (Franco, 2011). Sexual abuse is a potential causative factor in fecal incontinence. Children with fecal soiling, although not predictive of sexual abuse, had a higher rate of sexual abuse than children who had not been abused (Mellon, Whiteside, & Friedrich, 2006; Morrow, Yeager, & Lewis, 1996). It is unclear if fecal incontinence in sexually abused children results from pain with defecation following anal penetration that leads to withholding behavior with subsequent

encopresis or is used intentionally by the child as a protective mechanism to discourage access by the sexual predator.

Anthropometric and Nutritional Considerations

Obesity

There is a significantly higher rate of obesity in children with constipation and encopresis (Pashankar & Loening-Baucke, 2005; Teitelbaum, Sinha, Micale, Yeung, & Jaeger, 2009; Wagner, Equit, Niemczyk, & von Gontard, 2015). Fishman, Lenders, Fortunato, Noonan, and Nurko (2004) reported a higher incidence of fecal soiling in obese children. Of note, 50% of the soiling in their sample subjects was non-retentive. A study conducted by van der Baan-Slootweg, Liem, Bekkali, van Aalderen, Pels Rijcken, Di Lorenzo, and Benninga (2011) confirmed a higher incidence of constipation in children with obesity. A small percentage of those children had delayed colonic transit.

There is a well-known correlation between obesity and sleep apnea (Barone et al., 2009; Verhulst et al., 2007). Children with disordered breathing have an increased rate of nocturnal enuresis (Alexopoulos et al., 2006; Bascom et al., 2011; Brooks & Topol, 2003; Umlauf & Chasens, 2003). Children with voiding dysfunction have close to twice the rate of obesity found in the general population (Erdem, Lin, Kogan, & Feustel, 2006). Elevated BMI (including both overweight and obese categories) has been associated with a higher incidence of lower urinary tract symptoms, bladder-bowel dysfunction, and a poorer treatment response in children (Güven, Giramonti, & Kogan, 2007). Chang, Chiang, Lin, Hsieh, and Yang (2015) identified a higher risk of overactive bladder in children with an elevated BMI. Fraga and colleagues (2017) confirmed a significant association between obesity and a positive Dysfunctional

Voiding Symptoms Scale score. Findings from their study further defined the association of lower urinary tract function in obese children to symptoms of bladder emptying only. Arlen, Cooper, and Leong (2017) found obese children with a BMI greater than the 85th percentile were greater than 3 times more likely to fail treatment for symptoms of lower urinary tract dysfunction. Obese children with febrile urinary tract infection were more likely to have a higher grade of vesicoureteral reflux and more frequent occurrence of renal cortical defects than non-obese children (Byun et al., 2016). In addition, the body habitus in obese girls make them more prone to vaginal pooling of urine resulting in small volume daytime urinary incontinence.

Dietary considerations

Although ubiquitous in practice, there is no evidence to support the use of fiber in the active treatment of constipation in children (Tabbers et al., 2014). Outcomes in clinical practice, as yet not reported in the literature, suggests fiber, which acts as a bulking agent, can exacerbate constipation in the child who actively withholds stool or has anismus. There is preliminary evidence to support the use of probiotics including *Lactobacillus casei rhamnosus* Lcr35 and *Bifidobacterium breve* in the treatment of constipation in children (Bu, Chang, Ni, Chen, & Cheng, 2007; Tabbers, De Milliano, Roseboom, & Benniga, 2011).

Genetic Considerations

Numerous diseases and birth defects have a formal genetic linkage associated with abnormal bowel and bladder function. Formal genetic linkages have not been identified for constipation. However, there are a number of “syndromic forms of constipation” that may result from mutation in genes that affect the general physiology

of defecation and should be considered in the evaluation of the constipated child (Peeters, Benninga, & Hennekam, 2011). Formal genetic risks have been identified for nocturnal and diurnal enuresis (Von Gontard, Heron, & Joinson, 2011).

Summary of Pertinent Literature to Guide Theory Development

The literature identifies multiple physioanatomic, psychological, social, environmental, and genetic factors that contribute to incontinence and dysfunctional elimination in children and supports the existence of multiple, diverse, poorly understood associations between those factors. The anatomic model identifies obstruction at the level of the pelvic floor as the driver in bowel and bladder dysfunction. The bladder-distal gut cross-sensitization model identifies discreet afferent signals from the bowel and bladder transmitted through convergent dorsal root ganglia, and both superficial and deeper lumbosacral spine neurons as the driver for modulation of pelvic organ function. The neurocentric model identifies the brain as the primary driver of dysfunctional elimination by making the association between bowel, bladder, and psychiatric co-morbidities. The aversive stimulus model identifies the interplay of pain and development as the drivers in dysfunctional elimination in children. There is very little evidence elucidating the contribution of genetics to dysfunctional elimination.

Direct causes of neurogenic incontinence are better understood but the interplay of associated factors, most effective therapies, and therapy sequencing has not been established. A number of studies clearly elucidate the impact of dysfunctional elimination on quality of life. Very few studies address the contributions of environment and social considerations to incontinence and dysfunctional elimination. Prevention of

incontinence and dysfunctional elimination is not addressed. No unifying model promotes understanding of this issue.

Pediatric incontinence and dysfunctional elimination is a complex entity that negatively impacts the lives of a large number of children. Current practice and research addressing this issue is highly fragmented resulting in less than optimal care for this population. Preliminary review of the literature has demonstrated some possible linkages between the factors that comprise dysfunctional elimination, but reveal substantial holes. There is a need to integrate practice-related research into a comprehensive theory that would allow for a holistic and coherent approach to children with incontinence and dysfunctional elimination. A theory addressing pediatric incontinence and dysfunctional elimination is needed to serve as a guide for practice and future research.

Identification of a Metaparadigm to Guide Theory Development

Use of a metaparadigm to provide a global conceptual framework would be helpful in organizing a theory for children with incontinence and dysfunctional patterns of elimination. The metaparadigm would need to encompass the complexity of the different levels of interactions within and between organismic systems and organismic-environmental interactions within the context of a developing child. von Bertalanffy (1969) defined a living organism as a hierarchical open system that maintains itself in a dynamic equilibrium and evolves toward higher complexity by continuous exchange of matter with the environment that is attained and maintained through equifinal circular feedback processes. Gilbert Gottlieb, a developmental psychologist, derived the developmental-psychobiological metatheory of Probabilistic Epigenesis from Ludwig

von Bertalanffy's Systems Theory in which he conceived the developing organism as an open-system consisting of four levels of analysis with reciprocity of influences within and between each level (Gottlieb, 1991). The first three of the four levels of analysis or constructs are organismic and include genetic (DNA, RNA), neural (cytoplasm, cell, tissue, organs, organ systems, organism), and behavioral (prenatal and postnatal) levels. The fourth level of analysis is environmental, which is further subdivided into physical, social, and cultural groupings (Gottlieb, 1991; Gottlieb, 1998; Gottlieb, 2002; Gottlieb, 2007). Probabilistic Epigenesis provides constructs and relational statements that allow for the explanation and exploration of the multidimensional structural and functional properties of pediatric incontinence and dysfunction elimination within a developmental framework.

Probabilistic Epigenesis

Historical Overview

The historic definition of epigenesis is "the emergence of new structures and functions during the course of individual development" (Gottlieb, 2002, p. 158). Epigenesis was initially conceptualized as the predetermined, nonreciprocal development of the structure-function dyad whereby genetic activity determined structural maturation, which in turn determined function, activity, or experience in a feed-forward manner. In this view, the genome contains the blueprint that determines the master plan for the developing organism. Simplistically, the genetic activity (DNA → RNA → protein) → structural maturation → function pathway in development is thought to be unidirectional, encapsulated, and immune to the influence of supragenetic feedback (Gottlieb, 1991; Gottlieb, 1998; Gottlieb, 2001). Gottlieb (1991) proposed

epigenesis as probabilistic determination with active interaction and feedback among constitutive elements creating a bidirectional, hierarchical, constructive, reciprocal relationship or “coaction” among genetic activity; structural maturation; and function, activity, or experience. In this view, genes are inert and require supragenetic input to function during normal development. Simplistically, the genetic activity (DNA ↔ RNA ↔ protein) ↔ structural maturation ↔ function pathway is influenced by maturational processes and neural and behavioral functions. DNA is inert and requires intracellular signals or feedback from constitutive elements originating from outside the cytoplasm to turn DNA on or off in protein production, thereby regulating genetic expression. It is coaction above the level of DNA-RNA that drives cellular differentiation and function. The genome is a part of the development-physiological system responsive to intracellular, extracellular, and extra-organismic influences. Morphogenesis is not caused or controlled solely by genes, but rather genes enable morphogenesis, contributing to production of traits and features in the developing organism. Organisms with the same genome can demonstrate significant phenotypic variation under differing ontogenetic conditions (Gottlieb, 1998; Gottlieb, 2002; Gottlieb, 2007).

Constructs and Relational Statements

Gottlieb (2002) defined Probabilistic Epigenesis as:

...individual development characterized by an increase of complexity of organization – i.e., the emergence of new structural and functional properties and competencies – at all levels of analysis (molecular, subcellular, cellular, organismic) as a consequence of horizontal and vertical coactions among its parts, including organism-environment coactions (p. 159-160).

The constructs are levels of analysis and include genetic, neural, behavioral, and environmental. Horizontal coaction is defined as interactions that occur at the same level within an organism. Vertical coaction is defined as interactions that occur at different levels within the organism and between the organism and environment. Horizontal and vertical coactions are reciprocal, allowing constitutive elements to influence each other's structure and function within a developing system in a hierarchical and holistic manner. Behavioral and organic outcomes of development are a consequence of at least two specific components of coaction. Development is caused by interaction between at least two constitutive elements, not the elements themselves, and is temporal in nature as shown in Figure 1-1.

Gottlieb (2001) further defined the coaction between the constitutive elements that drive development as "experience," which includes function or activity at all levels of analysis. Experience serves three purposes, to (a) maintain function (maintenance function), (b) temporarily regulate or facilitate emerging features (facilitative function), and (c) induce or create features within a developing system that would not occur in the absence of that particular coaction (inductive function). Within the theoretical framework of Probabilistic Epigenesis, developmental causality is nonlinear and emergent and is therefore not obvious. Plasticity or malleability occurs at a cellular level as well as at neural, psychological, and behavioral levels in the developing organism. Plasticity of the nervous system and the developing organism's early experiences are the two determinants of behavioral adaptability. This plasticity allows coaction to drive phenotypic change or the creation of "behavioral neophenotypes" within the "norm-of reaction" range which represents all possible phenotypic outcomes for a genotype

exposed to all possible environments. The phenotypic change precedes and drives morphologic-physiologic change that eventually may drive genetic change (Gottlieb, 2002). Lastly, Probabalistic Epigenesis incorporates the principals of “equifinality” and “multipotentiality.” In equifinality, developing organisms can start at different points but reach the same endpoint; alternatively, organisms can share the same starting point and reach the same end point by different pathways. Multipotentiality maintains that each cell in the body has equal genetic potential because each cell contains the total genetic complement (Gottlieb, 2001). There is a wealth of biological experimentation in insects, animals, and humans that support Probabalistic Epigenesis and are clearly elucidated in various manuscripts (Gottlieb, 1991; Gottlieb, 1992; Gottlieb, 1998; Gottlieb, 2001; Gottlieb, 2002; Gottlieb, 2007).

The Applicability of Probabalistic Epigenesis to Pediatric Incontinence Research

The overarching principles contained within the constructs and relational statements of Probabalistic Epigenesis are broad, but are highly applicable to the derivation of a global theory of pediatric incontinence and dysfunctional habits of elimination and can be subjected to experimental confirmation. Review of the literature addressing pediatric incontinence and dysfunctional elimination demonstrates the complex interplay of factors including genetic, physioanatomic, behavioral, and environmental determinants within the context of emerging development that can be logically reformulated to fit the hierarchical constructs of Probabalistic Epigenesis. The relational statements proposing interaction and feedback among constitutive elements creating reciprocal, lateral, and horizontal coaction among genetic activity, structural maturation, and function promulgated within Probabalistic Epigenesis provide a logical

explanation for existing interactions and a framework for exploration of the multidimensional structural and functional properties of pediatric incontinence and dysfunction elimination.

The Integrative Model of Pediatric Incontinence and Dysfunctional Elimination

As a starting point in the development of a theoretical framework for pediatric continence, a restructured model of the Theory of Epigenesis will be used. Gottlieb's (1991) original metatheoretical model is depicted in Figure 1-1. While all encompassing, this theory was too abstract to be empirically tested. His theory was therefore substructured to allow formal evaluation of epigenetic trends testing horizontal and vertical interactions in the four constructs including genetic, neural, behavior, and environment as shown in Figure 1-2. The model looks at the vertical interaction of genetics, neural activity, and environment on behavior at a single point in time and the vertical and horizontal interaction of genetics, neural activity, environment, and behavior longitudinally. Behavior is determined by horizontal or vertical interaction of at least two constitutive elements within or between constructs. Each constitutive element may serve in the role of dependent variable, independent variable, or control. The specific interaction between organ systems contained within the neural constitutive element specific to incontinence and dysfunctional elimination is further defined in Figure 1-3.

The epigenetic principals of plasticity, equifinality, multipotentiality, norm-of-reaction range, and behavioral neophenotypes as defined by Gottlieb are essential principals in the new substructured theory. The substructured theory is comprehensive enough in scope to subsume the anatomic, bladder-distal gut cross-sensitization, neurocentric, and aversive stimulus models thus far elucidated in the literature and

provide context for as yet to be identified genetic and phenotypic contributions. Using Gibbs (1972) system of theory construction as modified by Dulock and Holzemer (1991), it is possible to clearly demonstrate theoretical and operational linkages between Probabilistic Epigenesis and proposed research addressing pediatric incontinence and dysfunctional elimination as shown in Appendix A. This substructured, new theory is parsimonious, allows for incorporation of multiple paradigms spanning genetic and epigenetic regulation of gene expression to environmental influences on treatment outcomes, facilitates temporal aspects of cross sectional and longitudinal design, is broad enough conceptually to encourage transdisciplinary and translational science, provides an integrated framework that views dysfunctional voiding and defecation dynamics as two expressions of the same entity, and functions to inform clinicians in the configuration of services and treatment of children with incontinence and dysfunctional elimination.

Pediatric incontinence and dysfunctional elimination is a complex entity that negatively impacts the lives of a large number of children with devastating effect. Current practice and research addressing this issue is highly fragmented resulting in less than optimal care for this population. Current research supports the existence of multiple, interrelated associations between dysfunctional voiding and defecation dynamics, suggesting both are different manifestations of the same syndrome that when integrated and embedded within the context of psychological, social, environmental, and genetic factors provide the beginnings of a comprehensive theoretical framework addressing incontinence and/or dysfunctional elimination in children. There is a need for a comprehensive theory that allows for a translational, transdisciplinary, and

coherent approach to children with incontinence and dysfunctional elimination. Such a theory could serve as a guide for practice and future translational research. New theories need to provide an expansive and dynamic guide that facilitates temporal exploration of symptom clusters, symptom trajectories, and interventional outcomes (Brant, Beck, and Miaskowski, 2010). Probabilistic Epigenesis provides a metaparadigm that allows for the derivation of a theory of pediatric incontinence and dysfunctional elimination within a developmental framework that provides a coherent, coordinated, and holistic approach to research and improves the outcomes and lives of effected children.

Proposed Program of Research

As a starting point in the pediatric continence program of research, the Integrative Model of Pediatric Incontinence and Dysfunctional Elimination will be used to evaluate epigenetic trends testing horizontal and vertical coaction in three of the four constructs including neural activity, behavior, and environment as shown in Figure 1-2. Genetic activity will be considered a constant backdrop. The research will explore the vertical coaction of environment and neural activity on behavior at a single point in time and the vertical and horizontal coaction of environment and neural activity on behavior longitudinally.

Problem

ACE therapy administered through a catheterizable stoma surgically placed in the cecum has helped children with intractable fecal incontinence attain continence for stool. There are a number of retrospective studies demonstrating the effectiveness of ACE therapy and several prospective studies that demonstrate improvement in quality

of life following an ACE procedure. There are case reports detailing morbidity and mortality associated with different flushing regimens. However, there are no prospective trials comparing the effectiveness of different flushing regimens on continence.

The catheterizable stoma used for the antegrade administration of enema solution is frequently made by bringing the appendix out through the abdominal wall or by placing a skin-level device (button) into the cecum. The appendix and cecum have significant amounts of gut-associated lymphoid tissue, have high concentrations of microbiota, and serve an essential immune function. ACE therapy administration through the appendix or into the cecum has the potential to disrupt the gut microbial ecosystem causing dysbiosis and immune dysfunction. The effects of such ACE administration on colonic microbiome and mucosal immunity have not been investigated.

Three aims with corresponding hypotheses have been identified as a starting point for a program of research and theory development in pediatric incontinence framed by the Integrative Model of Pediatric Incontinence and Dysfunctional Elimination. ACE therapy including identification of an optimal dosing regimen is the independent variable representing the Environment construct. The bowel, gut microbiome, electrolyte balance, and immune function represent the Neural Activity construct. Continence and quality of life represent the Behavioral Construct. This study will explore vertical coactions between Environment (ACE therapy) and Neural Activity (bowel) on Behavior (continence and quality of life). Exploration of horizontal coactions between Environment and Neural Activity include measurement of side effects, and changes in electrolyte balance, the gut microbiome, and markers of mucosal immune function.

Purpose, Aims, and Hypothesis

The purpose of this prospective pilot study was to compare two distinct flushing regimens, one high volume saline flush and one low volume USP glycerin flush, in the immediate postoperative period in children requiring ACE therapy. Findings from this study provided a comparative analysis of these two regimens serving as a starting point to guide practice and serving as a foundation for prospective, randomized, controlled trials. The aims of this study were to (a) identify the minimal administration frequency, titration time to reaching effective dose, and cost of two flushing solutions; (b) compare which solution at an optimum dose is delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence and quality of life; and (c) to collect and process stool for future analysis to determine if administration of antegrade enema solution through an appendicostomy/cecostomy affects gut microbiota and immune function.

Null Hypotheses for Aim 1

1. There will be no differences in frequency of administration necessary to gain and maintain continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (primary aim).
2. There will be no differences in titration time to reaching effective dose between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
3. There will be no differences in cost between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypotheses for Aim 2

1. There will be no difference in continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (primary aim).
2. There will be no difference in infusion time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
3. There will be no difference in procedural time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
4. There will be no difference in side effects between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
5. There will be no difference in parent/patient satisfaction as measured by the Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida (FIC QOL) between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypothesis for Aim 3

1. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on colonic microbiota in children requiring antegrade continence therapy.
2. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on mucosal immune function in children requiring antegrade continence therapy

In Chapter 2, research results from studies evaluating the effects of ACE flushing regimens on continence, side effects, and gut microbiota are reviewed. Chapter 3 reviews the design, measures, and analysis utilized in this study. Chapter 4 reports the findings from this study. Chapter 5 contains a discussion of findings.

Bidirectional Influences in Probabilistic Epigenesis

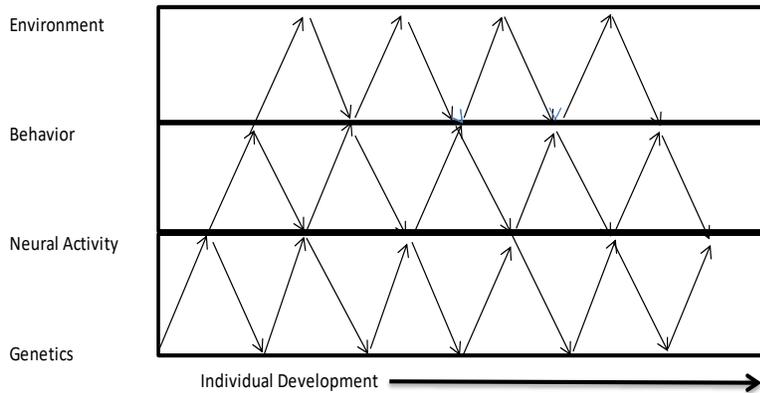


Figure 1-1. Gilbert Gottlieb's Model of Probabilistic Epigenesis. Depiction of the bidirectional coaction between levels of functioning over the course of development from "Individual Development and Evolution" by G. Gottlieb, 2002, Oxford University Press, New York, N.Y., p186. Copyright date by Taylor Francis Group LLC Books. Reprinted with permission.

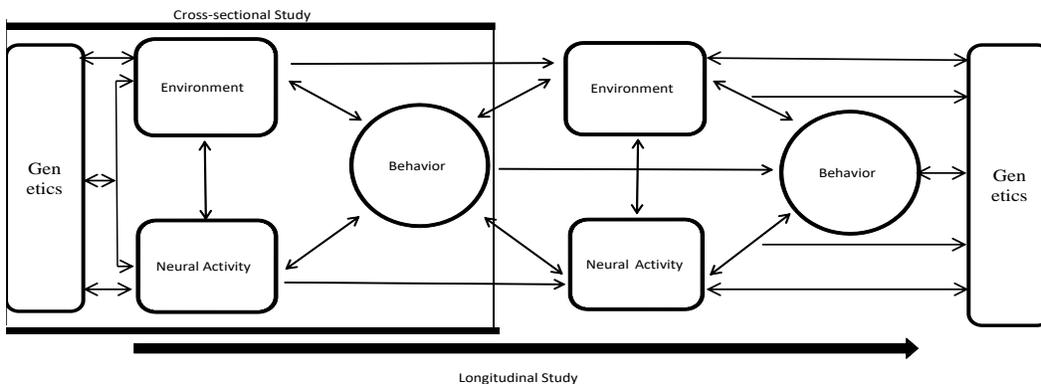


Figure 1-2. Reduced Model of Probabilistic Epigenesis adapted to test pediatric continence. Depiction of the bidirectional coaction between levels of functioning over the course of development modified to represent vertical interaction of genetics, neural activity, and environment on behavior at a single point in time and the vertical and horizontal interaction of genetics, neural activity, environment and behavior longitudinally. Adapted from "Individual Development and Evolution" by G. Gottlieb, 2002, Oxford University Press, New York, N.Y., p186. Copyright date by Taylor Francis Group LLC Books. Modified and reprinted with permission.

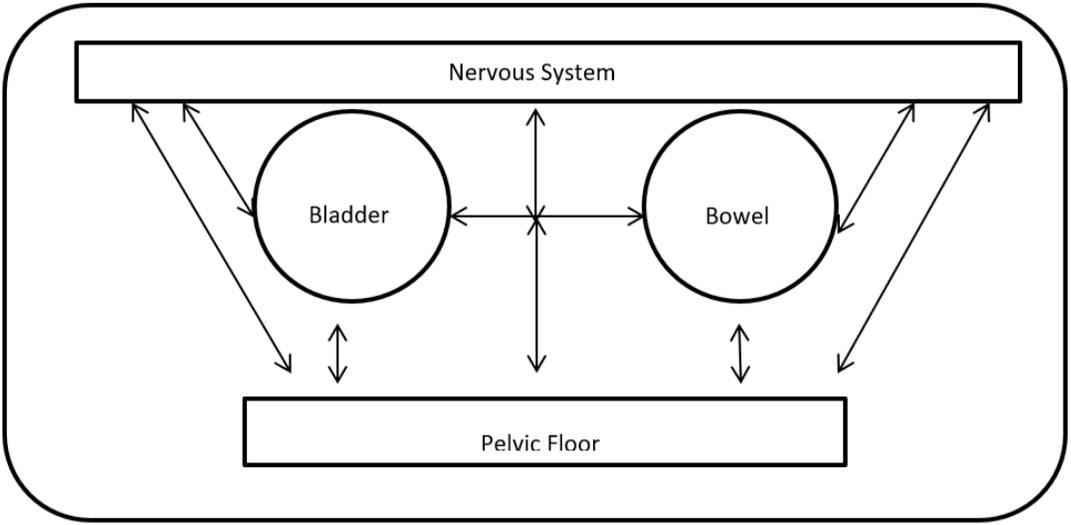


Figure 1-3. Model depicting specific interactions between organ systems contained within the neural constitutive element specific to incontinence and dysfunctional elimination depiction of the bidirectional coaction between levels of functioning over the course of development modified to specify the interactions between organ systems contained within the neural constitutive element specific to incontinence and dysfunctional elimination adapted from “Individual Development and Evolution” by G. Gottlieb, 2002, Oxford University Press, New York, N.Y., p186. Copyright date by Taylor Francis Group LLC Books. Modified and reprinted with permission

CHAPTER 2 LITERATURE REVIEW

Overview

Fecal incontinence in children past the expected time of toilet training has been associated with increased anxiety and depression, more social problems, worse school performance, an increased incidence of abuse and bullying, and a significantly poorer quality of life (Kaugars et al., 2010; Kovacic et al., 2015; Youssef, Langseder, Verga, Mones, & Rosh, 2005). It is particularly difficult to manage fecal incontinence using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumor, megarectum, and slow transit constipation. The Malone or antegrade continence enema (ACE) procedure was popularized over 20 years ago as a means of helping children with intractable fecal incontinence attain stool continence. A catheterizable stoma from the abdominal wall into the cecum is constructed using the appendix, a tubularized portion of the bowel, or a low-profile button device. The stoma allows for antegrade administration of enema solution into the colon.

Studies Addressing the Effectiveness of ACE Therapy in Promoting Continence

A number of retrospective studies evaluate overall continence rates in children following ACE therapy. Findings from these studies are highly variable. Short term reported success rates range from 55.7 to 98% with long term outcomes ranging from 14 to 41% abandonment rate at 5 years to a mean time to first relapse being as long as 121.9 +/- 29.7 months (Aspirot, Fernandez, Di Lorenzo, Skaggs, & Mousa, 2009; Bani-Hani, Cain, King, & Rink, 2008; Bassonet et al., 2014; Becmeur et al., 2008; Chu, Balsara, Routh, Ross, & Wiener, 2013; Church, Simah, Wild, Teitelbaum, & Ehrlich, 2017; Curry,

Osborne, & Malone, 1999; Chong et al., 2016; Dey et al., 2003; Dolejs, Smith, Sheplock, Croffie, & Rescorla, 2017; Driver et al., 1998; Freeman et al., 2014; Hoekstra et al. 2011; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Large et al., 2017; Levitt, Soffer, & Pena, 1997; Marshall, Hutson, Anticich, & Stanton, 2001; Matsuno et al., 2010; Mousa et al., 2006; Ok & Kurzrock, 2011; Peeraully et al., 2014; Randallet al., 2014; Siddiqui, Fishman, Bauer, & Nurko, 2011; Sinha et al., 2008; Thomas et al., 2006; VanderBrink et al., 2013; Yardley et al., 2009). The high variability in ACE effectiveness outcomes in these retrospective studies could have been due to a number of confounds. Study designs were uniformly retrospective and sample size was highly variable and often small. There was wide variability in the underlying organic and structural disorders in the study populations of children who received ACE therapy including, but not limited to, neurogenic etiologies including myelomeningocele, lipoma, tethering, caudal regression syndrome, spinal cord tumor or trauma, and transverse myelitis; Hirschsprung's disease; anorectal malformation including imperforate anus, perineal fistula, vestibular fistula, vaginal rectal atresia without fistula, cloaca, and Curarino triad; 22q11 syndrome; cerebral palsy; prune belly syndrome; intractable fecal incontinence; slow transit constipation; intractable functional constipation; and colonic pseudo-obstruction. The extensive variability in organic, structural, and functional etiologies could affect outcomes. Additional potential confounds include subject age at the time of surgery; type and placement of the stoma; colonic transit time: presence of outlet dysfunction; type and timing of post-operative complications; caregiver and child motivation, compliance, and education; co-morbid conditions such as autism; mitochondrial disease; metabolic disorders; chromosomal abnormalities; VACTERL;

psychiatric disorders, etc.; and flush solution type, volume, and frequency of administration. Kuizenfa-Wessel, Mousa, Benniga, and Di Lorenzo (2016) published an overview of existing literature excluding articles without a clear definition of addressing ACE outcomes, sample size less than 20, and patients over 25 years of age. They identified 21 articles that met inclusion criteria. In addition, they surveyed 23 pediatric gastroenterologists and surgeons worldwide with acknowledged expertise in ACE therapy. The authors concluded there are differing opinions regarding indications for the procedure, preoperative evaluation, preferred flushing regimens, and definitions of success.

Two retrospective studies evaluated ambulatory status as a potential confounding variable for achieving continence in children with spina bifida receiving ACE therapy. Vande Velde and colleagues (2007) evaluated 80 children ranging from 5 to 18 years of age and did not find an association between level of lesion or ambulatory status and continence rates. Large et al. (2017) evaluated 115 children greater than 8 years of age who had undergone an ACE procedure and found 65.1% of ambulatory children achieved full fecal continence compared to 44.2% of their non-ambulatory counterparts.

Two retrospective studies were identified that addressed age at the time of ACE stoma construction and initiation of flushing. Stenstrom, Graneli, Salo, Hagelsteen, and Arnbjornsson (2013) presented findings from a retrospective study of 21 of 164 children with anorectal malformation who underwent appendicostomy for bowel management. Median age at the time of stoma construction was 4 with a range of 1 to 6 years. Observation time ranged from 0.5 to 14 years. The goal was for every child with

anorectal malformation to achieve fecal continence by the start of school. The major indication for ACE stoma construction surgery was persistent fecal incontinence and failure to adhere to a retrograde enema regimen. Postoperatively, 15/17 children (88%) were continent for stool. Post-operative infection rate was reported at 29%, higher than infection rates reported in older populations. However, stomal stenosis (12%), surgical revision (6%), and retrograde stomal leakage (0%) were considerably lower than respective rates reported in older populations. Sixteen families were satisfied with the appendicostomy. The remaining family felt it was too early to evaluate. Freeman, Simha, Jarboe, Ehrlich, and Teitelbaum (2014) presented findings from a study of 35 children to determine if ACE stoma construction and initiation of flushing prior to starting school (< 6 years of age) would improve functional stooling and quality of life. Children were divided into two groups based on age at which ACE therapy was initiated. Families of children who had initiation of ACE therapy prior to 6 years of age were compared to families of children who had initiation of ACE therapy after 6 years of age. All were prospectively surveyed using a 17-item questionnaire addressing stool habits and a 7 item Pediatric QOL survey, both of which had been adapted from previous tools. Psychometric properties of the original and adapted tools were not addressed. Total stooling, defined continence, and stool pattern scores were calculated from the total survey. Of the 35 patients identified, 20 families could not be reached and one did not wish to participate, leaving 14 patients who were successfully surveyed. Of the 14 children surveyed, six were under 6 (3.68 +/-1.02) years of age and eight were older than 6 (10.30 +/- 1.02) years of age at the time of surgery. Absolute continence rates could not be determined based on reported results. However, stooling and continence

scores were significantly better in the <6 year old group. QOL scores showed significant improvement post-operatively in both groups, with the < 6 age group showing a significantly greater improvement over the ≥ 6 age group.

Many different ACE flushing regimens are prescribed in practice, including licorice root, mineral oil, treacle/milk mix, tap water, normal saline, twice normal saline, polyethylene glycol solution, phosphate soda solution, bisacodyl, and USP liquid glycerin (Bani-Hani et al., 2008; Becmeur et al., 2008; Chu et al., 2013; Dolejs et al., 2017; Hyde, Coulthard, Jaffray, Valley, & Harding, 2008; Marshall et al., 2001; Large et al., 2017; Matsuno et al., 2010; Siddiqui et al., 2011; Stenstrom et al., 2013; Yerkes et al., 2001; Youssef, Barksdale, Griffiths, Flores, & Di Lorenzo, 2002; Vande Velde, 2007) . A number of studies were identified that to some extent report flushing solution use, dosing, frequency of administration, and procedural time. A summary of this data is contained in Table 2-1.

Kuizenga-Wessel et al. (2016) reported survey data of 23 pediatric gastroenterologists and surgeons with expertise in ACE therapy. They found that 16 (70%) started the flushing regimen with saline; 5 (22%) tap water, polyethylene glycol, or sodium-phosphate; and only 2 (8%) a stimulant using either USP glycerin or bisacodyl. Nineteen respondents added a stimulant only when the initial antegrade solution was not effective. Respondents' preference for enema flush volume was variable. Forty-eight percent started at 10-20 mg/kg, 22% used 0-249 mL, 22% used 250-500 mL, and 8 % tailored the volume to the child. All but one respondent began the regimen with daily flushing. An infusion duration ranging from 0 to 15 minutes was preferred by 62% of respondents, with 38% preferring 16 to 30 minutes. A total

procedural time (inclusive of flush infusion ranging from 30 to 60 minutes following flush infusion was preferred by 65% of respondents, with 35% limiting procedural time to under 30 minutes.

A single prospective study was identified that addressed comparative effectiveness of ACE flushing regimens. This study utilized colonic manometry to compare motor response of three stimuli (meal, antegrade saline infusion, and antegrade bisacodyl administration) on the number of high-amplitude contractions and motility index in 13 pediatric patients. Findings in this study demonstrated that there was no significant difference in parameters after ingestion of a meal or saline infusion at 10 to 20 mg/kg with a maximum volume of 700 mL. Bisacodyl at a dose of 0.2 mg/kg with a maximum dose of 10 mg significantly increased the motility index and high-amplitude propagated contractions when compared to meal or saline (Gomez, Mousa, Liem, Hayes, & Di Lorenzo, 2010). Bani-Hani, Cain, King, and Rink (2008) identified timing of accidents as the most important factor in troubleshooting flushing regimen failure. Siddiqui et al. (2011) postulated an inverse relationship between increased commode time and long term ACE adherence. A number of prospective trials demonstrate significant improvement in somatic functions, psychosocial functioning, and quality of life (QOL) after an ACE procedure (Aksnes et al., 2002; Churchet al., 2017; Hoekstraet al., 2011; Mousa et al., 2006; Tiryaki, Ergun, Celik, Ulman, & Avanoğlu, 2010).

Identifying a successful flushing regimen is determined by individual clinician preference and often requires multiple attempts before success is achieved. There are no prospective studies comparing the effectiveness of type, dose/volume, or frequency of different flushing regimens in preventing incontinence to inform practice.

Studies Addressing Side Effects Associated with ACE Therapy

Several case reports detail morbidity and mortality associated with a particular flushing solution, including hypocalcemia and hyperphosphatemia, following retrograde administration of phosphate enemas in children (Helikson, Parham, & Tobias, 1997; Ismail, Al-Mutairi, & Al-Anzy, 2000), hypernatremia following retrograde administration of hypertonic saline (Schreiber & Stone, 1999), and water intoxication following retrograde enema therapy using tap water (Chertow & Brady, 1994). Solution composition, volume, retention time, and underlying electrolyte imbalances are all factors that increase morbidity and mortality (Yerkes et al., 2001). Several case series describe a variety of side effects of flushing regimens, including pain with stomal intubation, nausea, vomiting, abdominal cramping, sweating, dizziness, and pallor (Bani-Hani et al., 2008; Dey et al., 2003; King et al., 2005).

A single retrospective study evaluated the safety of a specific flushing solution. Yerkes et al. (2001) evaluated the safety of tap water antegrade flush in 71 patients using serum electrolytes obtained pre- and post-operatively as the dependent variable. The timing and place of the laboratory evaluation varied widely due to the retrospective nature of their study. The ACE flush was administered at home every day or every other day at volumes ranging from 300 to 1,000 mL. Clinically insignificant electrolyte abnormalities attributed to the flush included minor deviations in serum sodium or serum chloride in 18 patients. More significant hyperchloremia (107 to 113 mmol/L. with upper limits set at 105 mmol/L) was noted in 12 patients. Significant hypernatremia and hyperchloremia was noted in a single subject who used softened water to flush, which corrected when the flush was changed to untreated water.

A single prospective study was identified detailing and comparing adverse effects associated with different antegrade flushing regimens. ACE flush using twice normal saline has been associated with unpleasant symptoms including thirst and generally feeling unwell during the flush. Hyde et al., (2008) conducted a within subjects double-blind crossover study on four children comparing antegrade administration of three commercially prepared flush solutions: water, normal saline, and twice normal saline. Each solution was administered on separate days. Each flush was spaced two days apart to prevent the potential confound of carry-over effects. Each child was randomized to one of six treatment sequences to prevent the potential confound of order effects. The study protocol was complex. Blood pressure, pulse, peripheral toe temperature, and capillary refill were measured at baseline prior to initiation of flush and at 20, 40 and 60 minutes following flush administration. Blood was drawn at baseline, 20, and 60 minutes for hemoglobin, serum creatinine, electrolytes, albumin, total protein, osmolality, renin activity, and arginine-vasopressin concentration. Weight was measured before and after lavage with urine collected for electrolytes, creatinine, and osmolality, facilitating calculation of fractional excretion of water and sodium. Flush effluent was measured and analyzed for sodium and osmolality. Symptoms were recorded in real time. A paired t-test was used to analyze within-subjects data. Between subjects data was analyzed using an unpaired t-test. Findings in this study demonstrated no change in any of the measured parameters with saline flush. Using water to flush resulted in a transient decrease in plasma osmolality of 7.3 mmol/kg at 20 minutes (lower level than NS, $p=0.02$) and a decrease in urine sodium and osmolality not observed with administration of the other flushing solutions. Administration of twice

normal saline resulted in an increase in plasma sodium of 2.5 mmol/l at 20 minutes (higher than NS, $p=0.03$), a rise in plasma proteins of 2.3 g/l, and a decrease in the flush effluent sodium concentration by one-third, suggesting a shift of approximating 10 mL/kg of plasma water into the colonic lumen. The plasma sodium level increase of 2.5 mmol/l was insufficient to attribute the increase to sodium absorption alone. The drop in effluent sodium concentration was attributed to two processes including colonic absorption of sodium from the twice normal saline and water shifting from plasma to the colonic lumen. Two children reported thirst suggesting the potential start of dehydration. Thirst occurred only during the twice normal saline flush.

The same investigators also evaluated the composition of home-made saline and twice normal saline flush solution from five families. Each family collected five independent samples of both home-made flush solutions for sodium assay. Investigators found sodium concentrations in both the home-made normal saline and twice normal saline solutions were 45% higher than the target value with a wide within-family variation. Investigators in this study found 30 mL/kg of normal saline was as effective as 20 mL/kg of twice normal saline in achieving continence without the potential hazards of using a large volume of hypertonic solution (Hyde et al., 2007).

Studies Addressing Gut Microbiota

The catheterizable stoma used for antegrade administration of enema solution is frequently made by bringing the appendix out through the abdominal wall or by placing a button into the cecum. The appendix and cecum have significant amounts of gut-associated lymphoid tissue (GALT), have high concentrations of microbiota, and serve an essential immune function (Penders, Stobberingh, Van den Brandt, & Thijs, 2007).

The appendix has higher concentrations of microbial biofilms compared to other areas of the colon, serves as a safe-house for symbiotic gut flora, and functions to preserve gut microbiota through re-inoculation with normal flora following gastrointestinal infections (Bazar, Lee, & Yun, 2004; Bollinger, Barbas, Bush, Lin, & Parker, 2007; Gebbers & Laissue, 2004; Kawanishi, 1987; Smith et al., 2009).

The human gut contains 10^{14} bacteria (Jia, Li, Zhao & Nicholson, 2008). Bacterial composition varies along the bowel axis, with further differentiation of luminal or adherent microcolonies that lead to development of biofilms. Factors that influence microbial composition include pH, transit time, bile acids, pancreatic enzymes, mucus composition, nutrient consumption, medication, environment, bacterial adhesion capacity, and metabolic capacity. The most important function of the gut microbiome is colonization resistance, which is accomplished through competition for nutrients and secretion of bacteriocins (Penders et al., 2007). Microbiota function to degrade dietary substances and enhance digestive efficiency while providing nutrients to the microbes themselves. These microbes are essential for host physiology but in turn pose a threat of opportunistic invasion by resident bacteria with resulting pathologies.

The immune system maintains a delicate homeostatic and symbiotic balance protecting host-microbial ecosystem dualism through the mechanism of stratification and compartmentalization. Stratification minimizes direct contact between gut microbiota and the intestinal epithelial surface. Compartmentalization utilizes anatomic adaptation to confine bacteria that breach the mucosal surface to limit systemic immune system exposure. Immune system response to microbiota plays an important role in host vulnerability to disease. Host-microbial symbiosis and dysbiosis are extraordinarily

complex phenomena. The immune system controls the composition, diversity, and location of gut microbiota while the microbiota has a profound effect on lymphoid tissue formation and immune system development (Forchielli & Walker, 2005). Microbiota have been shown to have protective properties against autoimmune disease and, conversely, can cause inflammation and metabolic dysregulation in an immune compromised host (Hooper, Littman, & Macpherson, 2012; Nicholson et al., 2012).

Disruptions in gut microbiota due to diet, including infant feeding regimens, microbial inoculations, and antibiotics, can alter mucosal immunity and mechanisms involved in regulating immune tolerance outside the gastrointestinal tract (Noverr & Huffnagle, 2004). Microbiome composition imbalance has been associated with diverse disorders including cancer, inflammatory bowel disease (IBD), atopy, asthma, obesity, and autism (Barker, 2012). The appendix is a secondary lymphoid organ that is an important constituent of the mucosa-associated lymphoid tissue system; it has a pronounced function in children. Neonatal appendectomy in rabbits impaired mucosal immunity (Dasso & Howell, 1997). Long-term effects of appendectomy include moderate immune function changes in part due to a decrease in immunoglobulin production, particularly IgA; increased risk of Crohn's disease; and a moderately increased risk of acute myocardial infarction (Anderson, Olaison, Tysk, & Ekblom, 2003; Andreu-Ballester et al., 2007; Janszky, Mukamal, Dalman, Hammar, & Ahnve, 2011). ACE therapy administered through the appendix or into the cecum has the potential to disrupt the gut microbial ecosystem causing dysbiosis and immune dysfunction. Effects of appendiceal or cecal administration of ACE on colonic microbiome and mucosal immunity has not been investigated.

Significance and Innovation

A large body of literature demonstrates ACE therapy can be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in quality of life. However, findings regarding effectiveness are highly variable. This variability may be due to what is used to flush. No prospective trials compare the efficacy and adverse effects of different flushing regimens. No studies evaluate the effects of appendicostomy/cecostomy flush on gut microbiota. Findings from this study provided a comparative analysis of two different regimens and will serve as a starting point to guide practice and provide a foundation for additional prospective, randomized, controlled trials.

Table 2-1. Summary of Published Flush Solution, Dosing, Administration Frequency, and Procedural Time Outcomes

Authors	# of Subjects	Methods	Solution	Volume mL/kg	Total Volume (mL)	Infusion Time in Minutes	Procedure Time in Minutes	Frequency of Administration
Bani-Hani, A. H. et al., 2008	n = 236	Retrospective chart review	Tap water additives 10.5% mineral oil MiraLAX USP glycerin		100 - 1,000 □ 642			1 x day
Becmeur, F. et al., 2008	n = 22	Survey - patient recall			250 - 1000mL □ 700 mL	5 - 60 □ 25	10 - 90 □ 80	4 X week (qd - q1wk)
Chu, D.I. et al., 2013	n = 23	Retrospective chart review	Glycerin		30 mL of USP glycerin mixed in 50 mL of tap water followed by 30 mL of tap water		M 60	
Dolejs, S.C. et al., 2017	n = 93	Retrospective chart review	Normal saline + Bisacodyl if slow motility, USP glycerin, Polyethylene Glycol	IQR 16 - 30 □ 22 increase to IQR 18 - 35 □ 24				

Table 2-1. Continued.

Authors	# of Subjects	Methods	Solution	Volume mL/kg	Total Volume (mL)	Infusion Time in Minutes	Procedure Time in Minutes	Frequency of Administration
Large, T.et al.,2017	n = 115	Data mined from clinical records of patients in a separate QOL study	7% required additives including Polyethylene Glycol, Castile Soap, glycerin, castor oil and senekot				40 - 60 \bar{x} 45	
Levitt, M.A.et al., 1997	n = 20	Retrospective chart review	Normal saline				30 - 45	
Matsuno, D.et al., 2010.	n=25	Retrospective chart review	Tap water		400 - 1500		36.7 +/- 23.1	4.2 +/- 4.0 x wk Range 1-7 x wk
Peeraully, M.R., 2014	n=40	Retrospective	Combination of saline & phosphate enema solution in 85% of sample Variety of flush solutions in 15% of sample		808 +/- 612		20 - 60	1 x d in 77.5% of patients 1 x qod in 15% of patients

Table 2-1. Continued.

Authors	# of Subjects	Methods	Solution	Volume mL/kg	Total Volume (mL)	Infusion Time in Minutes	Procedure Time in Minutes	Frequency of Administration
Randall, J., 2014	n=203	Maintained a prospective data base 1998-2013	Normal Saline (48%) Klean-Prep (29%) Bisacodyl (22%) Not Specified (1%)					4 x wk Range 1–7 x wk
Siddiqui, A. A.et al., 2011	n=105	Retrospective chart review	Started on Saline (31%) Changed to Golytely if needed, if inadequate response used additives 34%, including Bisacodyle 28% or other 7% (glycerin, phosphosoda, magnesium citrate)	23 +/- 0.7	847 +/- 55	12.1 +/- 1.2	51.7 +/- 3.5	5 +/- 0.3 d/wk
Sinha, C.K., 2008	n=48	Retrospective Compared outcomes of 48 patients to the pooled outcomes of 676 patients from 24 studies published from 2002 – 2007	Bisacodyl Normal Saline Golytely Soap Water Glycerol and other flush combinations		180 - 3,000 M 516		5 - 60 M 42	

Table 2-1. Continued.

Authors	# of Subjects	Methods	Solution	Volume mL/kg	Total Volume (mL)	Infusion Time in Minutes	Procedure Time in Minutes	Frequency of Administration
Stenstrom, P.et al., 2013	n = 21	Questionnaire - patient recall	Saline, Klyx Movicol	\bar{x} 35 Range: 11-80	\bar{x} 85 Range: 200 - 3000		15 - 60 M 45	1 x day (3xd - 1 x wk)
Vande Velde, S.,et al., 2007	n = 16	Retrospective Chart review	Tap water				60 - 240 \bar{x} 150 weekly	
Yerkes, E. B.,et al., 2001	n = 71	Retrospective	Tap water		300 - 1,000 \bar{x} 550			1 x day to 1 x qod

CHAPTER 3 METHODOLOGY

Design

This prospective study utilized a repeated measures, single subjects alternating treatments A-B-C-B'-C'-B₁' withdrawal design in which all subjects were tested under all conditions and each subject acted as his or her own control (Gast, 2010; Janosky, Leininger, Hoerger, & Libkuman, 2009; Kazdin, 2011; Portney & Watkins, 2009). A within subjects cross-over design was embedded in the dosing B-C and maintenance B'-C'-B₁' treatment comparison phase of the study (Chow & Brady, 1994; Janosky, Leininger, Hoerger, & Libkuman, 2009; Jones & Kenward, 2003) . Subjects were randomly assigned to one of two treatment sequences to control for the possibility of order effects. The treatment was replicated across 5 subjects randomized to 2 treatment groups. The patient and investigator could not be blinded to flushing regimens contents due to the difference in dosing volume. The high-volume flushing regimen was comprised of normal saline alone. The low volume regimen was comprised of USP glycerin with a small volume of normal saline used as diluent. Volume and frequency of administration was structured to find the lowest dosing of each regimen sufficient to maintain continence. Baseline data A served as the control and was obtained pre-operatively. Ethics prohibited return to a no-treatment baseline phase as this would have resulted in multiple daily episodes of fecal incontinence. The first B - C phase of the study evaluated dose-response relationship and was used to identify the optimal dose and frequency of ACE administration for normal saline and USP glycerin with a very low volume of saline as diluent. When the optimal dose was identified, the child continued on that dose for two weeks to insure treatment stability and effectiveness. If

continence was not achieved within the dosing guidelines, the child was trialed on the alternate flushing regimen to determine if continence with minimal side effects could be achieved and if so, at what dose, but did not continue in the maintenance phase of the study. To prevent statistical bias from subject loss due to treatment failure, each child who completed the dosing phase was randomized to a second treatment sequence once they had achieved continence with minimal side effects in the dosing phase of the study. The last phase of the study (B'-C'-B₁') compared continence, presence and severity of side effects, quality of life, and flush effects on stool microbiome of both regimens administered for consecutive weeks each (B'-C') at optimal dose and administration frequency. The final two weeks of the study (B₁') consisted of reintroduction of the flush administered in the B' phase.

Rationale for Utilization of Within Subjects Designs

Any research design is a tool used to answer a question. Strategies, design choice, and use of design elements should be based on how best to answer the question at hand (Kazdin, 2011). The purpose of experimental design is to control the effects of random error and bias (Piantadosi, 2005). However, no design completely eliminates either. Whatever the chosen research method, good design and procedures are necessary to prevent confounding and improve causal inference (Shadish et al., 2002). Skillful statistical analysis cannot salvage significant design faults or increase the validity of a poorly designed study (Janosky et al., 2009). Skillful utilization of design elements can increase internal validity and causal inference (Cook & Campbell, 1979). Both single subject and between group research make and test predictions about treatment effects, the first by evaluating treatment effects on an individual, the second

by addressing group mean and variance (Kazdin, 2011). Single subjects and group designs, in their most rigorous form, decrease the plausibility that rival hypotheses resulted in the experimental outcome, improving the quality of inference. (Cook & Campbell, 1979; Kazdin, 2011; Shadish, Cook, & Campbell, 2002). A randomized controlled trial (RCT) is considered the gold standard for intervention research (Piantadosi, 2005). However, a RCT is not the only standard for causal inference (Kazdin, 2011). Reliance on large numbers makes application of a RCT with small groups or rare disease problematic (Janosky et al., 2009).

The focus of this research involved instillation of a solution through an appendiceal stoma. A procedure used for over a century and widely popularized over 20 years ago. Case reports and retrospective studies detail widely divergent effectiveness rates (Bani-Hani, Cain, King, & Rink, 2008; Dey et al., 2003; Mousa et al., 2006; Siddiqui, Fishman, Bauer, & Nurko, 2011; Yardley et al., 2009). The literature and involved clinicians have identified the need for prospective trials comparing ACE flushing regimens. Despite the identified need for prospective trials, only two have been identified to date. The first addressed comparative effectiveness of ACE flushing regimens utilizing colonic manometry to compare the motor response of three stimuli (meal, antegrade saline infusion, and antegrade bisacodyl administration) on the number of high-amplitude contractions and motility index in 13 pediatric patients (Gomez et al., 2010). The second compared safety parameters of a single flush of saline, twice normal saline, and tap water (Hyde et al., 2008). The lack of prospective trials is in large part because the small size and heterogeneity of this population does not lend itself to a large N study. Many

clinical questions go unanswered due to over reliance on RCT large N methodology (Kazdin, 2011). This population is an exemplar of that problem.

This study compared two flushing regimens utilizing a cross-over design embedded in a single subject A-B-C-B'-C'-B₁' design. Both methods are experimental and lay a foundation for causal inference (Chow & Liu, 2014; Elder, 1997; Portney & Watkins, 2009). In both designs the subject acted as his or her own control minimizing within subject variability and ensuring the highest possible degree of equivalence across treatment conditions, thereby, allowing greater precision and efficient estimates of treatment effects increasing internal validity and causal inference (Janosky et al., 2009; Piantadosi, 2005; Portney & Watkins, 2009). In both methods subjects were randomized to treatment sequence. Randomization decreased the threat of order effects in both methods, increased group equivalency in the cross-over design, minimized variability in measurement due to subject or period differences, and increased internal validity and causal inference (Chow & Liu, 2014; Jones & Kenward, 2003).

Single subjects design is an inductive, experimental methodology with controlled introduction and manipulation of an independent variable. Single subjects design promotes exploration of inter-subject variability without the introduction of error inherent in group methodology in the absence of subject homogeneity. This method allows for experimentation in situations in which a limited number of subjects are available, a circumstance that significantly limits the usefulness of large N studies. It allows for isolation of individual response to interventions and identification of valuable information from outliers that would be obscured or lost in group methodology. Single subjects design also allows for time series observations of response, providing continuous and

often a more accurate representation of the dependent variable of interest that may be compromised when the data are collected as an isolated snapshot in group methods (Elder, 1997).

This study was ideally suited to single-subject repeated measures design, because children requiring an ACE procedure comprise a very small population with widely disparate anatomic and physiologic causative factors making sample homogeneity difficult. Inclusion of heterogeneous subjects allowed differentiation of subject characteristics that could have impacted response to treatment. The design allowed for frequency, volume, and dose adjustment of each flushing regimen when indicated. The ability to adjust the treatment regimen facilitated dose response comparison and aided in identifying which flushing regimen required the minimal dose and administration frequency, was accomplished in the least amount of time, and had the fewest side effects while achieving continence. Because this design allowed for repeated measurement over time, it was particularly helpful when studying comparisons between several treatments and was more sensitive to variations in treatment response that might otherwise have been missed using group methodology (Gast, 2010; Janosky et al., 2009; Kazdin, 2011).

Subjects were limited to children who were scheduled for a cecostomy or appendicostomy, ensuring their gut was naïve to the effects of a flushing regimen and allowed for a true no-treatment baseline. Flush effects were reversible, which made this intervention amenable to a withdrawal design. There were no known carry-over effects associated with either flushing regimen that would have impacted treatment effect on continence. With regard to continence, the subject's bowel returned to a physiologic

baseline between treatments, negating the need for a wash-out period. Specimen collection facilitated comparison of treatment effects on gut microbiota, electrolytes, and stool calprotectin that occurred after an active wash out period at the completion of each flushing regimen, negating any carry-over effects. Given the pragmatic issues involved in answering the research question at hand, the chosen methods and design elements strengthened the demonstration of the counterfactual and made implausible potential threats to validity, lending credence to the assumption that intervention effects were due to the treatment and not random error or bias.

Inclusion and Exclusion Criteria

The goal in this study was to recruit six children, ages 3 to 12 years, recruited from subspecialty clinics at Nemours Children's Clinic (NCC) and the Pediatric Spinal Defects Clinic in Jacksonville, Florida. Five children, ages 3 to 7 years, were recruited. Children were selected by purposive sampling and included those who were scheduled to have ACE stoma construction and required regular antegrade enema administration to maintain continence. Children with preexisting electrolyte imbalance, chronic high rectal tone, quadriplegia, renal or cardiac disease, or those who required prophylactic antibiotics, could not communicate, or had significant cognitive delay that would interfere with their ability to fully participate in the study were excluded from the study. In addition, parents of participants were required to have English language competency and a willingness and ability to participate in administration or oversight of the flushing regimen and data collection for a minimum of twelve consecutive weeks.

Regulatory Considerations and Study Expenses

This study conferred no greater than minimal risk as categorized by the National Institutes of Health (National Institutes of Health [NIH], 1998). However, it involved vulnerable subjects and required full Institutional Review Board (IRB) approval with legal guardian informed consent by at least one parent and child assent for children age 7 and above (Knox & Burkhart, 2007; Pieper, 2008). In addition, although normal saline and USP glycerin have been used for over two decades in the clinical setting as ACE flushing solutions, administration of either solution through a ACE stoma is considered off-label use and therefore the study required FDA approval. It is a policy of NCC for any investigator conducting research involving patients seen in one of their facilities, to submit their proposal to the Nemours Children's Clinic Research Committee for review and approval of study design and analysis prior to submission to the IRB. The application for the Nemours Clinical Research Review Committee was successfully completed and approval was granted to proceed with the study following FDA and IRB approval. (Appendix J) The proposal was submitted to the FDA and an IND letter of exemption was obtained (Appendix K). The study was subsequently entered in the Clinicaltrials.gov website. An IRB application was completed for both UF and NCC. The UF IRB deferred to NCC because the study was to be completed at NCC. An IRB authorization agreement was completed, and the contract was signed by all relevant parties (Appendix L). As a part of the NCC IRB application process, a submission for research involving investigational drugs and biologics and management of research pharmaceutical products was completed and approved. (Appendix M). A submission was completed and successfully met the requirements for the Institutional Biosafety

Committee. Nemours IRB approval was obtained and renewed annually (APPENDIX N). The requirement for formal educational presentations to the clinical staff that would be involved with the children recruited in this study were successfully completed. The cost for five subjects and compensation to participants was approximately \$6029.34. An itemized account of costs is detailed in Table 3-1.

Subjects

Subject characteristics' data were collected and included: (a) age in years and months, (b) diagnosis, including specific description of level of spinal cord lesion or anal-rectal malformation when appropriate, (c) reason for ACE procedure, (d) surgical date for stoma formation, (e) type of stoma, (f) type of an enterostomal device, (g) comorbid conditions, and (h) results of previous pertinent test results, including upper gastrointestinal (GI) study with small bowel follow-through, unprepped barium enema, sitz marker study, and anorectal manometry. Parent characteristics included: (a) age, (b) ethnicity, (c) marital status, (d) family composition (including number of children or other individuals living in the household), (e) occupation, and (f) highest level of education.

Setting

Once parental consent and child assent were obtained, baseline data were collected daily for a minimum of 2 weeks prior to surgery. Baseline data included frequency and volume of episodes of fecal soiling, and frequency and severity of abdominal pain. Blood samples for electrolyte and stool for calprotectin and microbiota were obtained in the preoperative period. The initial stool specimens for analysis were collected prior to initiation of any pre-operative bowel prep. Postoperatively, the child

was randomly assigned to either the saline or USP glycerin protocol. A clinician from the surgical division at NCC met with the parent and child in the immediate postoperative period after surgical clearance had been obtained for initiation of the first flush. During the initial flush, the surgical clinician reviewed the flush protocol in detail, including materials and procedures. The child received the first antegrade infusion during that in-patient visit. A home visit was scheduled by the investigator for the initial home flush.

The reliability or accuracy and consistency of measurements were verified using interobserver agreement (IOA) during the first home visit. Gross method was used to calculate IOA comparing investigator and parent/child concurrent observations including flush time, procedural time, and number/level of soiling. IOA was calculated by dividing the smaller number by the larger number and multiplying by 100. At no point was there a significant discrepancy in observational accuracy, as demonstrated by a calculated IOA below 80% that would have required additional observer training to achieve a calculated IOA of 80% or higher (Gast, 2010). The initial plan was to ascertain IOA at the initial home visit and at each clinic visit. Procedural reliability was ascertained for each procedural variable to assure the intervention was being implemented as described in the methods section of the proposal. Procedural fidelity was calculated by dividing the number of observed behaviors by the number of planned behaviors and dividing by 100 (Gast, 2010). IOA and procedural fidelity for antegrade infusion and data collection techniques conducted by the parent was checked by the investigator and documented at the initial home visit. Procedural fidelity was completed prior to the start of each regimen change during the patients' clinic visit for sample collection. Error in

study design did not facilitate IOA during the subsequent clinic visits scheduled with each phase change.

Measures

Dependent variables at baseline included: (a) the number and severity of episodes of fecal soiling per day with fecal soiling scored based on volume of accidents with 0 = no accident, 1 = a small volume accident classified as a smear, 2 = a moderate volume accident not visible through clothing, and 3 = any accident visible through clothing, (b) frequency and severity of abdominal pain were recorded daily and measured using the Wong-Baker Faces Pain Rating Scale as the age-appropriate visual analog scale, (c) serum electrolytes, (d) stool for calprotectin, (e) quality of life measured by the FIC QOL, and (f) collection and processing of stool for future analysis utilizing molecular techniques for identification of 16SrRNA gene sequence in stool samples obtained pre-operatively to identify and quantify phylogenetic groups (Penders et al., 2007).

Dependent variables obtained post-operatively following initiation of cecostomy/appendicostomy flush in the dosing phase include: (a) flush administration time in minutes per flush, (b) total procedural time from start of flush to completion of colonic emptying in minutes per flush, (c) the number and severity of episodes of fecal soiling per day with fecal soiling scored based on volume of accidents with 0 = no accidents, 1 = a small volume accident classified as a smear, 2 = a moderate volume accident not sufficient in volume to be visible through clothing, and 3 = any accident of sufficient volume to be visible through the clothing, (d) timing of the accident when possible, (e) frequency and severity of abdominal pain was recorded daily and

measured using the Wong-Baker Faces Pain Rating Scale (WBFPRS) as the age-appropriate visual analog scale, (f) serum electrolytes collected at the end of each dosing phase, (g) stool for calprotectin collected at the end of each dosing phase, (h) collection of stool sample at the end of each dosing phase for downstream analysis utilizing molecular techniques for identification of 16SrRNA gene sequence to identify and quantify phylogenetic groups (Penders et al., 2007) and (i) quality of life measured by the FIQoL was to be completed at the completion of each maintenance phase. Only 1 of 5 subjects completed the maintenance phase so FIQoL data were not completed on 4 of 5 subjects. Fecal soiling score is detailed in Table 3-2. Dependent variables including type of sample or instrument, sample characteristics, and measurement and data level are explicated in Table 3-3.

Administration time in minutes per flush was defined as the time at which the tubing connected to the bag or syringe holding the flush solution was unclamped and the cecostomy fluid started to infuse into the patient to the time the infusion was completed (no more fluid left in the bag/syringe or tubing). The total procedural time was defined as the time the flush started to infuse into the subject and ended following passage of stool when the child had sat on the commode for 5 minutes with no additional stool passage. Both administration and total procedural times were measured using duration per occurrence direct observational with the recording completed by the parent. Total volume was recorded with each flush in mL. Accidents were defined as non-toilet elimination, which was tracked and tallied as the number of underwear soiled with stool with documentation of the severity of the accident and the estimated timing of

each accident using event recording. Dependent variables were measured and recorded by the parent using a data collection sheet specifically designed for this study.

Side effects were measured using the Wong-Baker FACES Pain Rating Scale. The WBFPRS has undergone extensive testing, is preferred by children, and has well established psychometrics in the pediatric population (Tomlinson, von Baeyer, Stinson, & Sung, 2010; Wong & Baker, 1988). The scale ranges from 0 (= very happy without pain) to 10 (= the worst pain imaginable). Each pain level is associated with a facial expression. The child is asked to choose the face that best describes his/her level of discomfort. The WBFPRS was used to evaluate the presence and severity of flush side effects including abdominal pain, abdominal cramping, and nausea. The parent was instructed to call if the child was having accidents or discomfort greater than a 4 on the WBFPRS associated with the flushing regimen. Documentation of severity of side effects was completed by the parent on a data-collection form.

The investigator kept results of all electronic communication from each subject that served as a research log that documented and detailed any event that caused a change in level, stability, or trend of the dependent variable not related to the intervention, for example, treatment with antibiotics or an intercurrent illness. The number of episodes of fecal soiling per week excluded accidents caused by viral, bacterial, or drug-induced enteritis; these were recorded and documented as confounds (Portney & Watkins, 2009).

The Fecal Incontinence and Constipation Quality of Life Measure in Children with Spinal Bifida (FIC QOL) was used to assess child and parental perception of social validity (Nanigian et al., 2008) The tool was administered preoperatively during the

baseline period and at the end of each flushing regimen in the comparative phase of the study. The FIC QOL is a 51-item questionnaire with established validity and reliability in families of children with spina bifida who are incontinent for stool. This instrument measures those aspects of daily living that are significantly impacted by fecal incontinence. Of the 51 items, four address subject and family demographics. The remaining 47 items are divided into seven groupings that include bowel program, diet, symptoms, travel and socialization, family relationships, caregiver support and emotional impact, and financial impact (Nanigian et al., 2008; Ok & Kurzrock, 2011). Only one subject was continent on both flushing regimens and completed the study, so the FIC QOL data were extremely limited. In addition to the FIC QOL, a simple qualitative question was directed to the children at the end of the study to ascertain which flushing regimen they prefer and why.

Materials

To ease access, each child had an appropriate low-profile button in place with the device specific access tube. Children with an appendicostomy had a Chait button in place with the device specific access tube. Materials for both flushing regimens are detailed in Appendix B. A monitored temperature storage area was identified for storage of the flushing solution. The cabinet was locked with restricted key access. The temperature in the storage cabinet was maintained between 15 and 30 degrees Centigrade and monitored on a continuous basis using a MCC USB 501-LCD thermometer which allowed for storage and downloading of temperature data. No excursions occurred in ambient temperature. A temperature monitoring log was kept. A dispensing log was kept detailing the solution, volume dispensed, and lot numbers each

time flush solution was dispensed to a subject. Funding was obtained to purchase the necessary equipment for isolating the microbiome DNA from samples to flash freeze for downstream identification of the 16SrRNA gene in stool samples.

High Volume Flush

For the high-volume flush, the normal saline was infused using a 1,000 mL enteral feeding bag with drip chamber and roller clamp. During the infusion, the bag was hung from an IV pole or a hook on the bathroom wall located 5 feet above the toilet seat on which the child sat during the flush. Tubing from the enteral feeding bag was hooked to the low profile device access tubing and primed with the high volume flush to remove all air in the tube prior to hooking the access tubing to the low profile device and infusing the solution. The step by step procedural directions for the parents were reviewed verbally and provided through written instructions. The written procedure is located in Appendix C. The written instructions were included in a parent notebook to reinforce parent teaching and served as a check-off list to document procedural integrity which was checked during the initial home visit and with each subsequent clinic visit at the time of every change in phase.

Low Volume Flush

For the low volume flushing regimen, a calibrated six ounce plastic container with a screw top was used to mix USP glycerin with normal saline. The mixed solution was then poured into a 60 mL catheter-tipped syringe attached to the low profile device access tubing. The tubing was primed prior to hooking it to the low profile device. The parent held the syringe containing flush solution approximately at the child's shoulder level while the flush was infusing. All components for each flush were at room

temperature prior to mixing and infusing the solution. A stop watch was used to measure the time taken to complete the procedure. The stop watch was started at the beginning of the infusion and was stopped when the child defecated and had not passed any additional stool for at least 5 minutes. The time in minutes and seconds from start to completion of the infusion was documented on a log designed specifically for this study. Following the flush, the tubing and bag or syringe was washed in warm soapy water, rinsed, and allowed to air dry. Step by step parental procedural directions are listed in Appendix D. The written instructions were included in a parent notebook to reinforce parent teaching and served as a check-off list to document procedural integrity which was checked during the initial home visit and with each subsequent clinic visit at the time of every change in phase.

Procedures

Each subject's baseline data were obtained preoperatively. Data at baseline (A) included continence data, serum electrolytes and stool calprotectin. The initial stool sample for microbiota analysis was obtained pre-operatively prior to initiation of any bowel prep. The child was randomized to one of the two treatment sequences pre-operatively. The process was restricted random assignment to force equal sample size and was accomplished using the SAS random number generator ensuring subject assignment results in equal group size.

High Volume Regimen

The high volume regimen (B) consisted of a normal saline flush at a starting dose of 10mL/kg infused every other day and adjusted as detailed in Table 3-4 until stability of target outcomes was achieved. At any point the investigator received notification that

the subject had had an episode of fecal soiling, the dosing strategy was increased by 5 mL/kg-increments with a subsequent increase in frequency, if needed, so as not to exceed a maximum dose of 500 mL administered daily for a child under five years of age and 1000 mL administered daily for a child over 5 years of age. If the child did not attain continence on the maximum dose, they were trialed on the alternate regime but did not proceed to the maintenance phase of the study.

If the child had side effects greater than 4 on the WBFPRS at the starting dose of 10mL/kg, flush volume was incrementally decreased as needed by 2.5 mL/kg to the lowest dose of 5 mL/kg daily as detailed in Table 3-5. The goal was to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize side effects resulted in episodes of fecal soiling or the child continued to have side effects greater than 4 on the WBFPRS at the lowest dose of administration, the child was trialed on the alternative flushing regimen, but did not proceed to the maintenance phase of the study.

Low Volume Regimen

The low volume regimen (C) consisted of USP glycerin diluted in normal saline prior to antegrade instillation through the low profile device. The child started on an every other day dose of 20 mL of USP glycerin and >20 mL of saline (used as diluent at a dose sufficient to allow the solution to easily infuse through the ACE access tubing) and adjusted as detailed in Table 3-4 until stability of target outcome was achieved. At any point the investigator was notified the child was having episodes of fecal soiling greater than one smear per week, the volume of USP glycerin was increased in 5 to10 mL increments with subsequent increase in frequency of administration, if needed, so

as not to exceed 40 mL of USP glycerin administered daily. If the child did not attain continence on the maximum dose of USP glycerin, he was trialed on the alternative flushing solution, but did not proceed to the maintenance phase of the study. If the subject was having side effects greater than 4 on the WBFPRS at the starting dose of 20mL of USP glycerin, the volume of the USP glycerin was decreased as needed by 5 mL increments until the child's symptoms were less than 3 on the WBFPRS or the lowest dose of 5 mL daily was reached as detailed in Table 3-5. The goal was to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize effects resulted in episodes of fecal soiling greater than one smear per week or the child continued to have side effects greater than 4 on the WBFPRS at the lowest dose of administration, the child was trialed on the alternative flushing solution, but did not proceed to the maintenance phase of the study.

Optimal Dose Regimen

Once the optimal dose was established, the child was maintained on that dose and frequency for at least 2 weeks or until stability in continence was achieved. The child was then scheduled to come into the clinic for a visit once the above criteria had been met at which time the child was flushed, labs were drawn, a stool sample collected, and the procedure for the next regimen was reviewed and procedural fidelity was obtained.

Regimen Comparative Phase

Following completion of the dose-response phase, the comparative portion of the study began by administering either the established effective dose of normal saline (B') or the established effective dose of USP glycerin (C'). Patients were randomized for a

second time to either a B'-C'-B₁' or a C'-B'-C₁' sequence. The last flush in the dose response sequence was stopped and the initial flush in the comparative treatment phase was introduced the following day at the previously established minimum effective dose and administration frequency. Children remained on treatment for 4 weeks at which point the treatment was withdrawn. Children were then placed on the next treatment in the sequence at the pre-established effective dose and frequency for 4 weeks. The second flush was then withdrawn and the initial flush in the sequence was reintroduced for an additional two weeks (B₁' or C₁'). At the conclusion of the study, the child was placed on the flushing regimen of his/her choice that maintained continence.

Specimen Collection

Stool samples were obtained during the baseline phase prior to any pre-operative bowel prep and additional stool samples were obtained at the completion of each phase. Stool calprotectin was completed on the preoperative sample and the postoperative samples collected at the completion of each dosing phase for a total of 3 samples. Stool collection for downstream microbiota analysis was obtained preoperatively and following the completion of each dosing and maintenance phase for a total of 5 samples. Blood samples for electrolytes were drawn prior to surgery and following surgery at the end of each dosing phase at the time stool samples were obtained for a total of three samples. Funding was secured and accounts with outside laboratories were set up. Blood samples and stool for calprotectin were sent for analysis. The investigator received bench training in the lab to process the stool samples for isolation of microbiome DNA. Stool samples for microbiota analysis were collected, labeled with patient identifier, date, time, and flush composition. Each stool

sample for microbiota analysis was processed by the investigator to purify and extract microbial DNA for sequencing, flash frozen to -80° C, and archived for future downstream-batch analysis using 16SrRNA as a taxonomic marker (Barker, 2012) as detailed in Appendix E. The procedural timeline is detailed in Figure 3-1.

Statistical Analysis

The strength of an interrupted time series design is that effects of the intervention under investigation can be repeatedly and reliably measured over time. Time series parameters include mean intercept level, slope, and additional non-linear changes in shape. If the treatment is effective, it will result in a change in parameters that reverse with treatment withdrawal. Independent variable impact on time series parameters is based on the degree of change elicited in level, slope, or cycle of the measures process. The ability to determine independent variable effects is a function of a stable baseline and the number of baseline, intervention, and post-intervention data points (Biglan, Ary, & Wagenaar, 2000; Janosky, Leininger, Hoerger, & Libkuman, 2009; Shadish, Cook, & Campbell, 2002). In this study, each regimen was administered at optimum dosing for a minimum of 2 weeks at the end of the titration phase. Each flushing regimen was administered for 4 weeks during the comparative phase for a total of 6 weeks per flushing regimen for those subjects who progressed, potentially yielding a total of at least 12 weeks or more of observations for those subjects who completed the study.

Data were used to facilitate analysis and dose response adjustment during the B-C or C-B dosing phase. Data were graphed on an equal interval line graph with the proportion of ordinate and abscissa scaled at a 2:3 ratio to ensure consistency of data

presentation and prevent data distortion during visual analysis. Dependent measures were placed on the ordinate scale with time-by-day on the abscissa scale. Separation of the baseline, both dose-response and the three comparative phases of the study were designated by a bold vertical line. Dosing regimen changes were designated by a thin dotted vertical line. Each phase change was labeled with solution name. Each dosing change was labeled with mL per dose and the total days of administration for the dose. Independent variable effects on target behaviors were analyzed using visual analysis. Analysis within each condition included: (a) condition length defined as the number of days contained within each phase, (b) level stability with a stability envelope calculated using the median and a stability criterion of 80% of the data points falling within 20% of the calculated median for the phase, (c) relative change in level, (d) absolute change in level, (e) estimation of trend direction using split middle method, and (f) identification of multiple paths within trends, if present (Gast, 2010; Hartmann et al., 1980; Ma, 2006; Kazdin, 2011; McCleary, Meidinger, & Hay, 1980; Portney & Watkins, 2009). Testing in multiple subjects allowed for analysis of replication of treatment effects. In addition to visual analysis of time series parameters, analysis was to include inferential procedures used to compare interventional effects and increase the reliability of visual methods analysis.

The independent variable was nominal and dichotomous. Dependent variables were comprised of either interval or ratio level measurements. The dosing and comparative phase of the study was a 2-treatment crossover design with each child receiving both treatments. Each child who completed the dosing phase of the study was to be randomly assigned to either a C'-B'-C₁' or B'-C'-B₁' treatment sequence with half of

the subjects allocated to each sequence (Portney & Watkins, 2009). The plan was to use inferential statistics for two-tailed hypothesis testing, which employs use of a sampling distribution combined with the laws of probability to make statistical inference regarding rejection or failure to reject the null hypothesis (Polit, 2010). Statistical testing is based on the hypothetico-deductive method where the null hypothesis states there is an absence of a relationship between the two populations at a given level of confidence. The object is to reject the null hypothesis at a fixed probability. The probability of committing an error is controlled through the level of significance. Alpha (α) is the level of significance, the probability level established as the risk of making a type 1 error, sets the threshold against which the p-value is measured, and is generally set at .05. Failure to reject the null hypothesis occurs if the probability value p is $> .05$ and the confidence interval contains zero. Confidence interval is used to estimate precision of the effect. Effect size is used to determine the magnitude of the treatment difference (Polit, 2010; Rempher & Silkman, 2007).

Initial consideration was given to testing the flushing regimen's effect on outcomes of interest that included number of soiling episodes, level of soiling, abdominal pain, procedural side effects, infusion time, procedural time, electrolyte balance, and fecal calprotectin, using a two-tailed, two-sample pooled variance t test with a significance level set at 0.05.

Because a cross-over design is comparing two treatment sequences, the groups are independent (Chow & Liu, 2014). Confounding by carry over and direct-by-period interaction is a potential with cross-over designs, which if present, can bias treatment effects (Jones & Kenward, 2003; Senn, 2002; Shuster, 2007). If inferential procedure

was to be used in data analysis, a decision had to be made regarding which test would best eliminate conditional bias and minimize variance. Jones et al. (2003) and Sen (2002) have suggested use of a one sample t test for analysis of cross-over designs ($y = \text{treatment two} - \text{treatment one}$). Shuster (2017) has advocated a two sample t test in the analysis of a randomized 2 treatment cross-over design irrespective of treatment order $y = (\text{period two} - \text{period one}) / 2$. Analysis of a one sample t test in a cross-over design ignores treatment ordering. Two sample t test analysis compares ordering and yields potentially useful data on carry over. When μ is the main treatment effect, and τ is carry over, results from a one sample or two sample t test will yield unbiased estimates of μ and variance when the sample size is equal and $\tau = 0$. If $\tau \neq 0$, the expected value of μ should be similar using either the one sample or two sample method. However, the one sample method does not account for carry over effects increasing variance. If sample sizes are unequal and $\tau \neq 0$ (conditional on sample size), the point estimates in the one but not the two sample t test will be biased. The decision was made to use the 2 sample method to lend precision in the presence of carry over effects, and precision and accuracy in the presence of unequal sample size when $\tau \neq 0$ (Shuster, 2009).

Due to small sample size, the treatment effects did not reach statistical significance so descriptive statistics were used including median, mean, range and standard deviation to describe effects of intervention on the outcomes of interest. Neither visual analysis nor inferential statistics alone will infer causality. Strictly speaking, correlation does not infer causation (Polit, 2010). Causal inference is primarily influenced by the research design. Visual analysis and statistical procedures are helpful in measuring the effects of potential causes (Kazdin, 2011). Appropriate use of

statistics aid in the inference of causality by quantifying the effect chance plays on conclusions (Hill, 1965). This study was prospective allowing for the determination of temporal precedence. Statistical and visual analysis was used to assess contiguity with respect to presumed cause and effect. Strategic use of design elements were used to limit alternative explanations for findings. The first phase of the study evaluated dose response which assessed biological gradient. The design assessed treatment response across multiple subjects, which provided support for consistency across contexts.

Social Validity

Attaining continence is a highly socially significant issue for any child but particularly difficult to attain using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumor, megarectum, and slow transit constipation. ACE therapy has been shown to be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in independence and quality of life. However, research findings regarding short and long-term effectiveness are variable (Aspirot, Fernandez, Di Lorenzo, Skaggs, & Mousa, 2009; Bani-Hani, Cain, King, & Rink, 2008; Bassonet al., 2014; Becmeur et al., 2008; Chu, Balsara, Routh, Ross, & Wiener, 2013; Church, Simah, Wild, Teitelbaum, & Ehrlich, 2017; Curry, Osborne, & Malone, 1999; Chong, Featherstone, Sharif, Cherian, Cuckow, Mushtaq, De Coppi, Cross, & Curry, 2016; Dey et al., 2003; Dolejs, Smith, Sheplock, Croffie, & Rescorla, 2017; Driveret al., 1998; Freemanet al., 2014; Hoekstra,Kuijper, Bakx, Heij, Aronson, & Benninga, 2011; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Large, Szymanski, Whittman, Misseri, Chan, Kaefer, Rink and Cain, 2017; Levitt, Soffer, &

Pena, 1997; Marshall, Hutson, Anticich, & Stanton, 2001; Matsuno, Yamazaki, Shiroyanagi, Ueda, Suzuki, Nishi, Hagiwara, & Ichiroku, 2010; Mousa et al., 2006; Ok & Kurzrock, 2011; Peeraully et al., 2014; Randal et al., 2014; Siddiqui, Fishman, Bauer, & Nurko, 2011; Sinha et al., 2008; Thomas et al., 2006; VanderBrink et al., 2013; Yardley et al., 2009).

This variability may be due to what is used to flush. In addition, there are no comparative studies documenting the time necessary to complete the flushing procedure. Sitting on the commode for one hour versus 20 minutes represents a major difference in age-appropriate expectations for a 6-year-old child. In a child, procedural time may be inversely related to adherence and negatively impact effectiveness of the procedure. Currently, the flushing regimen for each child is based on clinician preference and can be a lengthy process of trial and error. Identifying effective dose and frequency of commonly used flushing solutions or medication regimens and comparing effectiveness of the different flushing regimens will benefit any child requiring ACE therapy promoting continence at an earlier stage in their therapy and cost savings for the family by decreasing costs associated with ineffective trials and additional time spent in protective garments. This study allowed implementation of ACE flush by the parent and, eventually, the child in the home after research support was withdrawn.

Social importance and acceptability of the treatment mediates the relationship between the prescribed therapy and implementation. Treatment acceptability and parental willingness to carry out the procedure is directly related to the likelihood of procedural fidelity and effectiveness. (Jones, Eyberg, Adams, & Boggs, 1998; Kazdin,

2011). Upon completion of the study, a simple question was directed to the children to ascertain which flushing regimen they preferred.

Threats to Validity

Causal inference is predicated on covariation in the independent and dependent variables. The source of that covariation is central to inferring cause (Cook & Campbell, 1979). Confounding or threats to internal validity occur when the covariation is not due to the independent variable, but to another variable not accounted for in the causal pathway (Susser, 2001). Multiple threats to internal validity can operate simultaneously (Cook & Campbell, 1979). A central task of any researcher is to identify and trenchantly examine all the potential confounds, assess the plausibility of the identified threats, and determine how best to deal with them (Shadish et al., 2002). A sound study is designed to limit plausible alternative explanations achieved through utilization of proper control that strengthens the inference that the outcome is due to treatment (Janosky et al., 2009). Reliability is essential to validity. In single subject research, emphasis is placed on the reliability of measurement and consistency in implementation of procedures. Reliability of the individual collecting data was verified using interobserver agreement (IOA) at the initial home visit. Procedural fidelity was ascertained to assure interventions were reliably implemented with each phase change (Kazdin, 2011; Gast, 2010).

Typology used for assessing potential sources of confounding include threats to: (a) statistical conclusion validity, (b) internal validity, and (c) construct validity (Portney & Watkins, 2009). Assessment of potential threats to statistical conclusion validity are detailed in Appendix F. Assessment of potential threats to internal validity are detailed in Appendix G. Assessment of potential social threats to internal validity are detailed in

Appendix H. Assessment of potential threats to construct validity are detailed in

Appendix I.

Table 3-1. Itemized Estimate for Maximum Flushing Costs For 6 Subjects

	Normal Saline	USP Glycerin	Diluent	BMP + Draw Fee	Stool Calpro- tectin	Lido- caine Tube	Tega- derm Box	Stipend
Cost per Unit	\$3.65/L	\$126.55 / 3.78 L	\$3.65/ L	\$11.98 + \$3.00	\$123.45	\$32.86/ Tube	\$18.76/ Box	\$25.00
Estimate Units	1 L/d x 70 d	60 mL/d x 70 d	60 mL/d x 70 d	3	3	4	2	6
Total Cost/ Subject	\$255.55	\$140.61	\$15.33	\$44.94	\$370.35			\$150.0 0
Total Cost/ Six Subjects	\$1,533.00	\$843.66	\$91.98	\$269.64	\$2,222.10	\$131.44	\$37.52	\$900.0 0

BMP - Basic Metabolic Panel

Table 3-2. Level of Soiling

Code	Description
0	No soiling
1*	Smear of Stool
2*	Moderate volume accident not visible through clothing
3*	Any accident large enough to be visible through clothing

*Change dose with any soiling

Table 3-3. Dependent Variables

Sample/Instrument	Variable	Measurement	Data Level
Blood	BMP	Electrolyte Balance	Ratio
Stool	Calprotectin	Mucosal Inflammation	Ratio
Stool	Colonic microbiome	Metagenomic Profiling 16SrRNA	Ratio
FIC QOL	Parent/child quality of life	Symptoms Rating Scale	Ordinal
WBFPRS	Abdominal Pain	Symptom Rating Scale	Ordinal
WBFPRS	Procedural side effects	Symptom Rating Scale	Ordinal
Stop Watch	Infusion Time	Minutes	Ratio
Stop Watch	Procedural Time	Minutes	Ratio

Table 3-4. Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Maintain Continence

Normal Saline (High volume regimen)	USP Glycerin + Normal Saline as Diluent (Low volume regimen)
B1 = 10mL/kg maximum dose 1000 mL qod	C1 = 20 mL + > 20 mL qod
B2 = 10mL/kg maximum dose 1000 mL q3d	C2 = 20 mL + > 20 mL q3d
B3 = 10mL/kg maximum dose 1000 mL qd	C3 = 20 mL + > 20 mL qd
B4 = 15mL/kg maximum dose 1000 mL qod	C4 = 25 mL + > 40 mL qod
B5 = 15mL/kg maximum dose 1000 mL q3d	C5 = 25 mL + > 40 mL q3d
B6 = 15mL/kg maximum dose 1000 mL qd	C6 = 25 mL + > 40 mL qd
	C7 = 30 mL + > 50 mL qod
	C8 = 30 mL + > 50 mL q3d
	C9 = 30 mL + > 50 mL qd

*Change dose with any soiling > Level 0

Incrementally increase volume of saline or glycerin until maximum dose is reached

Stopping rule for saline is a maximum of 500 mL flush volume for children 5 years of age and under and 1,000 mL flush volume in children over 5 years of age who do not achieve continence at maximum dose

Stopping rule for USP glycerin is incontinence at a maximum dose of 40 mL

qd – every day

qod – every other day

q3d – every third day

Table 3-5. Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Minimize Side Effects

Normal Saline*	USP Glycerin + NS as Diluent*
(High volume regimen)	(Low volume regimen)
B1 = 10 mL/kg maximum dose 1000 mL qod	C1 = 20 mL + > 20 mL qod
B7 = 7.5 mL/kg maximum dose 1000 mL qod	C10 = 15 mL + > 15 mL qod
B8 = 7.5 mL/kg maximum dose 1000 mL q3d	C11 = 15 mL + > 15 mL q3d
B9 = 7.5 mL/kg maximum dose 1000 mL qd	C12 = 15 mL + > 15 mL qd
B10 = 5.0 mL/kg maximum dose 1000 mL qod	C13 = 10 mL + > 10 mL qod
B11 = 5.0 mL/kg maximum dose 1000 mL q3d	C14 = 10 mL + > 10 mL q3d
B12 = 5.0 mL/kg maximum dose 1000 mL qd	C15 = 10 mL + > 10 mL qd
	C16 = 5 mL + > 5 mL qod.
	C17 = 5 mL + > 5 mL q3d
	C18 = 5 mL + > 5 mL qd

*Change dose with any side effects > 3 on the WBFPRS or with any soiling > Level 0
 Incrementally decrease volume of saline or glycerin until minimum dose is reached
 Stopping rule for saline is a continued pain or incontinence at a minimum dose of 5.0 mL/kg
 Stopping rule for USP glycerin is continued pain or incontinence at a minimum dose of 5.0 mL/
 qd – every day
 qod – every other day
 q3d – every third day

	Baseline		Dose Response Phase					Flush Effectiveness Phase													
Weeks:	1	2	3	4	5	6	7	8	9	10+	11	12	13	14	15	16	17	18	19	20	
Randomize		X								X											
Order:																					
A	NT	NT																			
B-C			B	B	B	B	C	C	C	C											
C-B			C	C	C	C	B	B	B	B											
B-C'-B ₁ '											B'	B'	B'	B'	C'	C'	C'	C'	B ₁ '	B ₁ '	
C-B'-C ₁ '											C'	C'	C'	C'	B'	B'	B'	B'	C ₁ '	C ₁ '	
Visits ^a :																					
Hospital			X																		
Home				X																	
Clinic						X				X				X					X	X	
Biomarkers:																					
Stool	X					X				X				X					X		
BMP	X					X				X				X					X		
SIM	X					X				X				X					X		
Instruments:																					
FICQOL	X													X					X		
QQ																				X	
Measures:																					
Soiling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Abd Pain	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Admin T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Proc T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ISE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cost	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

A - Baseline

NT -No treatment

B - Saline dose-response phase

C - USP Glycerin dose-response phase

B' – Initial trial of saline effectiveness phase

C'- Initial trial of USP glycerin effectiveness phase

B₁'- Second trial of saline effectiveness phase

C₁'- Second Trial of USP glycerin effectiveness phase

BMP – Basic Metabolic Profile

SIM – Stool immune marker

Visits^a- Procedural fidelity and inter-rater reliability will be measured at each visit

FIC QOL – Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida

QQ – Qualitative question asked of the child at the end of the study

Abd Pain – Abdominal Pain

Admin T – Administration time

Proc T – Procedural time

ISE – Infusion side effects

Figure 3-1. Procedural timeline.

CHAPTER 4 FINDINGS

Subject Characteristics

Five subjects between the ages of 3 and 7 years were recruited to participate in the study. All subjects were male. Two subjects were Caucasian, one was Hispanic, one was African American and one was Asian and had recently been adopted from China. Four of the five subjects had lumbosacral myelomeningocele and were ambulatory. The remaining child had cord transection below T-10 from a motor vehicle accident and was wheelchair bound. All five subjects had a patulous anus and had failed retrograde suppository administration or retrograde enema therapy using an assistive device.

Aims

The primary aims of this study were to determine (a) if there was a difference in minimal administration frequency, (b) titration time to reaching effective dose, and (c) which solution at an optimum dose was delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence. Secondary aims were to (a) compare quality of life metrics, (b) determine the cost difference between two flushing solutions, and (c) collect and process stool for future analysis to determine if administration of antegrade enema solution through an appendicostomy/cecostomy affects gut microbiota and immune function. Visual analysis was used to analyze within-subjects data. Sample size was too small to produce statistically significant findings using inferential statistics. Between subjects data were analyzed using descriptive statistics including median, mean, standard deviation, and range. A power analysis was conducted to estimate the necessary sample size needed in a future study to minimize

the probability of committing a Type II error ($1 - \beta$) with the significance criterion $\alpha = 0.5$ and power = .80. To correct for data dependence among means, a correlation between two means was calculated and applied to make a direct comparison of effect sizes

Null Hypotheses for Aim 1

Frequency of Administration

Null Hypothesis 1.1. There will be no differences in frequency of administration necessary to gain and maintain continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (primary aim).

One hundred percent of subjects (five of five) required daily flushing with saline. Sixty percent (three of five) required daily flushing with USP Glycerin. Twenty percent (one of five) maintained continence on every other day flushing with USP Glycerin. Twenty percent (one of five) maintained continence on every third day flushing with USP Glycerin. Of those subjects who achieved continence on saline during the dosing phase (two of five), 100% of subjects (two of two) required daily flush administration with saline to maintain continence, only 50% of subjects (two of four) required daily flushing with USP glycerin to maintain continence. A visual comparison of the administration frequency of normal saline versus USP glycerin is graphically represented in Figure 4-1. Inferential statistics did not yield statistical significance.

Titration Time

Null Hypothesis 1.2. There will be no difference in titration time to reach effective dose between two different ACE flushing regimens using normal saline and

normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Titration time between subjects and between flush solutions was highly variable. Analysis excluding titration times for the subjects who did not achieve continence revealed titration times ranged from 52 to 53 days for the two subjects who initially achieved continence on saline during the dosing phase of the study. Individual subject data and descriptive statistics are detailed in Table 4-1. A visual comparison of titration time for normal saline versus USP glycerin including all subjects is found in Figure 4-2.

Cost Burden

Null Hypothesis 1.3. There will be no difference in cost between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Normal saline flush was more expensive than USP Glycerin flush both on a per dose and per week basis. The average cost per saline flush was \$2.59 as compared to \$1.19 per flush of USP glycerin. Using the USP glycerin resulted in an average reduction of 54% in cost per dose. Average weekly cost for the saline flushing regimen was \$18.16 as compared to \$4.89 for USP glycerin. Using USP glycerin resulted in an average reduction of 73% in cost per week. Cost per subject is detailed in Table 4-2. The difference in cost did not reach statistical significance.

Null Hypotheses for Aim 2

Continence

Null Hypothesis 2.1. There will be no difference in continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (primary aim).

There was a difference in continence rates between the two flushing regimens. The difference did not reach statistical significance. Descriptive statistics were calculated on the last data point in the final phase of each flush. Only 20% of subjects (one of five) gained and maintained continence on saline. Eighty percent of subjects (four of five) gained continence on USP Glycerin. Severity of daily soiling was greater on saline when compared to glycerin. Power analysis conducted using data from this study with $\alpha = 0.5$, power of .80, correlation between two means of .598, and effect size of 1.554 estimated a sample size of 11 would be needed to minimize the risk of a Type II error to (20%). Individual subject data and descriptive statistics are detailed in Table 4-1. The graphed continence data used in visual analysis to evaluate absolute frequency of incontinence is detailed in Figures 4-3 through 4-7 with graphed severity data detailed in Figures 4-8 through 4-12. Within subjects calculations including stability and trend graphs are located in subject specific Appendices O through S.

Infusion Time

Null Hypothesis 1.2. There will be no difference in infusion time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

The infusion time of USP glycerin was less in 80% of subjects (four of five) when compared to normal saline. Infusion time data were included in procedural time graphs and calculations.

Procedural Time

Null Hypothesis 1.3. There will be no difference in procedural time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Descriptive statistics were calculated on the last data point in the final phase of each flush. Procedural time with normal saline was less in 60% of subjects (three of five) when compared to USP glycerin. Procedural time was equivalent for normal saline and USP glycerin in 20% of subjects (one of five). Procedure time on USP glycerin was less in 20% of subjects (one of five) when compared to normal saline. Individual subject data and descriptive statistics and are detailed in Table 4-1. The difference in procedural time did not reach statistical significance. The graphed data used in visual analysis to evaluate procedural time is detailed in Figures 4-13 through 4-17. Within subjects calculations for procedural data including stability and trend graphs are located in subject specific Appendices O through S.

Side Effects

Null Hypothesis 2.4. There will be no difference in side effects between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Descriptive statistics were calculated on the last data point in the final phase of each flush. The difference in pain between the two flushing regimens did not reach

statistical significance Side effects were minimal with only 20% of subjects (one of five) experiencing consistent cramping with USP glycerin. The cramping was 4 or less on the WBFPRS, was consistently relieved with defecation, and did not necessitate dosing adjustment. Of interest, this is the only subject that documented consistent pain at baseline. No subjects had consistent cramping with normal saline flush. One subject had a significant episodes of abdominal pain secondary to constipation that improved with flushing. One subject had a single significant degree of cramping on a higher than prescribed dose of glycerin that resolved with defecation and did not recur. Only 20% of subjects (one of five) had a vagal response to USP glycerin. The response was severe enough the child was dropped from the study. The child had started taking iron supplementation, had missed several flushes, and was quite constipated. The child was cleaned out with Golytely and placed back on USP glycerin without further side effects. It is interesting to note that constipation appeared to be the major cause of side effects. Individual subject data and descriptive statistics are detailed in Table 4-1. The graphed data used in visual analysis to evaluate side effects is detailed in Figures 4-3 through 4-17. Within subjects calculations for side effects data including stability and trend graphs are located in subject specific Appendices O through S.

Quality of Life

Null Hypothesis 2.5. There will be no difference in parent/patient satisfaction as measured by the Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida (FIC QOL) between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Only one of five subjects achieved and maintained continence on saline. The remaining four subjects either did not progress to or complete the maintenance phase of the study. By study design, the FIC QOL tool was administered in the comparative phase of the study and therefore was not completed by 80% of subjects. Thus, any difference in parent/patient satisfaction between flushing regimens could not be assessed.

Electrolytes

There were no abnormalities in electrolytes associated with either normal saline or USP glycerin flush. A visual comparison of the effects of saline and USP glycerin flushing solutions on serum electrolytes including sodium, potassium, chloride, carbon dioxide, calcium, blood urea and nitrogen, and creatinine are graphically represented in Figures 4-26 through 4-31.

Stool Calprotectin

Null Hypothesis 3.2. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on mucosal immune function in children requiring antegrade continence therapy

Stool samples for calprotectin were obtained directly following saline flush. One subject had a significant rectal prolapse and did not pass stool following the saline flush completed in clinic prior to transition to glycerin, so levels are available in only four of five subjects for saline. Of the four remaining samples, calprotectin levels were elevated in 75% of subjects (three of four) and decreased in 25% of subjects (one of four) in response to saline. Changes in calprotectin levels on saline ranged from a decrease of 31% to an increase of 2,550% when compared to baseline. In one subject, levels

exceeded the upper limits of normal on saline, but did not reach a level of clinical concern that would necessitate stopping ACE therapy.

Stool samples for calprotectin were obtained in 100% (five of five) of subjects following USP glycerin flush. Calprotectin levels were elevated in 80% of subjects (four of five) and decreased in 20% of subjects (one of five) on glycerin. Changes in calprotectin levels on glycerin ranged from a decrease of 58.6% to an increase of 379% compared to baseline levels. Individual subject data and descriptive statistics are detailed in Table 4-1. Stool calprotectin levels are graphically presented in Figure 4-32.

Child's Flushing Preference

Three of five (60%) subjects preferred normal saline; two (40%) had no preference. None of the subjects who preferred saline could articulate their reasons for doing so.

Table 4-1: Individual Subject Data and Descriptive Statistics

	Titration Time Days Saline	Titration Time Days Glycerin	Continenence Rates Per Day Saline *	Continenence Rates Per Day Glycerin *	Dosing Frequency Saline	Dosing Frequency Glycerin	Procedure Time Minutes Saline *	Procedure Time Minutes Glycerin *
KJ001**	53	46	0	0	Daily	Daily	22	57
KJ002***	54	37	1	0	Daily	Q3d	20	90
KJ003***	52	33	1	0	Daily	QOD	42	102
KJ004**	12	79	4	1	Daily	Daily	35	35
KJ005**	39	53	4	0	Daily	Daily	50	30
Mean	42	49.6	2	0.2			33.8	62.8
Median	52	46	0	0			35	57
Range	12 to 54	33 to 79	0 to 4	0 to 1			20 to 50	30 to 102
SD	17.85	18.19	1.87	0.45			12.85	32.24

*Data calculated on last data point in final phase of each dosing regimen

**Randomized to start on Normal Saline

***Randomized to start on USP Glycerin

Table 4-1: Continued

	Pain Severity WBFPRS Saline *	Pain Severity WBFPRS Glycerin*	Stool Calprotectin Saline	Stool Calprotectin Glycerin	Weekly Cost Dollars Saline	Weekly Cost Dollars Glycerin
KJ001**	0	0	413.4	217.4	21.70	10.92
KJ002***	0	0	148.9	34.7	25.55	1.9
KJ003***	0	2	54.1	211.2	17.92	1.95
KJ004**	0	0	119.1	57.9	12.81	10.92
KJ005**	0	0	Missing	277.4	12.81	7.77
Mean	0	0.4	183.88	159.72	18.16	6.69
Median	0	0	134 119.1 to	211.2	17.92 17.92 to	7.77
Range	0 to 0	0 to 4	413.4	34.7 to 277.4	25.55	1.90 to 10.92
SD	0	0.89	158.05	107.03	5.58	4.54

*Data calculated on last data point in final phase of each dosing regimen

**Randomized to start on Normal Saline

***Randomized to start on USP Glycerin

COMPARITIVE FREQUENCY OF FLUSH ADMINISTRATION

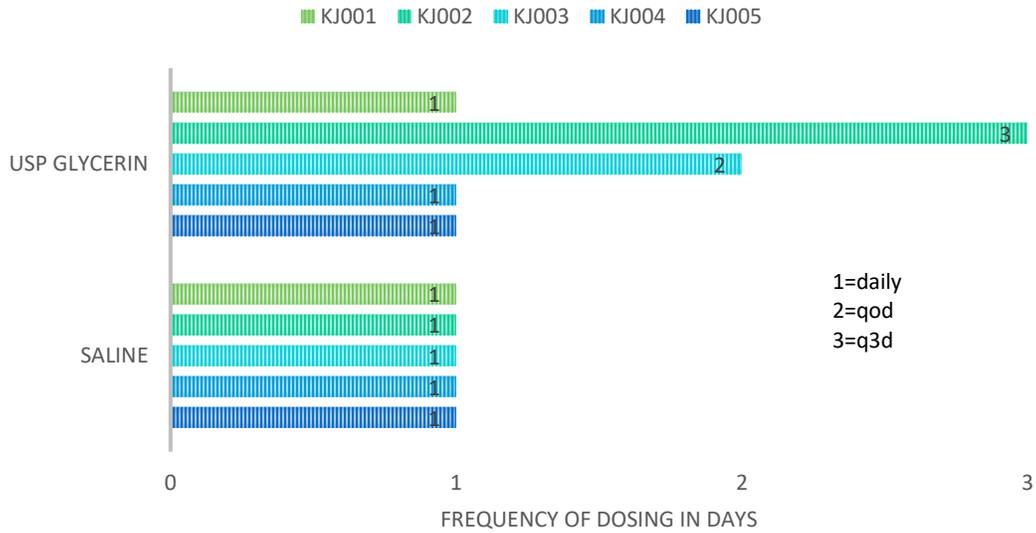


Figure 4-1: Frequency of flush administration

TITRATION TIME TO CONTINENCE

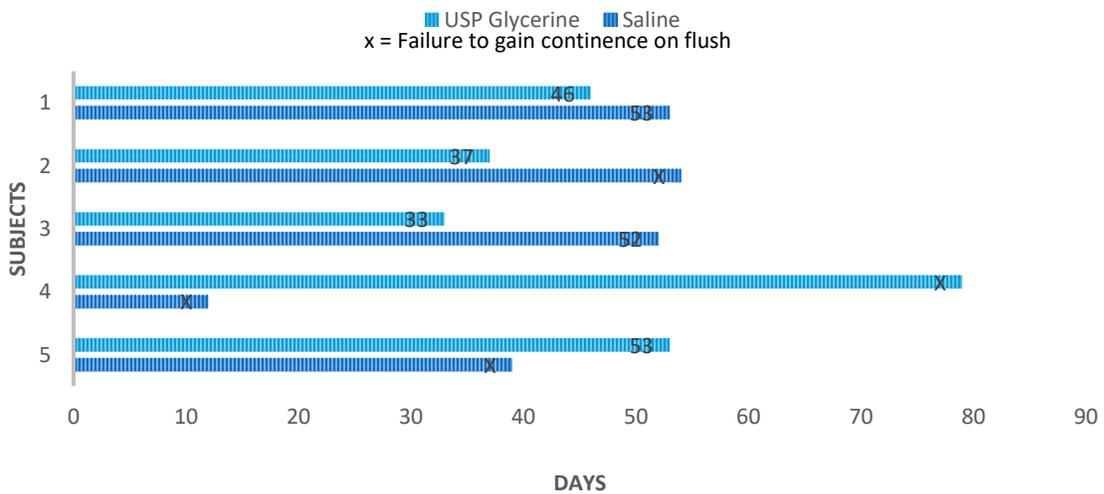


Figure 4-2: Titration time to achieve continence

Table 4-2 Cost Comparison Normal Saline vs USP Glycerin Flush

Subject #	NaCl 0.9% Dose (mL/day)	USP Glycerin + Dose & Diluent (mL/day)	Cost/Dose NaCl 0.9%	Cost/Dose USP Glycerin	Weekly Cost NaCl 0.9%	Weekly Cost USP Glycerin
KJ001	850	40 mL + 60 mL/d	\$3.10	\$1.56	\$21.70	\$10.92
KJ002	1000	25 mL + 30 mL/q3d	\$3.65	\$0.95	\$25.55	\$1.90
KJ003	700	20 mL + 30 mL/qod	\$2.56	\$0.78	\$17.92	\$1.95
KJ004	500	40 mL + 60 mL/d	\$1.83	\$1.56	\$12.81	\$10.92
KJ005	500	30 mL + 30 mL/d	\$1.83	\$1.11	\$12.81	\$7.77

*Cost calculation based on pricing of normal saline at \$3.65/L and USP glycerin at \$33.48/L

qod – every other day

q3d – every third day

KJ001 ABSOLUTE FREQUENCY OF INCONTINENCE

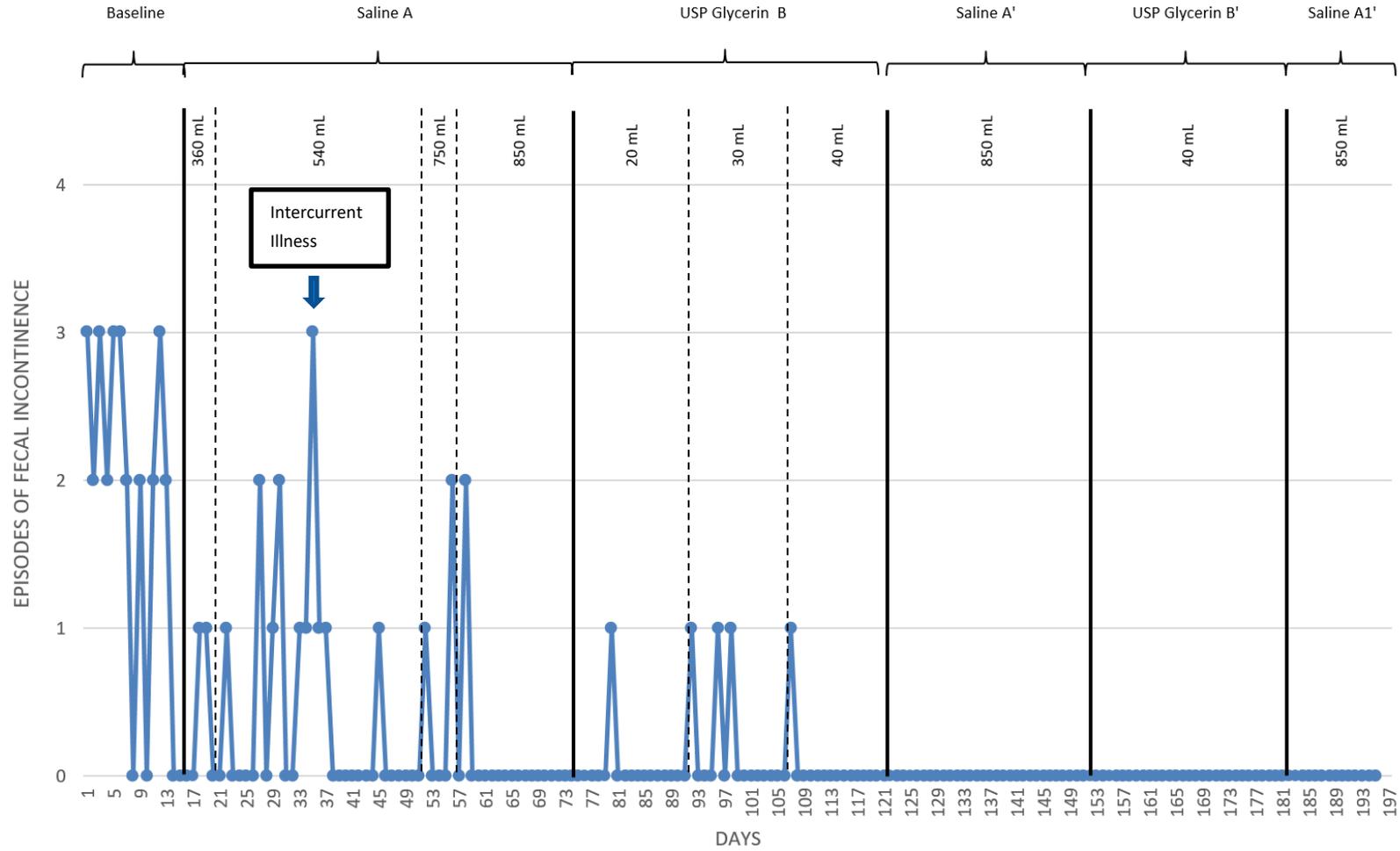


Figure 4-3: KJ001 Absolute frequency of incontinence graph

KJ002 Absolute Frequency of Incontinence

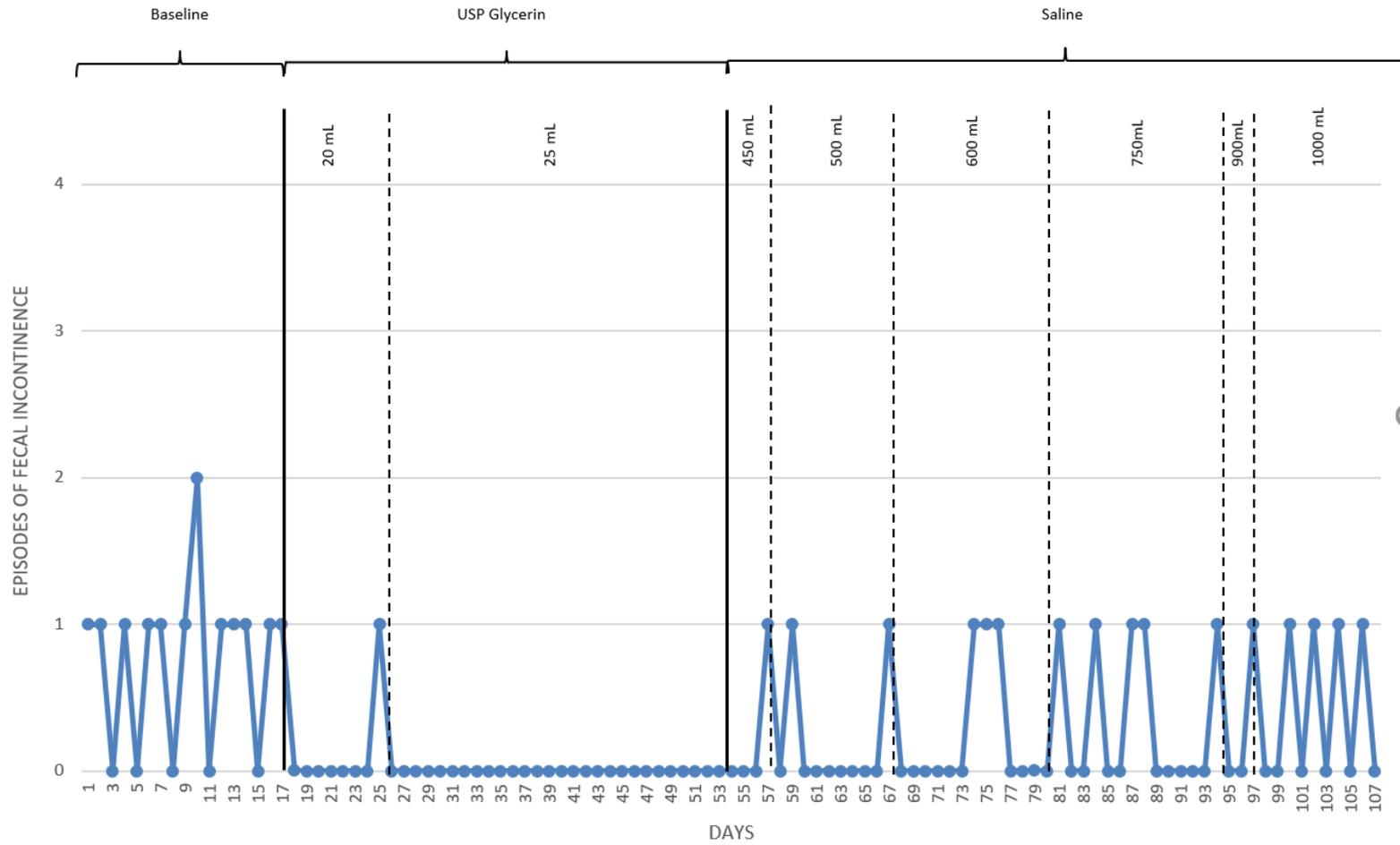


Figure 4-4: KJ002 Absolute frequency of incontinence graph

KJ003 ABSOLUTE FREQUENCY OF INCONTINENCE

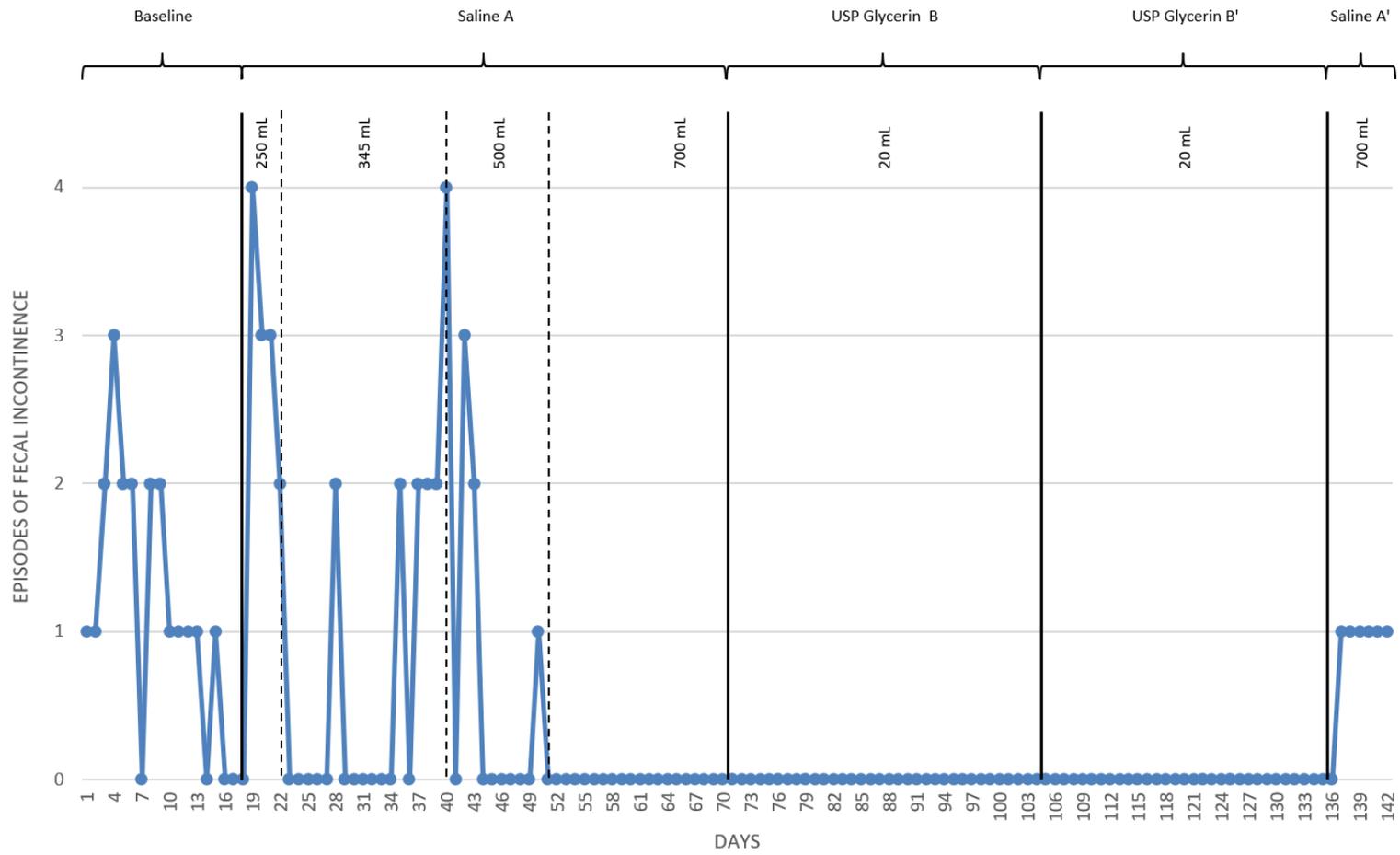


Figure 4-5: KJ003 Absolute frequency of incontinence graph

KJ004 ABSOLUTE FREQUENCY OF INCONTINENCE

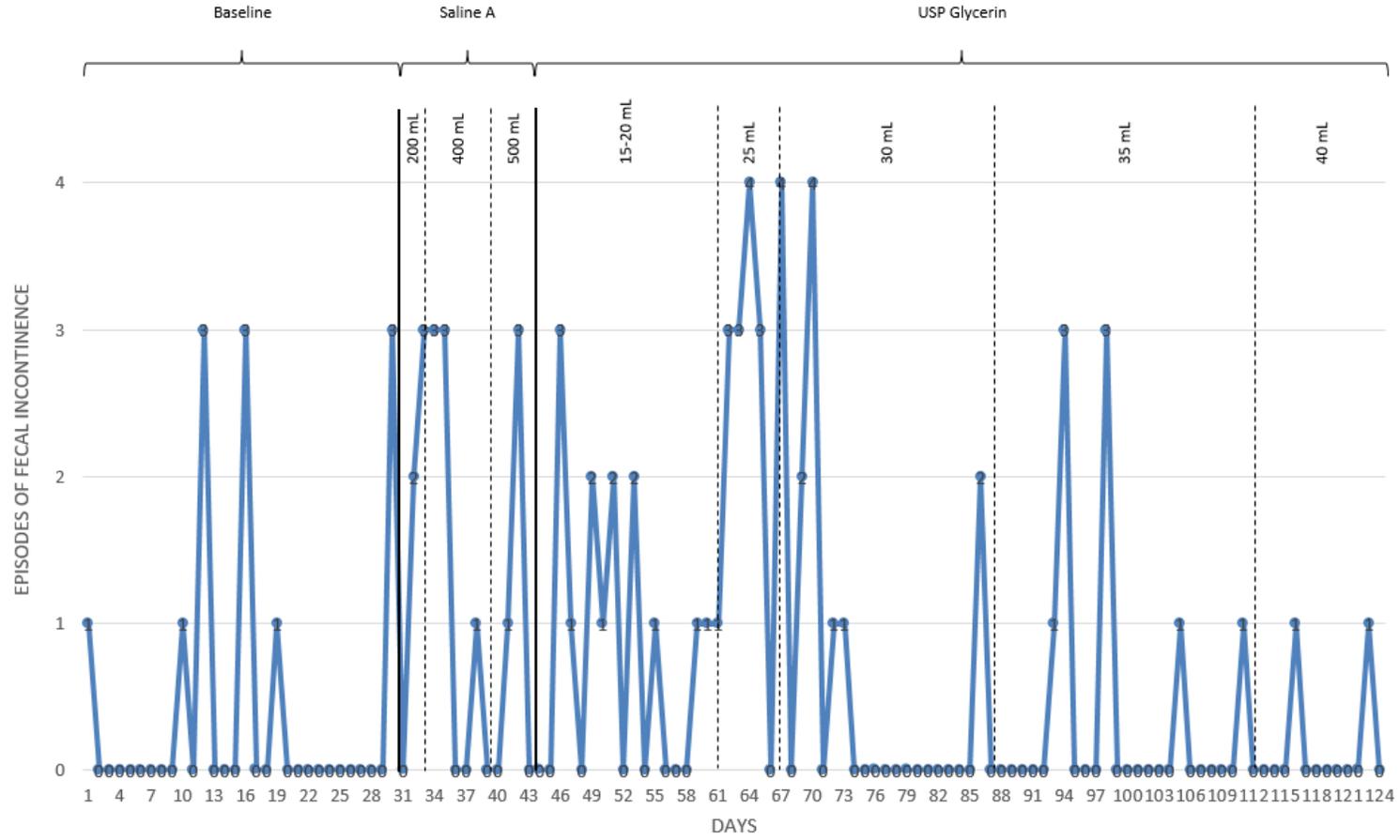


Figure 4-6: KJ004 Absolute frequency of incontinence graph

KJ005 ABSOLUTE FREQUENCY OF INCONTINENCE

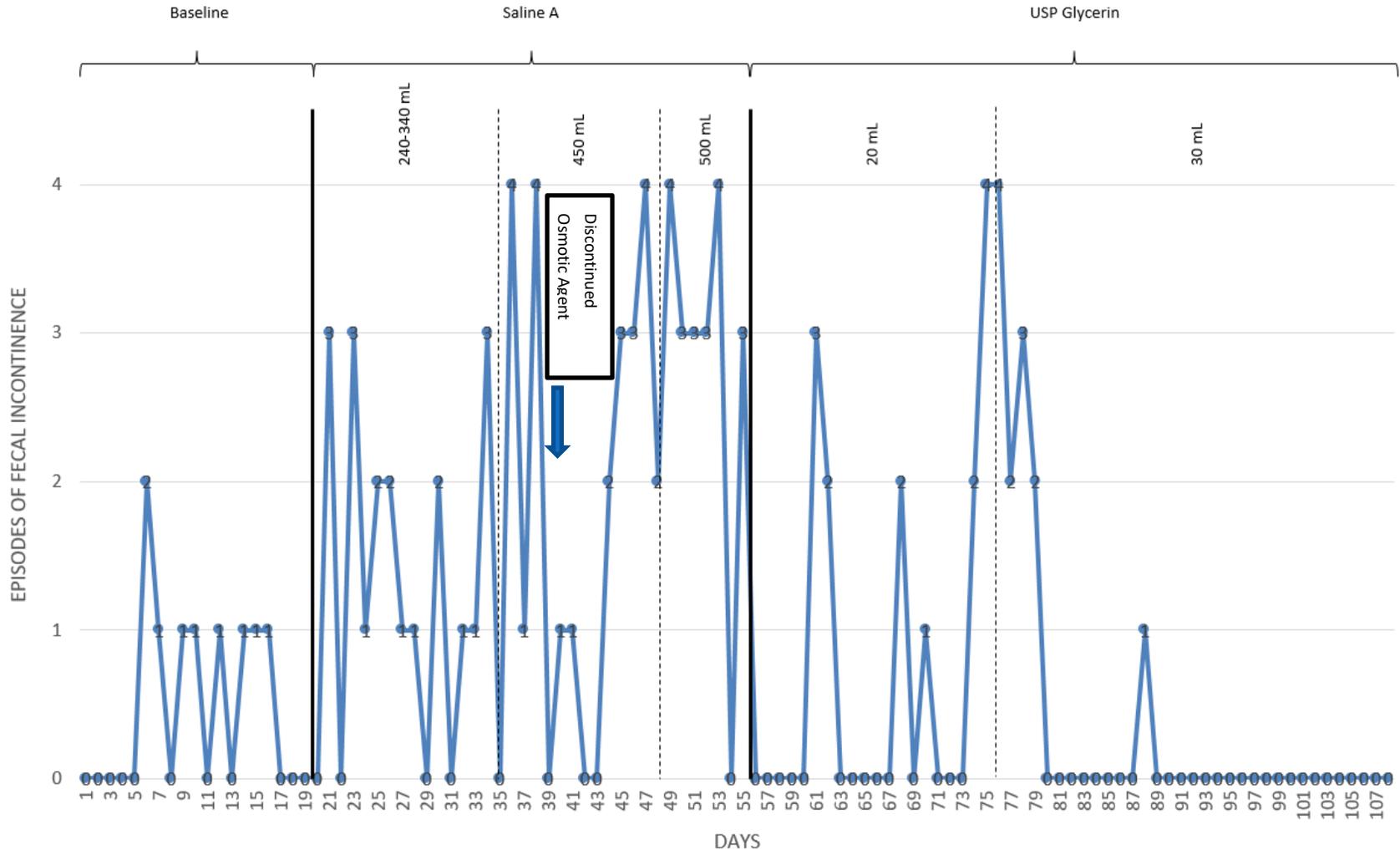


Figure 4-7: KJ005 Absolute frequency of incontinence graph

KJ001 FREQUENCY AND SEVERITY OF INCONTINENCE

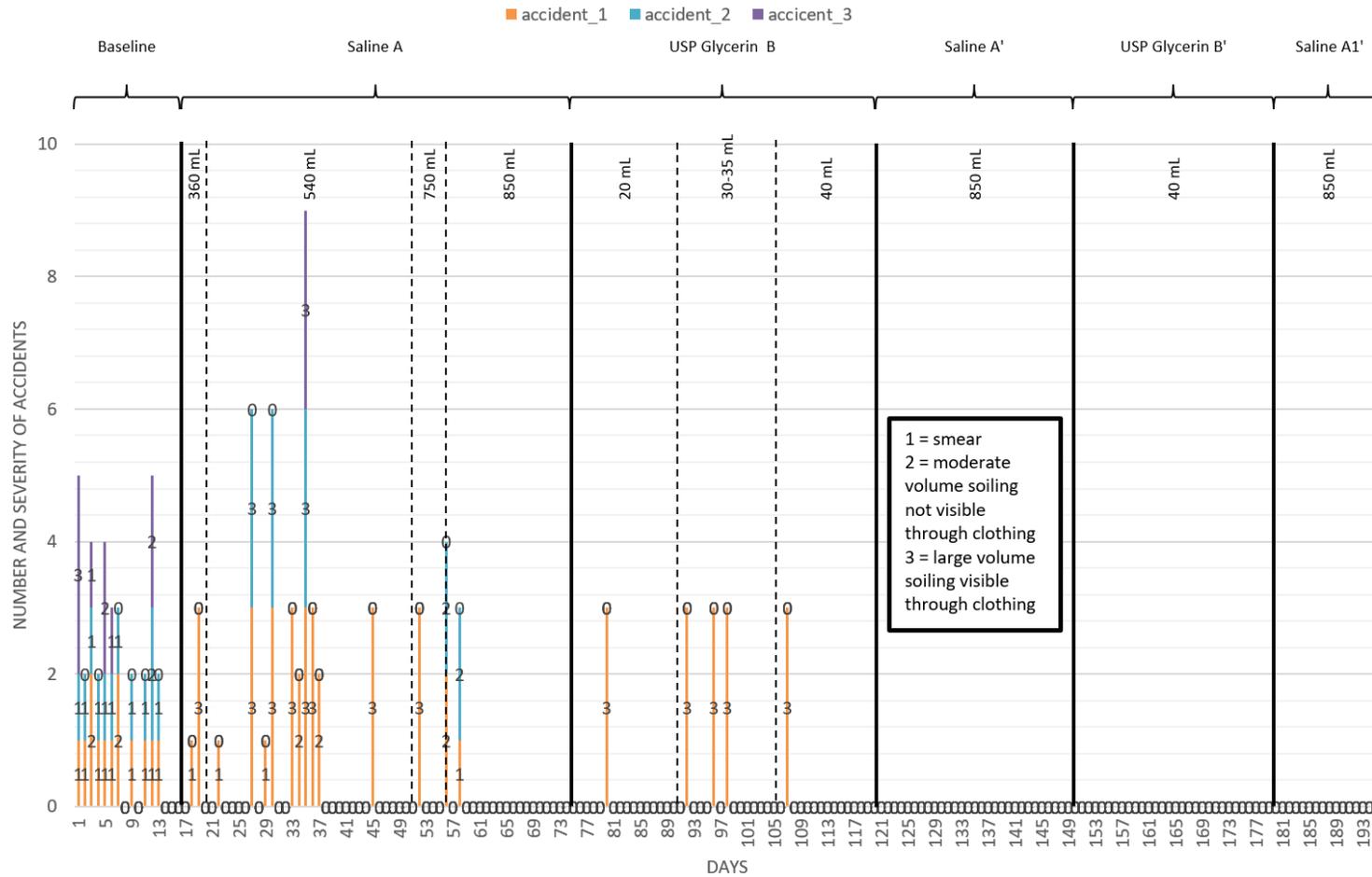


Figure 4-8: KJ001 Frequency and severity of incontinence graph

KJ002 FREQUENCY AND SEVERITY OF INCONTINENCE

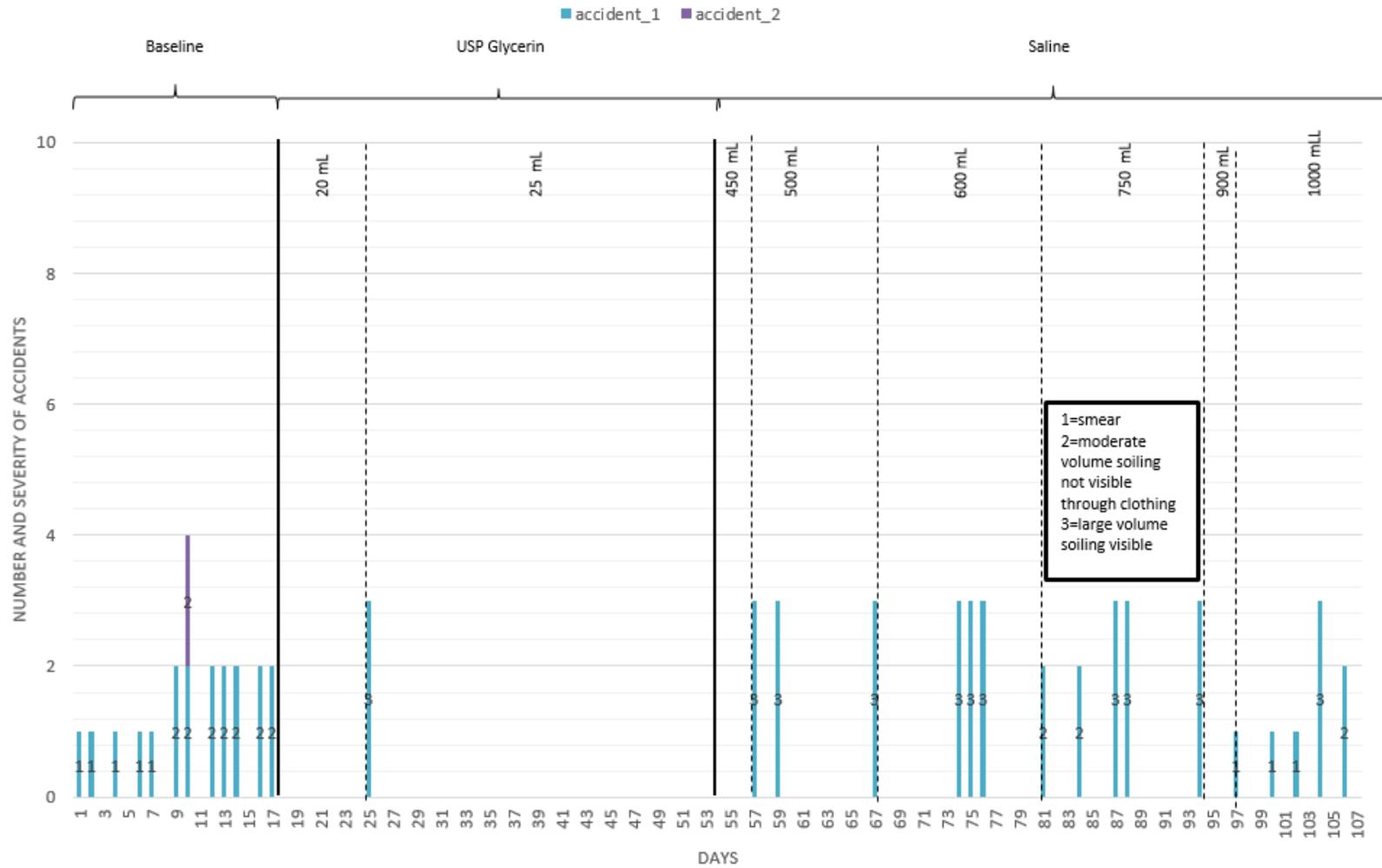


Figure 4-9: KJ002 Frequency and severity of incontinence graph

KJ003 FREQUENCY AND SEVERITY OF INCONTINENCE

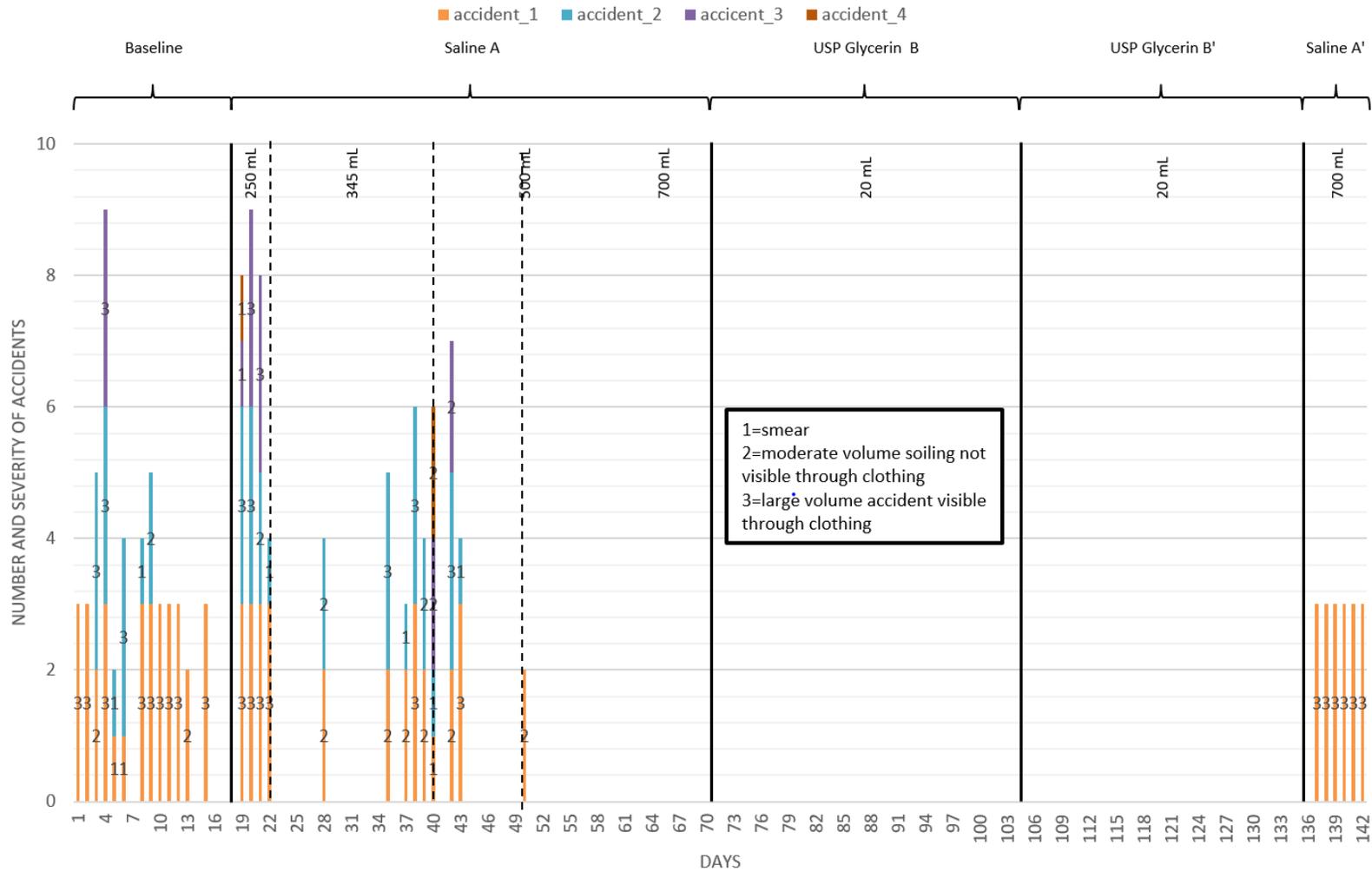


Figure 4-10: KJ003 Frequency and severity of incontinence graph

KJ004 FREQUENCY AND SEVERITY OF INCONTINENCE

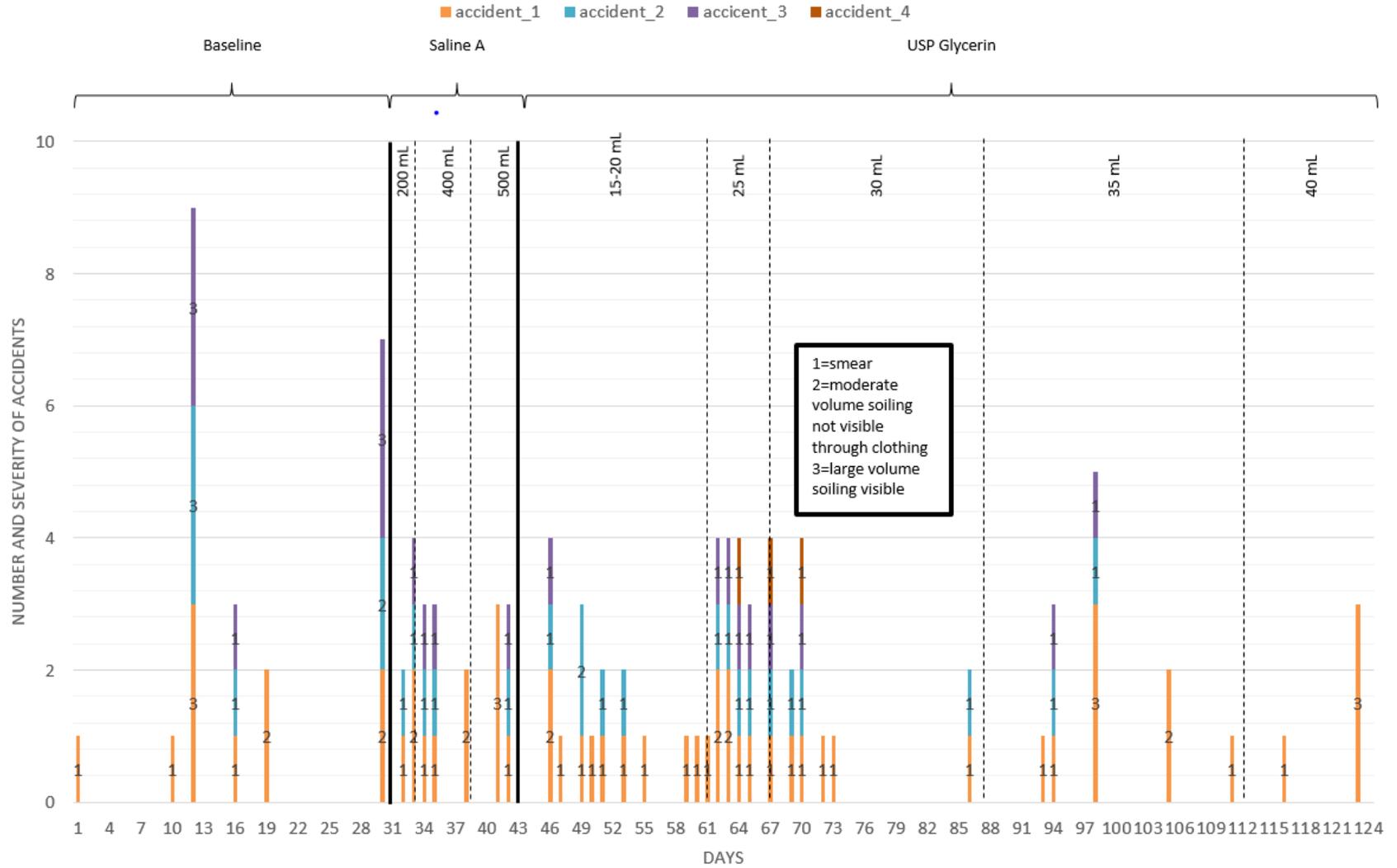


Figure 4-11: KJ004 Frequency and severity of incontinence graph

KJ005 FREQUENCY AND SEVERITY OF INCONTINENCE

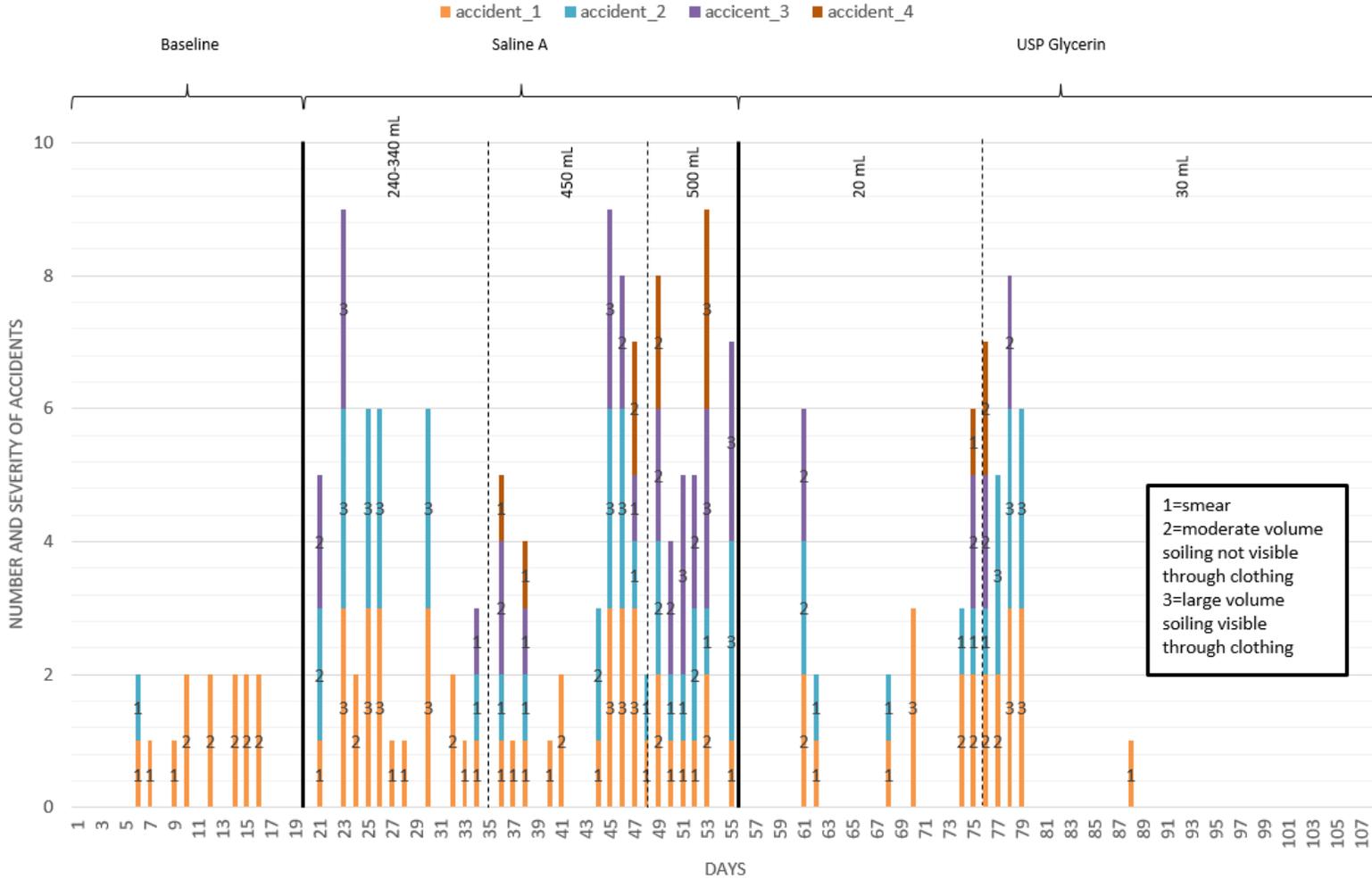


Figure 4-12: KJ005 Frequency and severity of incontinence graph

KJ001 PROCEDURAL TIME

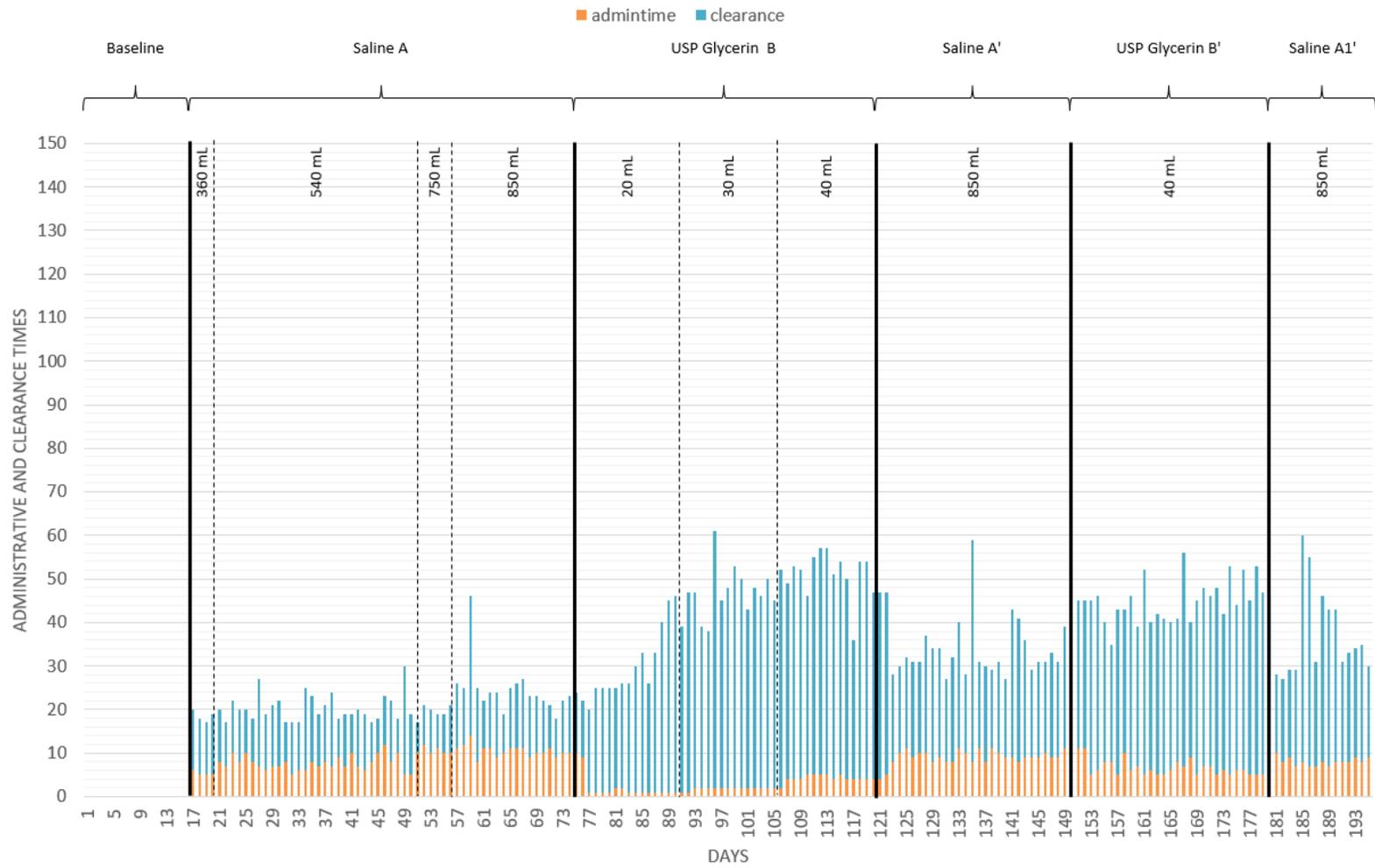


Figure 4-13: KJ001 Procedural time graph

KJ002 PROCEDURAL TIME

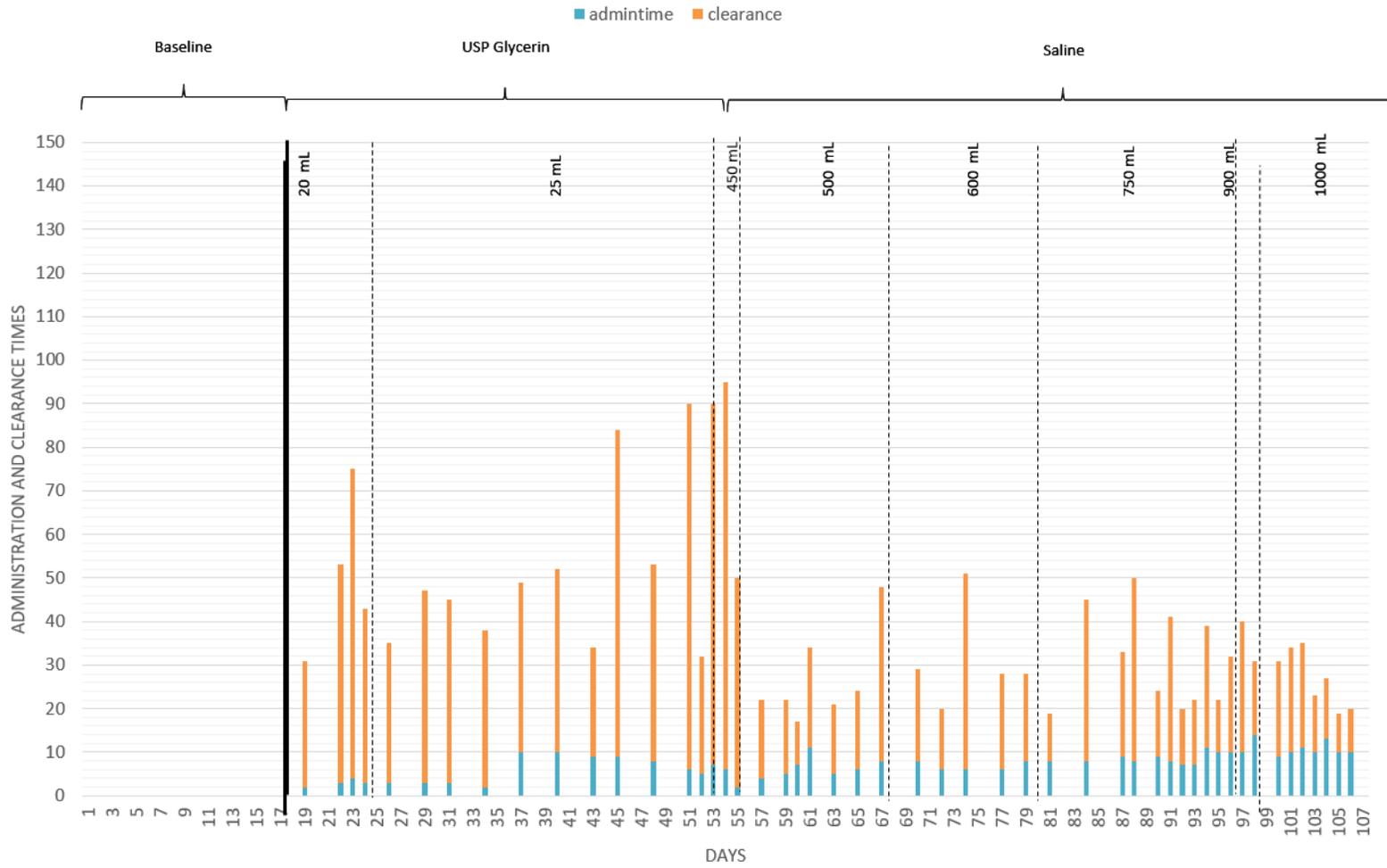


Figure 4-14: KJ002 Procedural time graph

KJ003 PROCEDURAL TIME

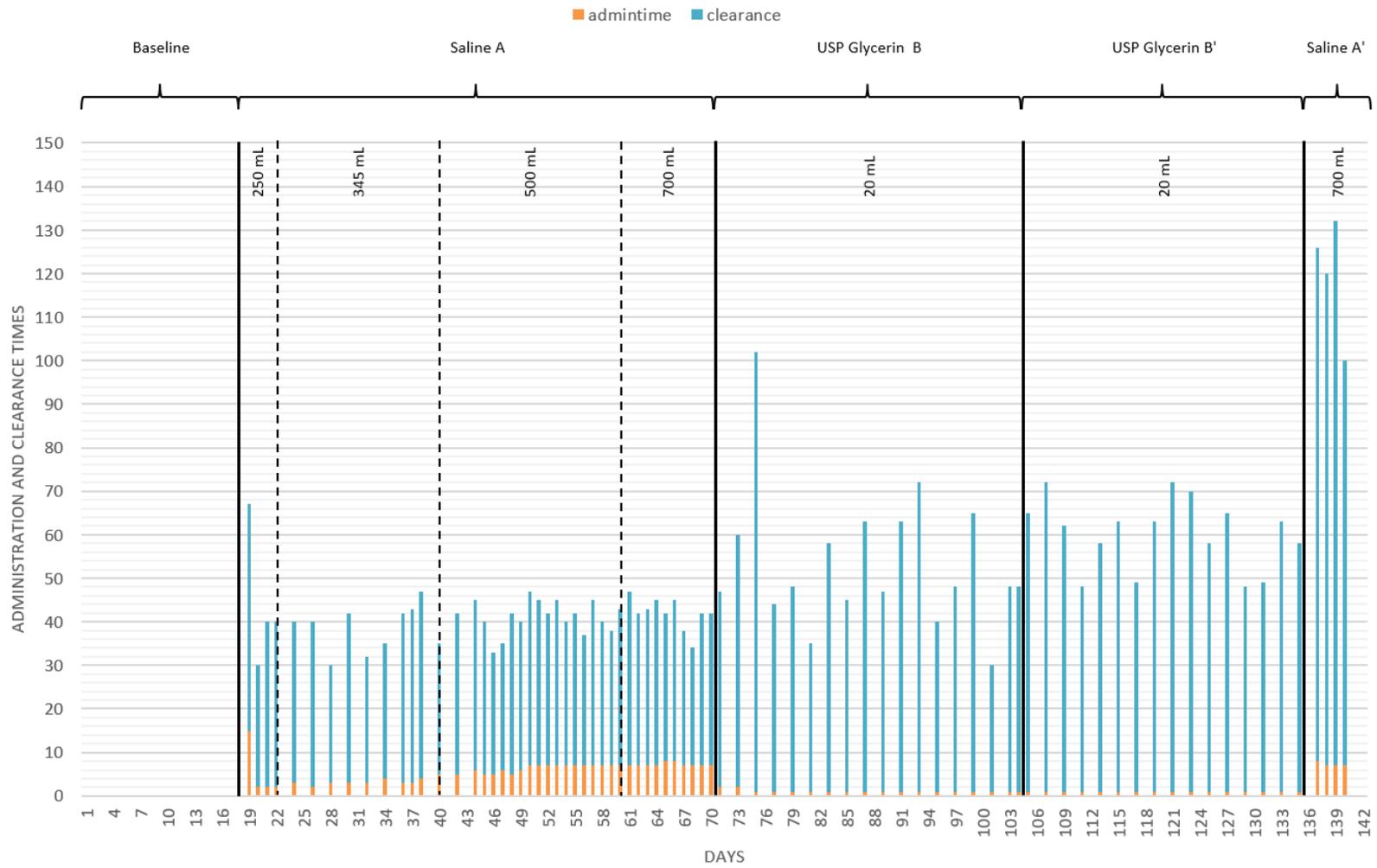


Figure 4-15: KJ003 Procedural time graph

KJ004 PROCEDURAL TIME

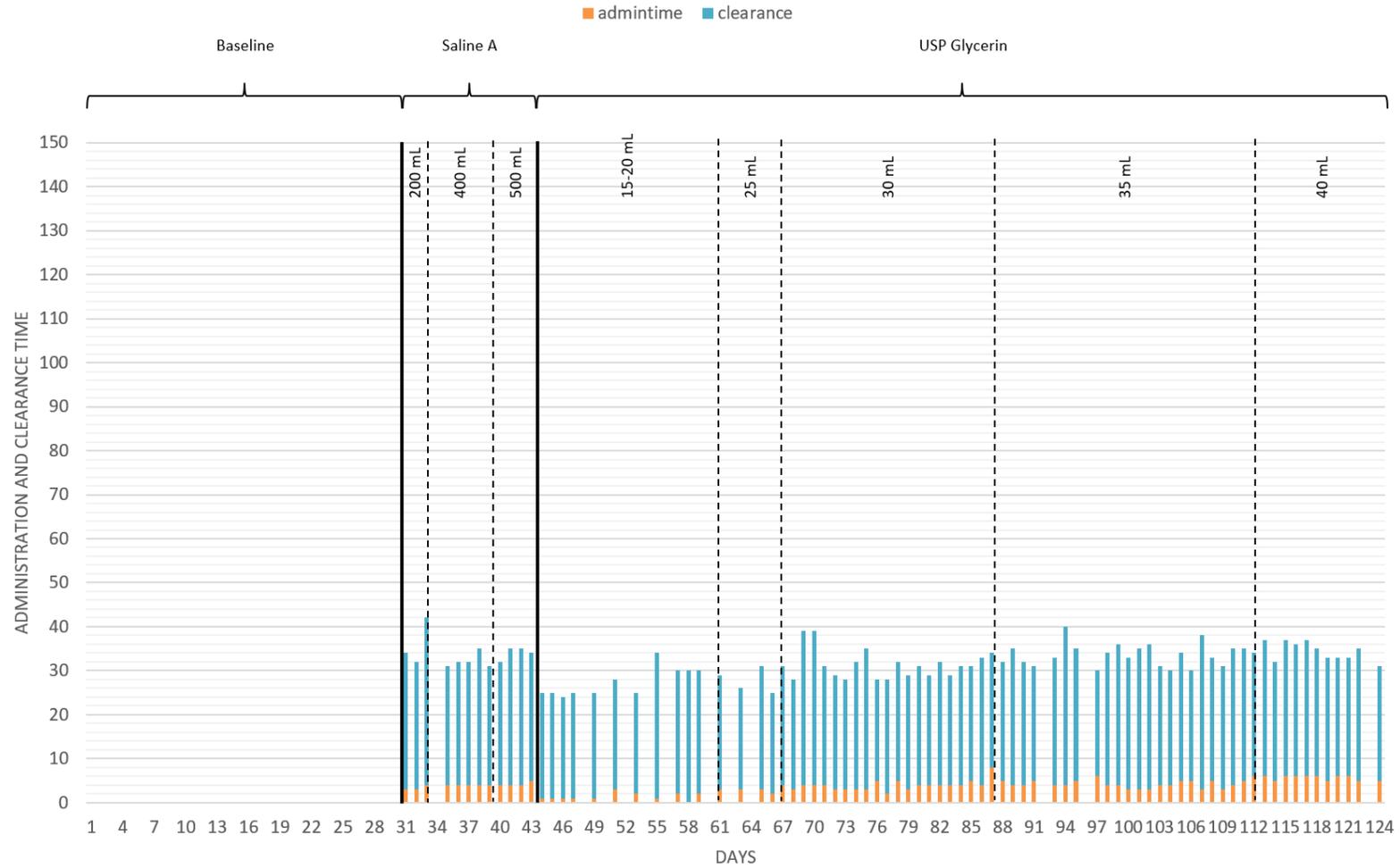


Figure 4-16: KJ004 Procedural time graph

KJ005 Procedural Time

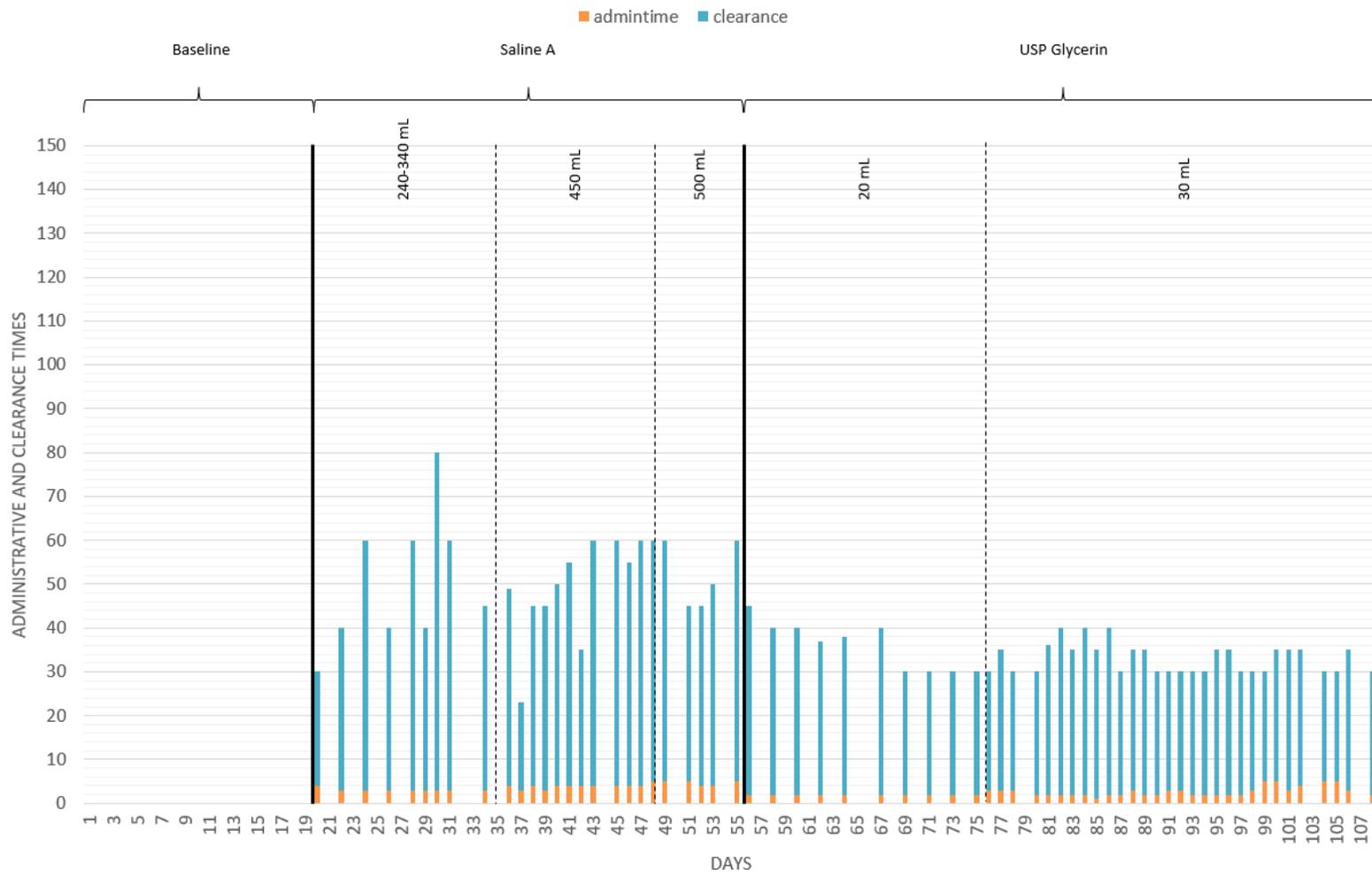
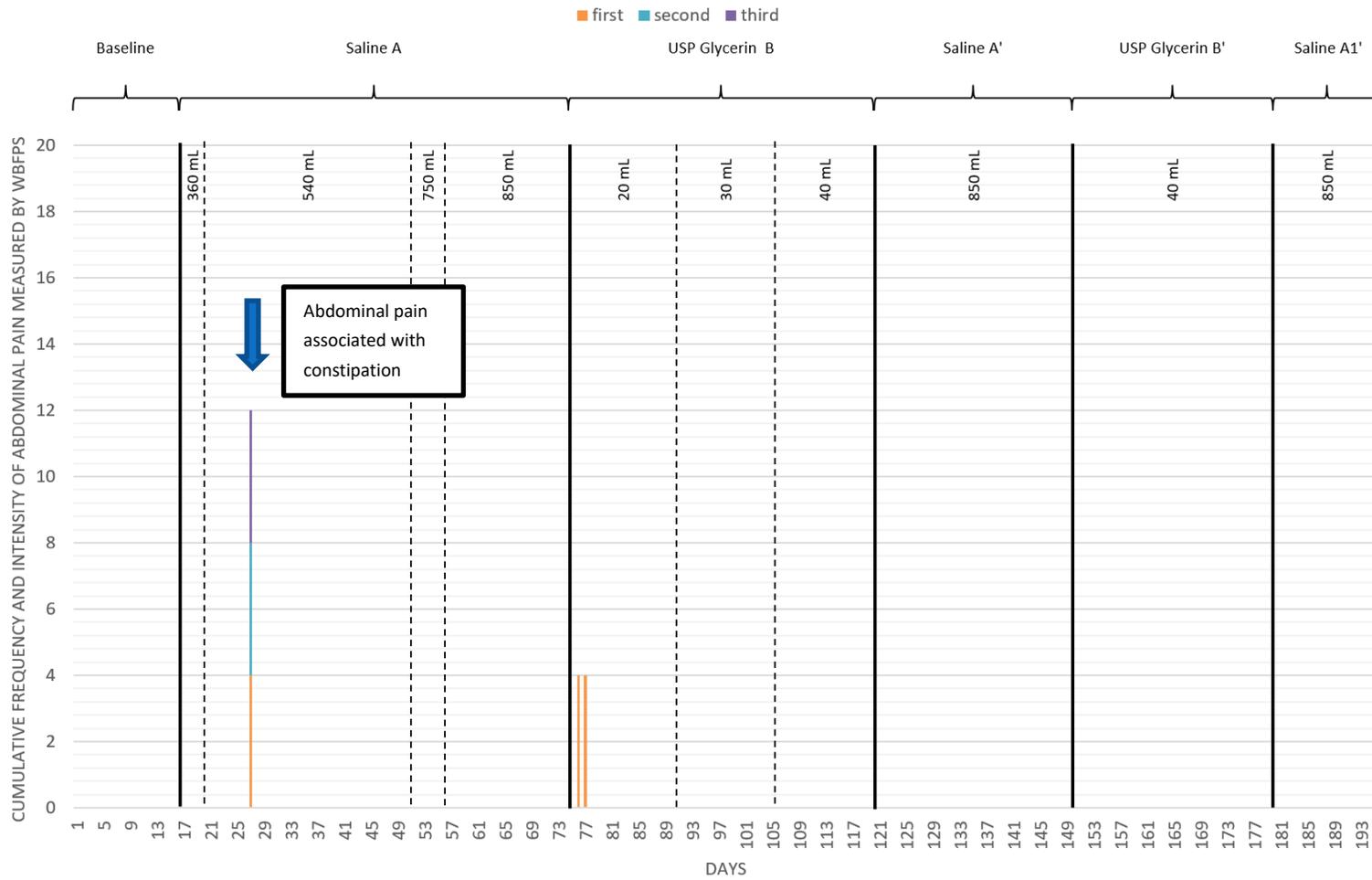


Figure 4-17: KJ005 Procedural time graph

KJ001 ABDOMINAL PAIN



Note: Severe episode of abdominal pain due to constipation – relieved with defecation following flush

Figure 4-18: KJ001 Abdominal pain graph

KJ001 CRAMPING FROM FLUSH

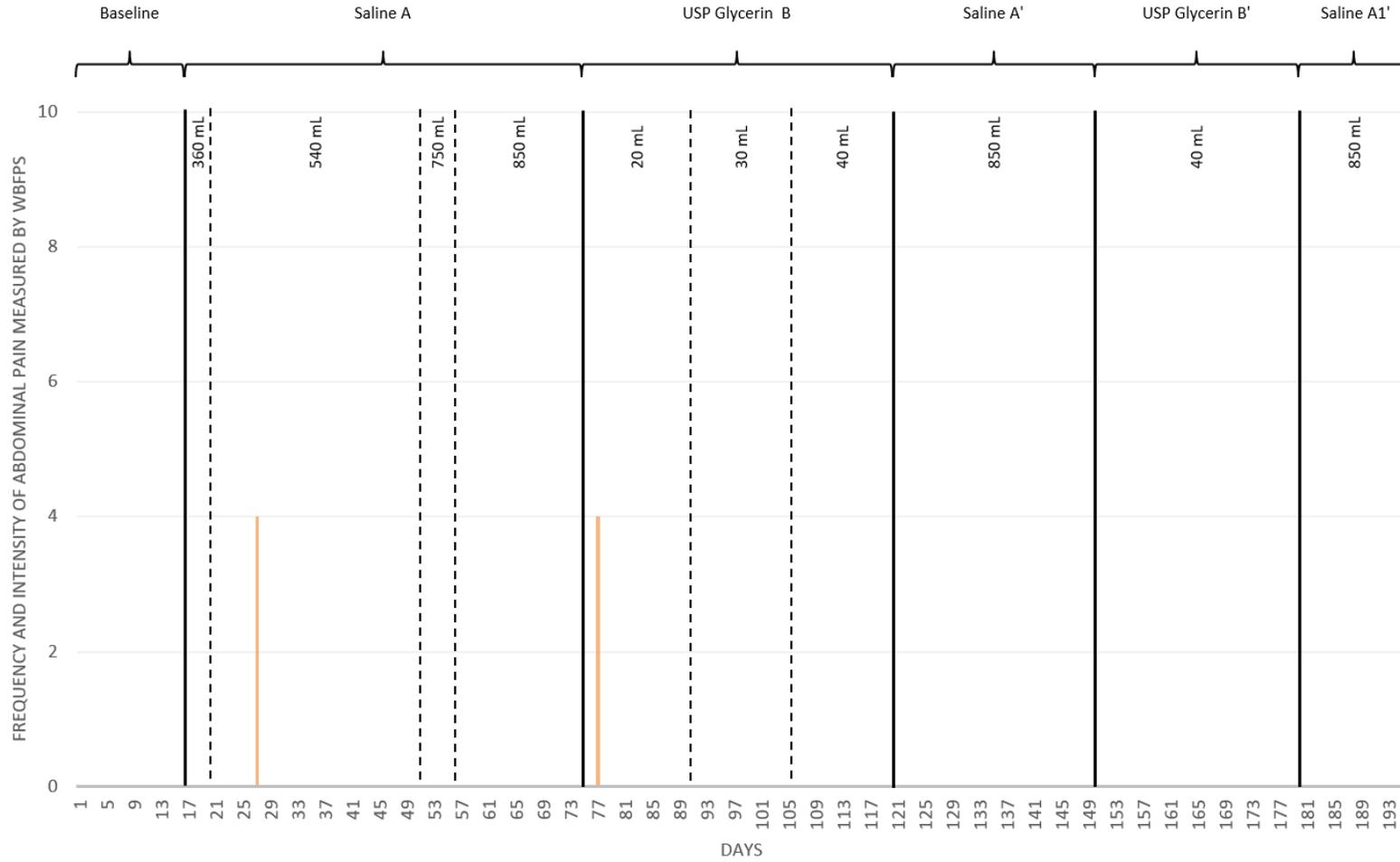


Figure 4-19: KJ001 Cramping from flush graph

KJ002 ABDOMINAL PAIN

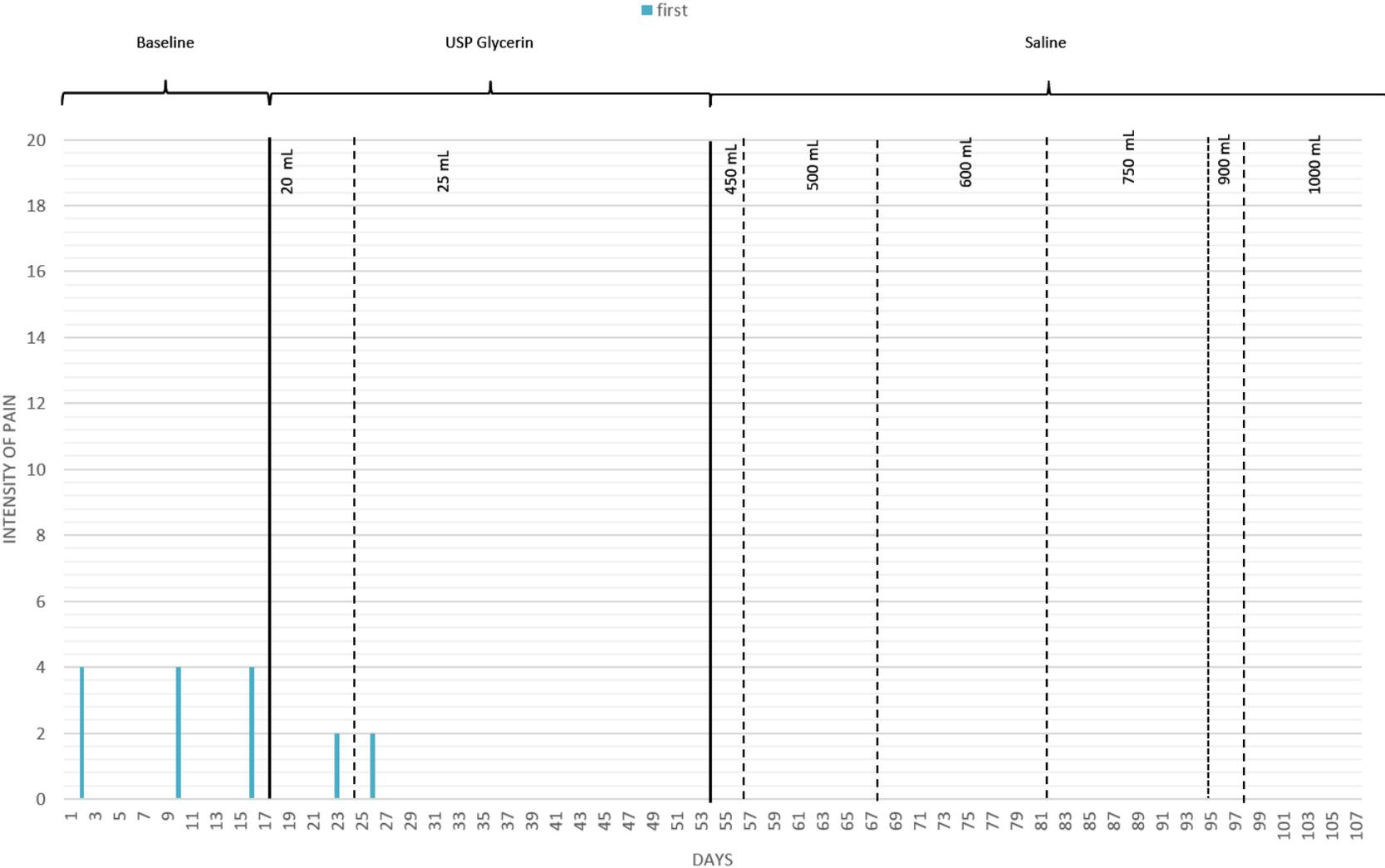
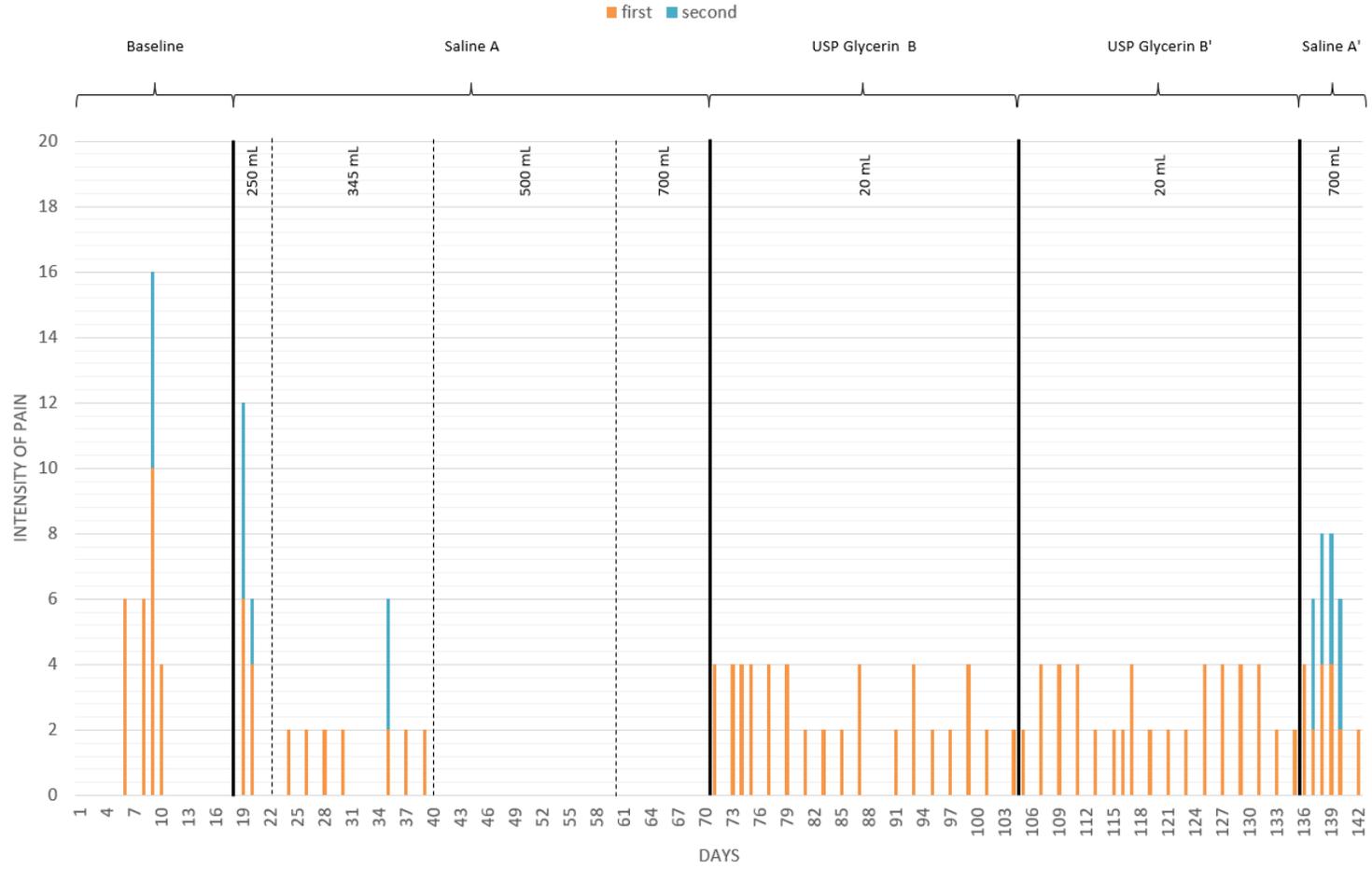


Figure 4-20: KJ002 Abdominal pain

KJ003 ABDOMINAL PAIN



Note: Significant abdominal pain in baseline may indicate existing predisposition to abdominal discomfort

Figure 4-21: KJ003 Abdominal pain

KJ004 CRAMPING FROM FLUSH

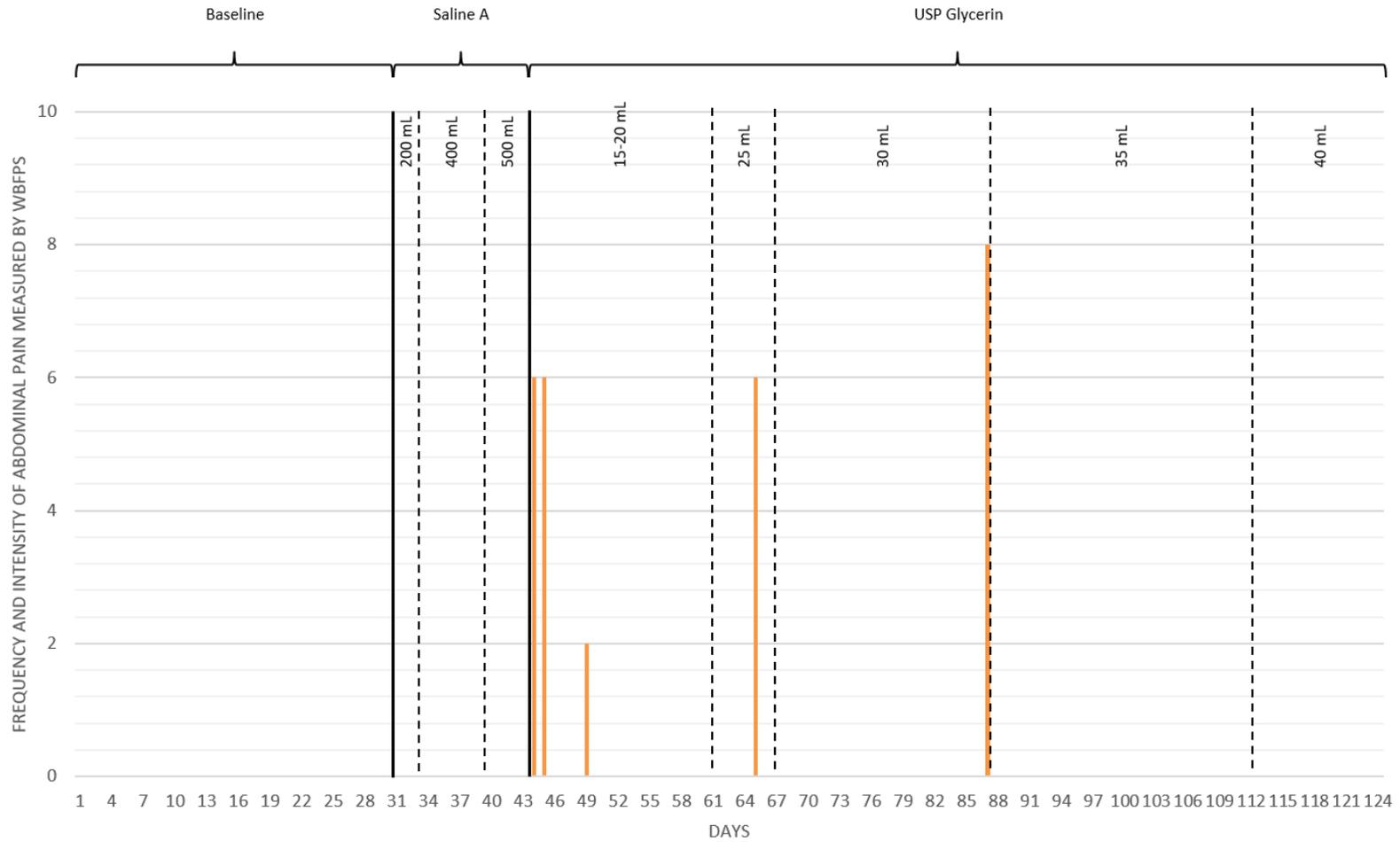


Figure 4-23: KJ004 Cramping with flush

KJ005 CRAMPING FROM FLUSH

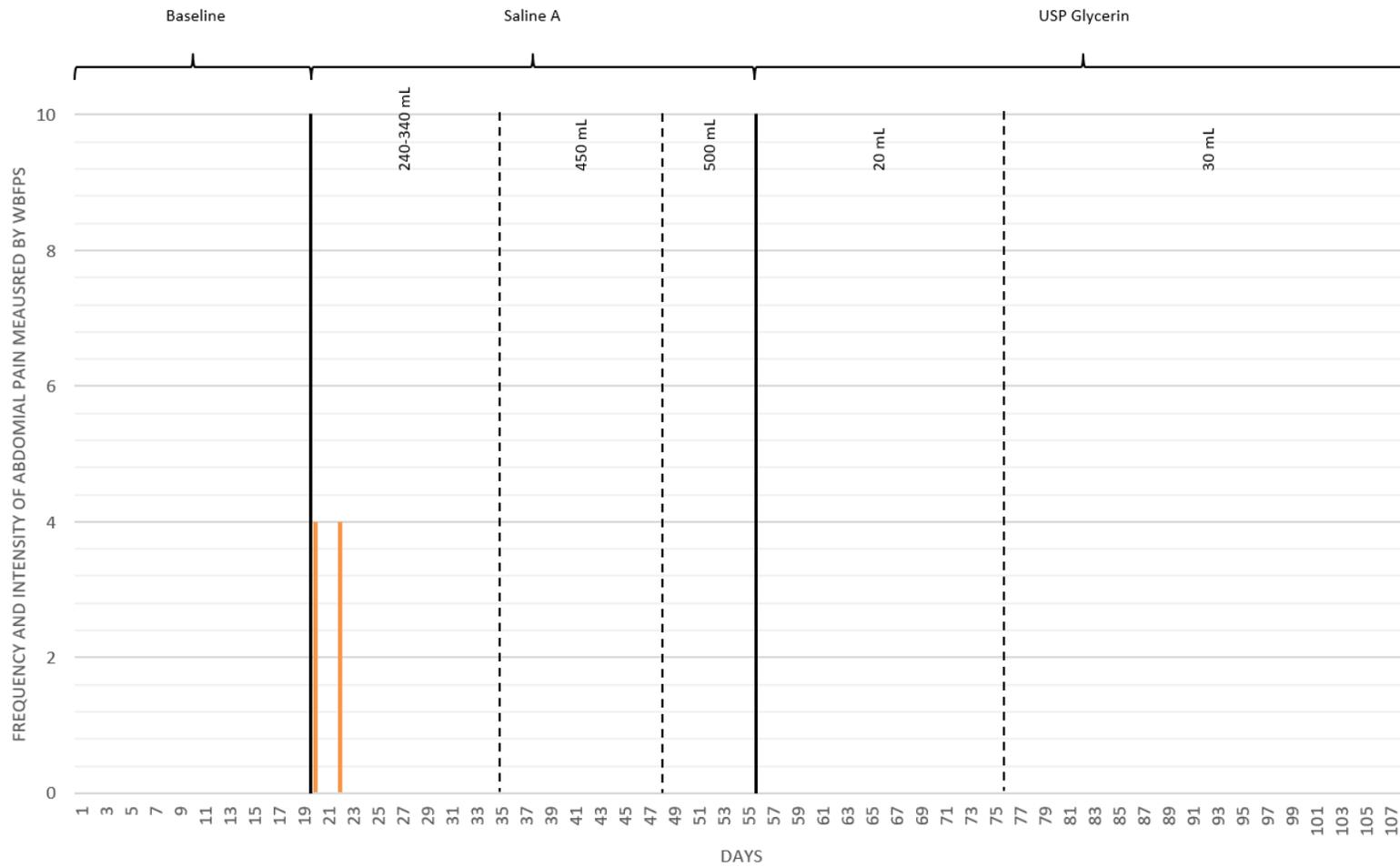


Figure 4-24: KJ005 Cramping with flush

KJ005 VAGAL SYMPTOMS

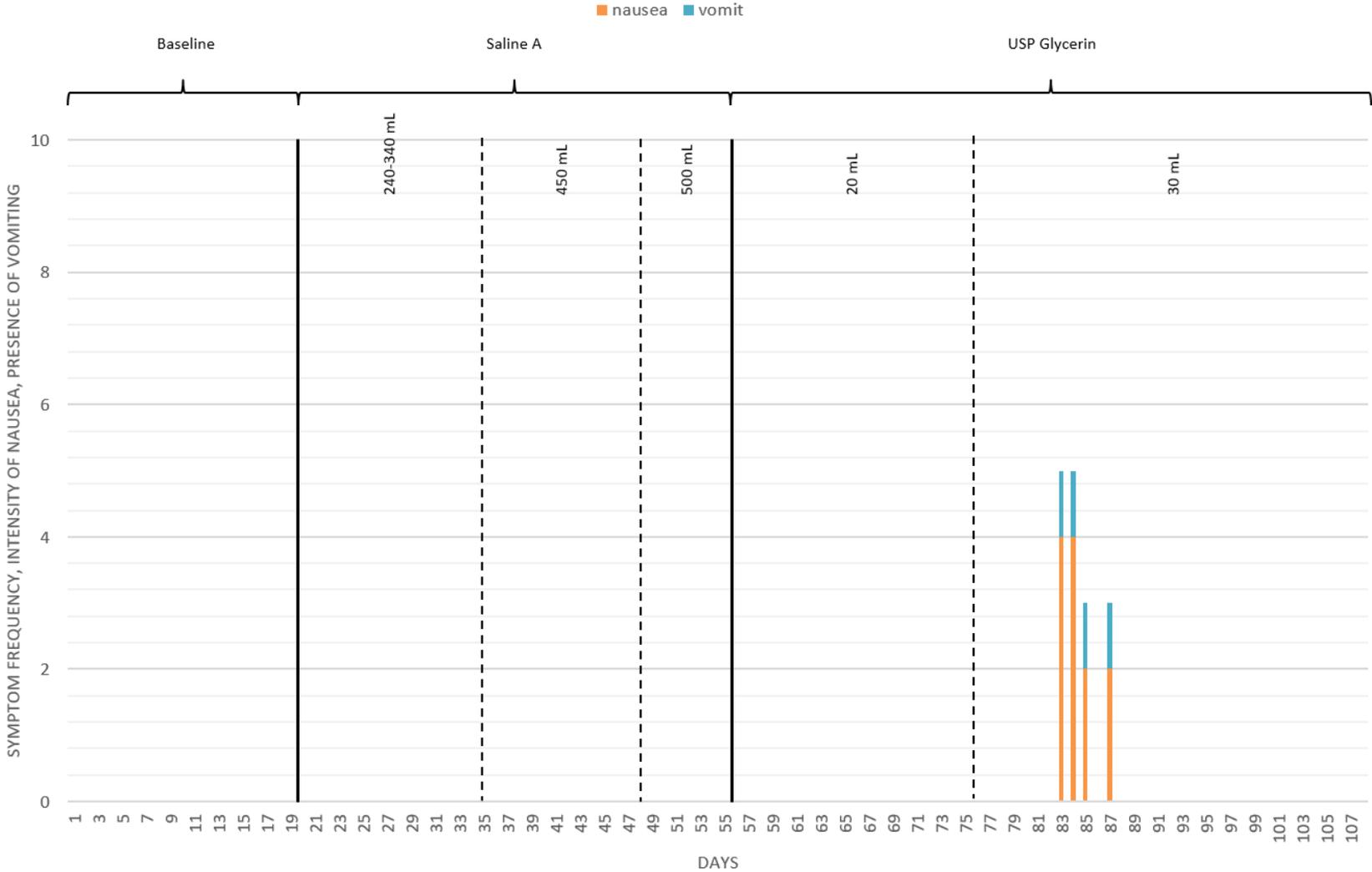


Figure 4-25: KJ001 Vagal symptoms graph

SERUM SODIUM LEVELS

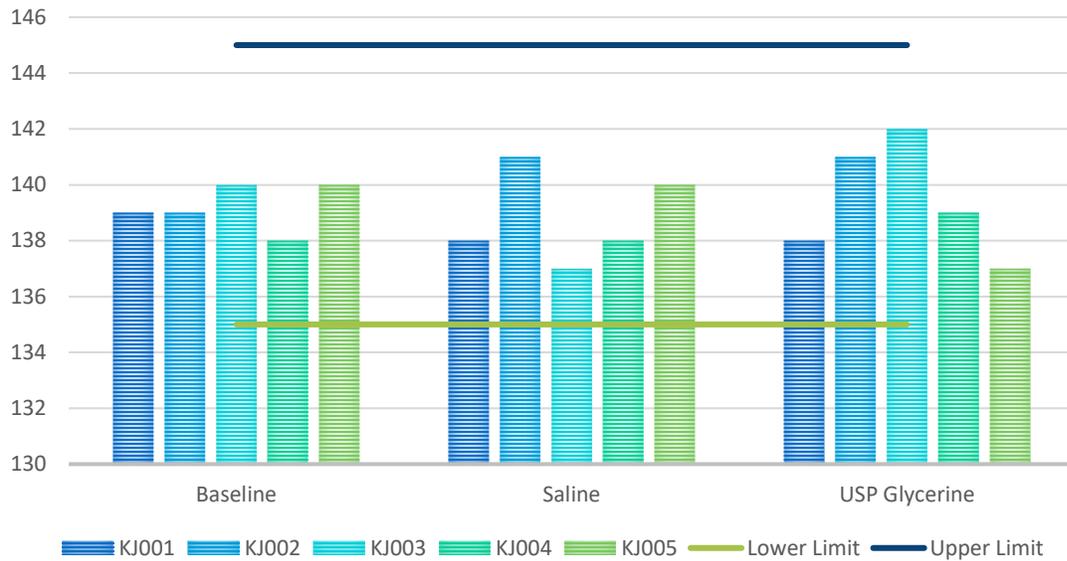


Figure 4-26: Serum sodium levels

SERUM POTASSIUM LEVELS

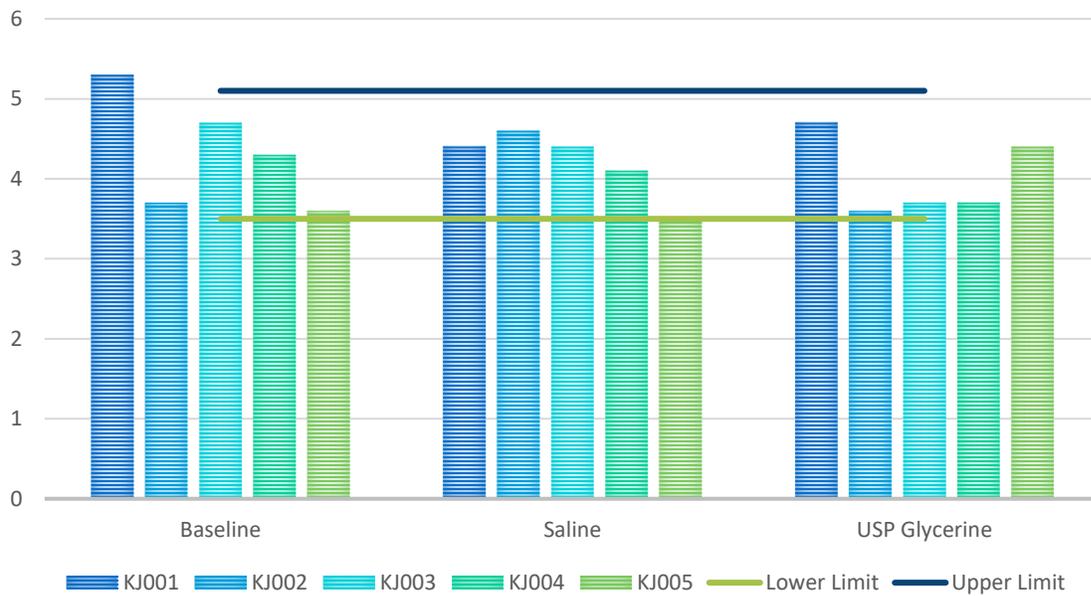


Figure 4-27: Serum potassium levels

SERUM CHLORIDE LEVELS

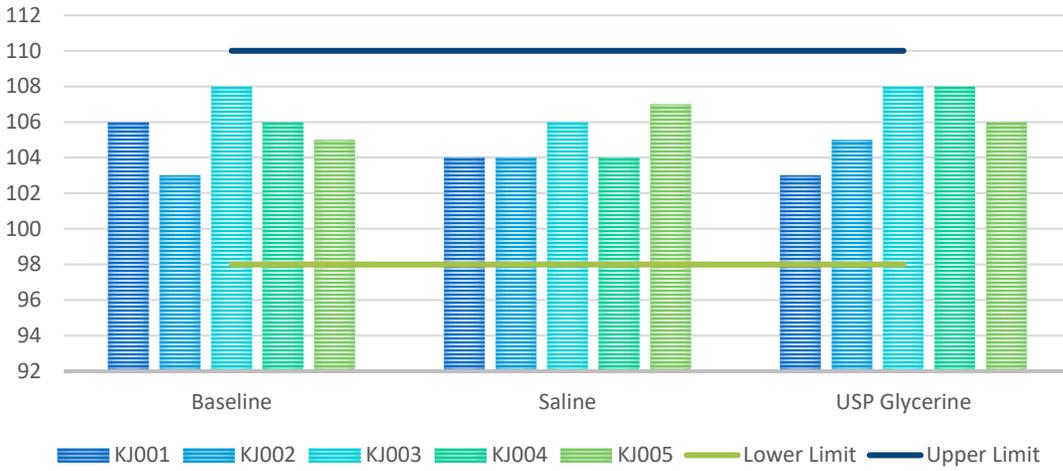


Figure 4-28: Serum chloride levels

CARBON DIOXIDE LEVELS

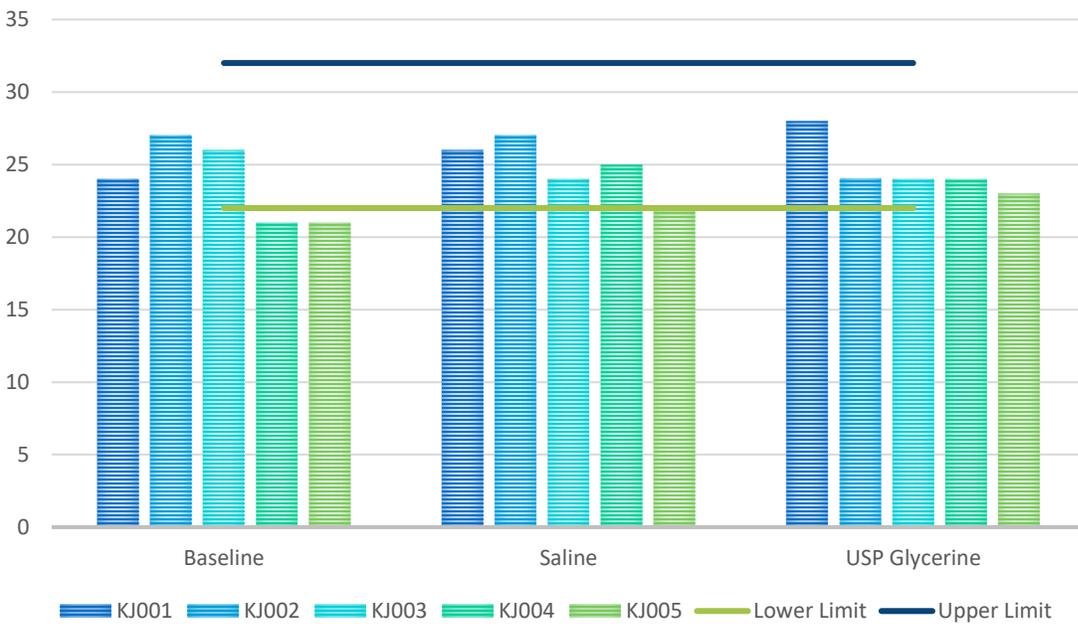


Figure 4-29: Serum carbon dioxide levels

BLOOD UREA NITROGEN LEVELS

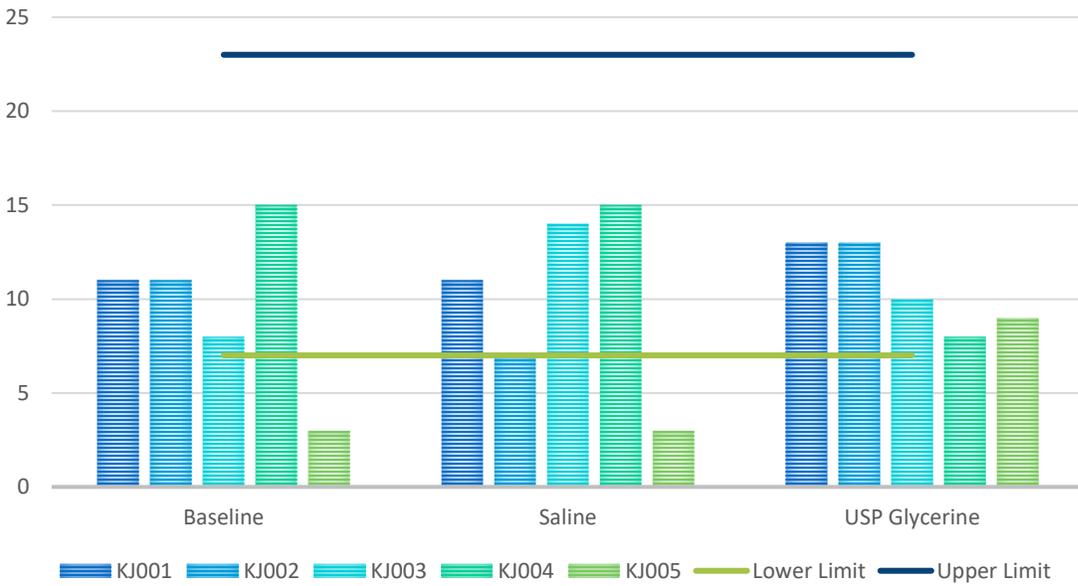


Figure 4-30: Blood urea nitrogen levels

CREATININE

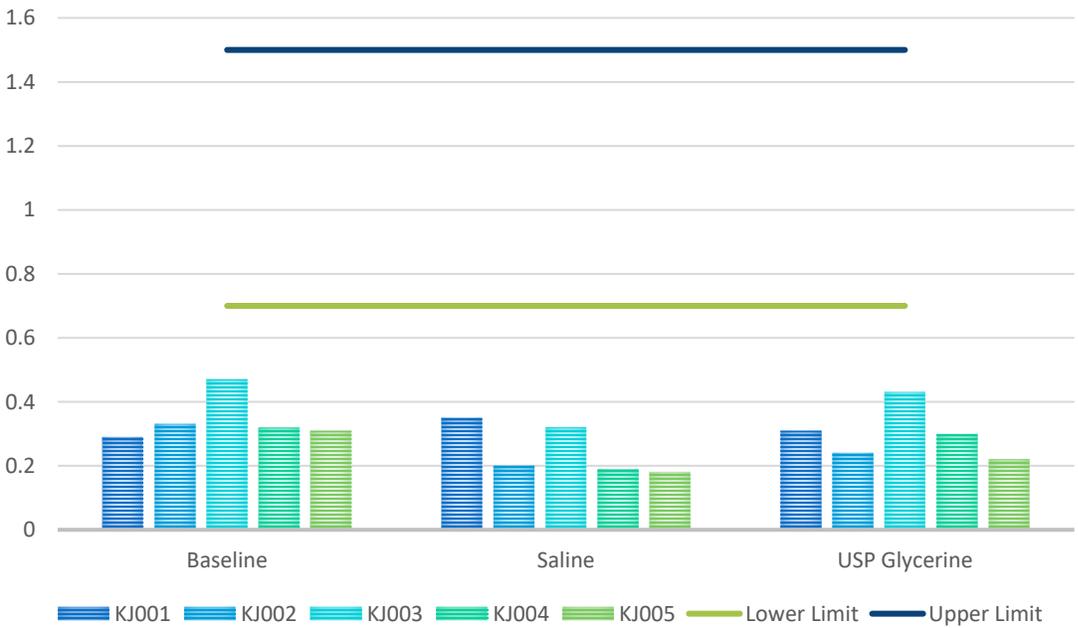


Figure 4-31: Serum creatinine levels (low levels normal in children)

CALCIUM LEVELS

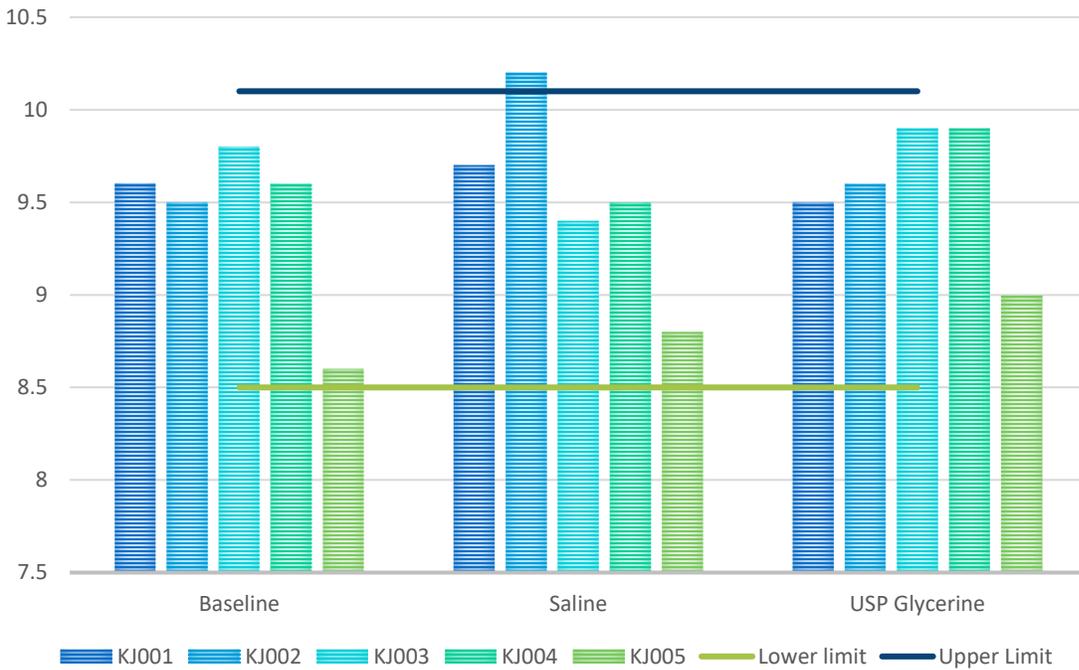


Figure 4-32: Serum calcium levels

STOOL CALPROTECTIN LEVELS

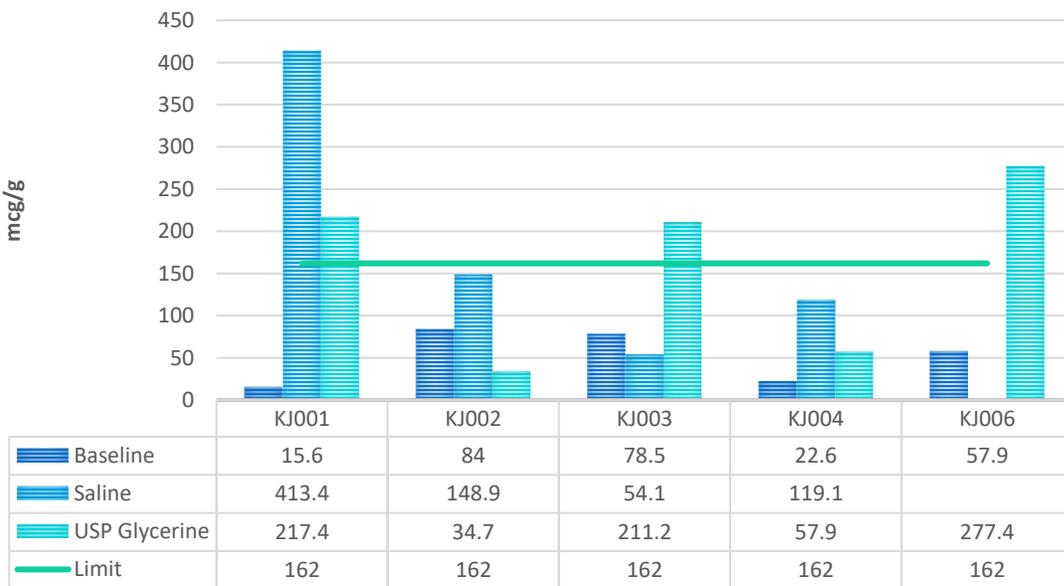


Figure 4-33: Stool calprotectin levels

CHAPTER 5 DISCUSSION AND CONCLUSIONS

Aimes Specific Discussion of Outcomes

Frequency of Administration

The ability to minimize frequency of flush administration is a clinically significant issue. One goal of ACE therapy is to maximize effectiveness while minimizing procedural intrusiveness. Minimizing flush frequency is one means to decrease the time commitment required to maintain continence. In the single study reporting outcomes of antegrade administration of USP glycerin, 84% of subjects required an every other day or less flush frequency to maintain continence (Chu et al., 2013). In this study, all five subjects required daily dosing when using normal saline, with only one of five achieving and maintaining continence on saline. Of the four subjects who gained and maintained continence on USP glycerin, 50% required an every other day or less flush frequency. Failure of subjects to gain continence on saline negated the utility of the results.

Minimizing the time required to achieve predictable continence on any flush regimen has implications for procedural acceptability on the part of the child and quality of life for both the child and family. Compared to the Chu et al. (2013) data, findings from this study may underestimate the flush frequency required to gain and maintain continence using USP glycerin. Findings in this study did not reach statistical significance due to sample size, but may have clinical utility. Building on previously reported outcomes, use of USP glycerin may result in predictable continence at a decreased frequency of administration in male children under 8 years of age with low lying spinal cord lesions, thereby decreasing the overall time burden and cost associated with a successful ACE flushing regimen. Further studies are needed to

confirm administration frequency, providing clinically useful information that has potential to guide clinical care and improve procedural acceptability.

Flush Titration Time to Continence

Sawin and Thompson (2009) reported themes from a qualitative study documenting seven parents' stress and struggle in finding an effective bowel management program for their children with spina bifida. The parents described a long journey complicated by the uncertainty of soiling and lack of responsiveness on the part of health care providers. Parents described the devastating impact of soiling on the affected children, including embarrassment, continual battering of self-esteem, and school issues. They described the constant struggle and stress caused by the unpredictable soiling. Finally, they described either immense frustration with failure to find an effective bowel management program or life changing joy when they found a program that resulted in predictable continence for their child.

The importance of expeditious achievement of predictable continence for affected children and their families cannot be overstated. Unfortunately, findings in this study were confounded by numerous factors, including medications, illness, dietary indiscretion, and failure in the timely reporting of episodes of fecal incontinence to the investigator, resulting in prolongation of the titration period and/or delays in dosing adjustment. Time to continence was further complicated by only two of five subjects achieving continence on saline during the dosing trial. These confounds render flush titration time to continence data irrelevant.

Cost Burden

Saline is the costlier antegrade treatment regimen when compared to USP glycerin. Because only 1/5 children achieved continence on saline, the results are confounded. However, it is conceivable that those children who failed on saline may have achieved continence on higher doses, increasing the cost differential. The argument could be made for mixing saline at home, considerably decreasing the cost of the saline flushing solution. Of concern, Schreiber et al. (1999) reported a death due to fatal hypernatremia following inaccurate home compounding and flush administration of a twice normal saline solution concentration. Hyde et al., (2008) prospectively evaluated the composition of homemade saline and twice normal saline flush solution from five families and found sodium concentrations in both the homemade normal saline and twice normal saline solutions were 45% higher than the target. Multiple conversations with peers working at different pediatric institutions suggest few continue to allow home compounding of saline due to electrolyte imbalance secondary to improper solution composition. While home compounding saline may reduce flush solution cost, it has been associated with increased morbidity and mortality which can be prevented with the use of premixed standardized flush solutions. Findings in this study do not reach statistical significance due to sample size, but in all five subjects, administration of saline was more expensive than USP glycerin, suggesting use of USP glycerin instead of saline may reduce the cost burden associated with ACE flushing regimens.

Fecal Continence

Findings from this study demonstrated a difference in the effectiveness of normal saline and USP glycerin flushes in achieving continence. Only two of five subjects

gained continence on normal saline during the dosing phase and only one of five (20%) maintained continence on normal saline in the maintenance phase. Four of five subjects gained and maintained continence on USP glycerin (80%).

The continence rate achieved on USP glycerin in this study is less than the 95% continence rate reported by Chu and colleagues in 2013. The dosing range was similar in both studies (continence dose \bar{x} = 28.75mL and Mdn = 27.5mL in this study versus a \bar{x} = 29mL, and Mdn = 30mL in the Chu study). The protocol in this study utilized normal saline as a diluent versus tap water in the Chu study. The difference in reported success rates in the two studies may be due in part to the difference in sample size (5 versus 23), subjects' age (3 to 7 years versus a mean age of 8.8 years), and the timing of data collection. In this study failure was defined as a single accident at maximum flush volume, whereas subjects in the Chu study had to have been on therapy for greater than 6 months and could have failed multiple times prior to reaching the 6-month mark. As an exemplar of that point, the single child that failed on glycerin during this study is now continent on glycerin, raising the overall success rate to 100% on glycerin flush measured at greater than 6 months follow-up.

The failure of subjects in this study to gain and maintain continence on saline differs from reported outcomes in previous studies. Published ACE effectiveness outcomes on saline were highly variable but uniformly higher than those reported in this study. It is difficult to place the findings from this study within the context of reported outcomes from previous retrospective studies. This in part stems from differing definitions of what constitutes continence, procedural and dosing considerations, sample size and composition, wide variability in diagnoses leading to the need for ACE

therapy, and publication content and clarity. Two retrospective studies were identified that reported continence rates using normal saline without additives. Siddiqui et Al. (2011) reported a success rate of 33% on saline at flush volumes ranging from 845 +/- 68 mL in 66 patients with age range of 11.2 +/- 5 years at time of antegrade stoma construction. Becmeur (2008) reported a success rate of 64% on flush volumes ranging from 250mL to 1000 mL (mean 700 mL) in 29 patients, 18 males and 11 females, ranging in age from 3 to 21 years in patients with a wide variety of diagnoses leading to the need for ACE therapy.

Kuizenga-Wessel et al. (2016) reported survey data of 23 pediatric gastroenterologists and surgeons with expertise in ACE therapy. Respondents' preference for enema flush volume was variable. Forty-eight percent started at 10-20 mg/kg, 22% used 0-249 mL, 22% used 250-500 mL, and 8% tailored the volume to the child. The higher rate of failure of subjects to gain and maintain continence on saline in this study may have been due to limiting the volume of the saline flush to 500mL in children 5 years of age or younger and 1000 mL to children over 5 years of age although saline flush volume reflected current standard of care. In addition, other factors that may have resulted in a discrepancy include a homogenous sample with regard to gender and diagnosis as compared to a wide variety of diagnoses represented in other studies, a younger sample age range, and a more restrictive definition of continence with immediate determination of flush failure at the time of any soiling on maximum dosing.

Kuizenga-Wessel et al. (2016) found that 16 (70%) started the flushing regimen with saline; 5 (22%) tap water, polyethylene glycol, or sodium-phosphate; and only 2

(8%) a stimulant using either USP glycerin or bisacodyl. Nineteen respondents added a stimulant only when the initial antegrade solution was not effective. The importance of achieving continence in the immediate post-operative period following an ACE procedure should not be underestimated. Findings in this study did not reach statistical significance but are clinically relevant. It may be reasonable for clinicians caring for a male child under eight years of age with a neurogenic bowel and patulent sphincter secondary to a spinal cord lesion lower than T-10, to consider starting the child on USP glycerin as opposed to saline.

Infusion and Procedural Time

In a survey of expert clinicians conducted by Kuizenga-Wessel et al. (2016), all but one respondent began the regimen with daily flushing. An infusion duration ranging from 0 to 15 minutes was preferred by 62% of respondents, with 38% preferring 16 to 30 minutes. A total procedural time ranging from 30 to 60 minutes following flush infusion was preferred by 65% of respondents, with 35% limiting procedural time to under 30 minutes. Although findings in this study suggest saline has the lower procedural time in minutes (\bar{x} =33.8, Mdn=35) when compared to USP glycerin (\bar{x} =62.8, Mdn=57), because only one of five subjects gained continence on saline, the difference in procedural time is clinically irrelevant. Because the timing of accidents did not occur directly following the flush, but occurred the following day prior to the start of the next day's scheduled flush, the decreased procedural time does not seem to be related to the poorer flushing outcomes on saline.

Side Effects

Several case series describe a variety of side effects from different flushing regimens, including pain with stomal intubation, nausea, vomiting, abdominal cramping, sweating, dizziness, and pallor (Dey et al., 2003; King et al., 2005; Bani-Hani et al., 2008). One of five children experienced persistent cramping with USP glycerin flush. The cramping was short in duration and immediately relieved with passage of stool. It is interesting to note that this child experienced abdominal discomfort in the baseline phase of the study, which may indicate a predisposition to abdominal pain. Of interest, the limited episodes of pain and cramping during saline administration either occurred in the immediate post-operative period (one subject) or were correlated with episodes of constipation (two subjects).

One child failed USP glycerin due to vagal symptoms. The child had missed several scheduled flushes and started on elemental iron supplementation. In addition, his osmotic agent was stopped while he was on normal saline in an effort to achieve continence and had not been restarted. USP glycerin was stopped and he was cleaned out with Golytely. He had no vagal symptoms on USP glycerin prior to the missed doses and successfully returned to a glycerin flushing regimen with the addition of an osmotic agent following the clean out without recurrence of vagal symptoms, suggesting a significant colonic stool burden secondary to missed doses and medication may have been responsible for the vagal symptoms.

Electrolytes

The bowel is a substantive absorptive surface. Instillation of solution into the cecum has the potential to cause electrolyte imbalance. Deaths have been reported due

to electrolyte imbalance from retrograde and antegrade enema solutions (Schreiber & Stone, 1999). Several case reports detail morbidity and mortality associated with flushing solutions, including hypocalcemia and hyperphosphatemia following retrograde administration of phosphate enemas in children (Helikson, Parham, & Tobias, 1997; Ismail, Al-Mutairi, & Al-Anzy, 2000) and water intoxication following retrograde enema therapy using tap water (Chertow & Brady, 1994). Solution composition, volume, retention time, and underlying electrolyte imbalances are all factors that increase morbidity and mortality (Yerkes et al., 2001).

Hyde et al., (2008) prospectively demonstrated clinically significant changes in serum electrolytes associated with antegrade enema administration of tap water and twice normal saline. No clinically significant electrolyte imbalance was associated with antegrade administration of saline. Outcomes in this study confirm previous findings that antegrade administration of normal saline does not result in electrolyte imbalance. A finding from this study that has not been previously reported is the absence of electrolyte abnormalities with antegrade administration of USP glycerin. Neither normal saline nor USP glycerin flush at the doses and frequency of administration used in this study resulted in any abnormality in serum electrolytes, increasing clinician confidence that both solutions may be safely administered as ACE flush solution in the well hydrated child with normal transit time,

Stool Calprotectin

Previous research has not explored the effects of ACE therapy on gut health. All subjects had an elevation of stool calprotectin levels in response to at least one flushing regimen. The highest increase in stool calprotectin was seen following saline flush.

Elevations above baseline suggest the presence of a low lying inflammatory process. In no instance did elevations reach a level of clinical concern requiring cessation of the study. Further study is needed to better define the long-term course and clinical relevance.

Quality of Life

A number of prospective studies have demonstrated the positive impact of ACE therapy on the quality of life for children and their families (Aksneset al., 2002; Bower, 2008; Kaugerset al., 2010; Ok & Kurzrock, 2011; Youssef et al., 2005). Subject attrition due to treatment failure resulted in incomplete survey completion.

Study Limitations

Both the limited number of enrolled subjects and failure of the majority of those enrolled to progress to the study maintenance phase, thereby limiting the number of collected data points, posed a substantial threat to statistical validity resulting in insufficient power. Reliability of measures, reliability of treatment, and heterogeneity of subjects did not pose a threat to statistical validity. Although there was no voluntary loss of subjects, a consequence of treatment failure was substantial attrition, resulting in an A-B-C design instead of an A-B-C-B'-C'-B₁' design as intended. Failure of subjects to complete the study was inductively relevant but deductively posed a threat to internal validity. Only one subject completed the study and was continent on both solutions, so replication of treatment effects did not occur in the majority of subjects. Failure of replication weakened the design, increasing the plausibility that other factors may have influenced the outcome. A legitimate argument could be made that confidence in the outcomes would have been significantly strengthened by allowing the three subjects

who initially failed on saline to progress to the maintenance phase of the study to determine if their failure on saline could be replicated. However, ethics prohibited the reintroduction of a treatment they had previously failed that had a high probability of causing recurrence of soiling.

Intercurrent illness, medications, dietary indiscretion, and, to a limited degree, inconsistency in treatment adherence, posed historical threats to internal validity. Interobserver agreement (IOA) was completed at protocol initiation, but could not be completed at every change in phase due to a study design flaw. Procedural fidelity was completed at each phase change, to some degree offsetting instrumentation threat to internal validity.

The shortened length of data collection and follow-up could have posed a threat to construct validity of cause and effect. However, the length of data collection on a single treatment was sufficient to reduce the potential for carry-over effects. Order effects were minimized by randomization. There were no identified social threats to internal validity. Inability to blind to treatment increased the risk of experimental bias.

Single subjects methodology is often used to evaluate operant behavior. Fecal incontinence in the children in this study was secondary to neurogenic bowel. Maintaining continence was not under their volitional control and could not be achieved without extraordinary intervention. This in some ways complicates use of traditional within subjects visual analysis, where timing for the institution of treatment and treatment changes are based on behavioral stability, trend, and trend direction. Issues such as a stable baseline or whether the rate of incontinence is accelerating or decelerating in the baseline phase is not applicable to this population. All subjects were

incontinent at baseline. The purpose of the study was to assess if subjects could gain continence on one or both flushing regimens. Any single episode of fecal incontinence or pain greater than a 4 on the pain scale were unacceptable and resulted in an immediate dosing change, making the traditional methods of visual analysis such as data stability and trend relevant only in those instances in which the subjects gained and maintained continence in the presence of minimal side effects.

Implications for Future Research and Practice

Probabilistic Epigenesis as a Framework for Pediatric Incontinence Research

Probabilistic Epigenesis provided a theoretical framework for the study of ACE therapy in children. The overarching principles contained within the constructs and relational statements of Probabilistic Epigenesis appear to be highly applicable to the study of pediatric incontinence and dysfunctional habits of elimination. The restructured model facilitated experimental confirmation. Findings from this study suggest there is both a vertical and horizontal coaction of the constructs of Environment (ACE flush) on Neural Activity (electrolyte balance, and gut health as measured by calprotectin), and Behavior (continence). Further studies are needed test the applicability and fit of Probabilistic Epigenesis in pediatric incontinence research.

Implications for Future Research

Findings from this study are promising but the sample size was insufficient to reach statistical significance. Further study is needed. The children who require ACE therapy to maintain continence constitute a small population. The case can be made to use inductive methodology utilizing single subjects design with replication between subjects. The limited population makes the study of ACE therapy well suited to within

subjects methodology. The case can be made to compare flushing regimens deductively using a cross over design. The study of ACE therapy is well suited to a cross over design, which facilitates obtaining the same number of observations with fewer patients and the same precision of estimation with fewer observations resulting in a significant savings in resources when compared to a parallel group design. One of the major disadvantages of a cross over design is carry-over, which if not accounted for, may lead to errors in interpretation. The impact of carry-over can be minimized by incorporation of a wash out period and use of appropriate statistical analysis. Using a two-sample method versus a one sample method produces a smaller variance and eliminates conditional bias when sample sizes differ by order. Calculating treatment effects by ignoring the washout and post washout values and using the last data point (post-test) differences only will substantially decrease variance. Increased precision and efficiency in analysis will allow estimation of treatment effects in the presence of a smaller sample size thereby saving resources (Jones et al., 2003; Senn, 2002; Shuster, 2017). Future research comparing flushing ACE flushing regimens would be well served by utilization of a cross-over design embedded in a single subjects design and analyzing the cross-over using a two-sample method and post-test differences.

Conducting a multicenter study would accelerate enrollment, thereby producing information on treatment effects in a shorter time frame. The design would benefit from simplification of the titration scheme which could conceivably shorten the study duration and minimize the burden of data collection on families.

Implications for Clinical Practice

The results of this study did not reach clinical significance but the effect size suggests clinical utility. This study is the first study to prospectively compare two antegrade flushing regimens. Findings of interest in this study included (a) comparative treatment outcomes with substantial treatment failure on normal saline when compared to USP glycerin, (b) decreased frequency of administration in two subjects using USP glycerin versus saline, (c) discomfort with USP glycerin flush in a single subject that did not occur with any subject on normal saline flush, (d) saline was the costlier flushing regimen, (e) no evidence of electrolyte imbalance with either flushing regimen, and (f) elevations in stool calprotectin compared to baseline that did not reach a level of clinical concern requiring cessation of the study, but is of interest and will require further study to better define course and determine relevance.

Conclusions

This study compares two ACE flushing regimens. The ACE procedure has been used for well over two decades. Case reports and retrospective studies detail widely divergent effectiveness rates. The literature and involved clinicians have identified the need for prospective trials comparing ACE flushing regimens, but none have been undertaken to date. This is in large part because the small size and heterogeneity of this population does not lend itself to a large N study. Many clinical questions go unanswered due to over reliance on randomized controlled trial large N methodology. This population is an exemplar of that problem. Use of a cross-over design embedded in single subjects methodology has started to provide clinically useful information that could ultimately improve care for children with neurogenic bowel on ACE therapy.

Both single subject and between group research make and test predictions about treatment effects, the first by evaluating treatment effects on an individual, the second by addressing group mean and variance (Kazdin, 2011). Single subjects and group designs, in their most rigorous form, rule out or make implausible rival hypotheses for the experimental outcome, improving quality of inference (Cook & Campbell, 1979; Kazdin, 2011; Shadish, Cook, & Campbell, 2002). In this study, the sample size was too small to deductively determine inferential significance. Due to early treatment failure within the bounds of ethical considerations, the inductive case for which flushing regimen is more effective could not be made by replicating the intervention within subjects. However, the argument could be made that replication of findings between subjects has started to inductively suggest that in male children under 8 years of age with neurogenic bowel due to low lying spinal cord lesions, USP glycerin can be safely administered and may be more effective than normal saline in achieving continence.

APPENDIX A THEORETICAL AND OPERATIONAL LINKAGES FOR RESEARCH ADDRESSING INCONTINENCE

CONCEPTUAL	Constructs	Genetic Activity	Neural Activity	Behavior	Environment	
THEORETICAL	Concepts	Family History of Incontinence or dysfunctional elimination	Bowel, bladder & pelvic floor function; assessment of psychological comorbidities that interfere with functional patterns of elimination	Toileting behaviors	Physical and Social Environment including: home and school, child and parental assessment of social environment, and Quality of Life	
EMPIRICAL INDICATORS	Measures	Complete a three generation family tree specifically detailing constipation, encopresis, anorectal malformation, IBD, neurogenic bowel, spinal cord anomalies, urinary frequency, urgency, UTI, diurnal enuresis, nocturnal enuresis, neurogenic bladder, midline birth defects, psychiatric comorbidities in family members of children with incontinence and dysfunctional elimination	Bowel - Rectal manometry Bladder - Urodynamics Pelvic Floor - EMG Screening for psychological comorbidities using the Vanderbilt Assessment Scale - 55 questions assessment tool. Screens for ADD/ADHD, conduct disorder, anxiety, OCD, ODD & depression	Daily elimination diary in children who are neurologically intact & who have neurogenic incontinence Identify stool consistency in neurologically intact children using the pictorial Bristol Stool Scale	Number, accessibility, cleanliness, and privacy of restroom facilities. Assessment of child's response to the social environment using "The Way I Feel About Myself" Piers-Harris Children's Self-Concept Scale Assessment of parents' response to the child's incontinence using the Parenting Stress Index Short Form	Assess child with urinary incontinence quality of life using the Pediatric Urinary Incontinence Quality of Life (PIN-Q) tool Assess both the parent of and child with neurogenic bowel and antegrade enema administration quality of life using the Fecal Incontinence and Constipation on QOL tool There is no tool with established psychometrics for non-neurogenic children with constipation and encopresis
	Scores	Obtain frequency counts & identify associations between family and child with regard to predisposing factors, type of incontinence, & type of dysfunctional elimination; compute family member prevalence; analyze associations between parental and child manifestations with calculation of odds ratios	Bowel- Rectal Manometry: anal canal length/cm; max squeeze mm/Hg; first sensation & critical volume in mL/air balloon insufflation; presence of rectoinhibitory reflex Bladder- Urodynamics: vesical, abdominal, detrusor & urethra pressure in cmH2O at first, normal, strong & urgent desire; compliance in mL/cm H2O; Valsalva leak prs cmH2O; compliance; maximum infused capacity, detrusor activity, residual volume mL Uroflow parameters of maximum flow mL/s, voiding time in seconds, flow curve assessment, and continuity voiding stream Pelvic Floor - evaluate for synergic or dyssynergic coordination of bowel or bladder contraction and pelvic floor relaxation Vanderbilt scale 0-3; ADD items 1-9, ADHD items 10-18, ODD items 48-55, CD items 48-55, anxiety/depression items 41-47 & 48-55.	Neurologically intact - Daily elimination diary including: fluid intake in mL/kg; timing in 0 to 240 hrs & minutes and volume in mL of each void; timing of each bowel motion in 0 to 2400 hrs & minutes; document stool consistency using the Bristol Stool Scale, & timing and # of accidents for urine and stool Neurogenic Bowel/Bladder - Daily elimination diary including: fluid intake in mL/kg; timing in 0 to 2400 hrs & minutes and volume in mL of urine output with each catheterization; # and timing of accidents for urine; use of retrograde/ antegrade enemas: volume in mL/kg and composition of flush, time in total minutes to complete flush, number and timing of stool accidents	Restrooms counted and rated in cleanliness & privacy on a 5-point ordinal scale Piers-Harris Children's Self-Concept Scale is a 6 category, 80 item dichotomous yes-no scale questionnaire Parenting Stress Index Short Form is a 36 item questionnaire using an ordinal scale ranging from Strongly Agree to Strongly Disagree	PIN-Q consists of a 20 item ordinal scale 0-4 scale with FICQOL is a 7 grouping, 51 item ordinal scale questionnaire

C - T - E format abstracted from Gibbs (1972) and modified by Dulock and Holzemier (1991)
Note - all listed questionnaires have well established psychometrics for this population

APPENDIX B
REQUIRED MATERIALS FOR FLUSHING PROCEDURE

Required Flushing Materials

Flushing Regimen	Materials
Normal Saline	<ul style="list-style-type: none"> One liter containers of Normal Saline (NS) premixed to 0.09% One 1,000 mL enteral feeding bag with drip chamber and roller clamp One Christmas tree adapter connected to the end of the enteral feeding bag tubing A hook hung on the wall beside or behind the commode at a height of 5' from the floor One appropriate access tube for Chait or Mickey low profile device Warm tap water and mild liquid dish detergent to wash equipment after each use One stop watch One clip board with data collection sheets and pen attached with string to the clipboard
USP Liquid Glycerin mixed with Normal Saline	<ul style="list-style-type: none"> One appropriate access tube for Chait or Mickey low profile device One 60 mL catheter tipped syringe One 8 ounce bottle with screw top to mix liquid glycerin and Normal Saline One liquid bisacodyl enema solution in pre-packaged 10 mg aliquots One 10 mL syringe for measuring liquid glycerin solution One liter containers of NS premixed to 0.09% One stop watch One clip board with data collection sheets and pen attached with string to the clipboard

APPENDIX C
PROCEDURAL INSTRUCTIONS FOR COMPLETING SALINE FLUSH

Steps	Parental Instructions for Saline Flushing Regimen
1	Assemble equipment: One liter container of Normal Saline(NS) premixed to 0.09% Each container will be marked by the investigator with ACE flush contents and a lot number One 1,000 mL enteral feeding bag with drip chamber and roller clamp One Christmas tree adapter connected to the end of the enteral feeding bag tubing A hook hung on the wall behind the commode at a height of 5' from the floor One appropriate access tube for Chait or Mickey low profile device Stop Watch Clip Board with data collection sheets and pen attached with string to the clipboard
2	Warm tap water and mild liquid dish detergent to wash equipment after each use
3	Make sure flushing solution is at room temperature
4	Insert the Christmas tree adapter into the end of the end of the feeding bag tubing making sure the fit is secure
5	Make sure the roller clamp is closed on the feeding bag tubing
6	Unscrew cap from the top of the feeding bag by rotating in a counter clockwise direction
7	Pour room temperature liquid from the 2 liter mixing bottle into the feeding bag
8	Screw the cap to the top of the feeding bag by turning in a clockwise direction until secure
9	Hold the feeding bag with liquid in onehand at shoulder height and put the end of the tubing in the sink
10	Using your other hand slowly loosen the roller clamp on the feeding bag tubing until the liquid fills the tube and then reclamp by tightening the roller clamp to stop the flow of liquid
11	Hang the feeding bag on the wall hook above the commode
12	Position child comfortably on commode using a toilet seat insert and a foot stool if needed
13	Give child toys/books for distraction
14	Hook the end of the Christmas tree adapter into the access tubing and secure the access tubing to the button
15	Unclamp the roller clamp to start the flow of the flushing solution and immediately start the stop watch
16	If your child complains of cramping or discomfort, slowly tighten the roller clamp to slow the flow of the flush
17	When the flush solution has infused, write the time from the stopwatch onto the record sheet under "Flow Time" but do not stop the stopwatch
18	Once the flush has infused, disconnect the tubing and rinse with a mixture of mild dish soap and tap water followed by tap water alone and hang back on the hook to air dry
19	Once your child has passed a bowel motion and 5 minutes has gone by without any additional stool output, stop the watch and record the time on the record sheet under column "Completion Time"

APPENDIX D
PROCEDURAL INSTRUCTIONS FOR COMPLETING USP GLYCERIN FLUSH

Steps	Parental Instructions for USP Glycerin and Normal Saline*
1	<p>Assemble equipment:</p> <ul style="list-style-type: none"> One appropriate access tube for Chait or Mickey low profile device 60 mL catheter tipped syringe One 8 ounce bottle with screw top to mix glycerin and NS USP Glycerin dispensed in a resealing container appropriate for liquids Glycerin container will be marked by the investigator with ACE Flush and a lot number One 30 mL syringe for measuring liquid glycerin One liter container of NS premixed to 0.09% One stop Watch One clip Board with data collection sheets and pen attached with string to the clipboard <p>Warm tap water and mild liquid dish detergent to wash equipment after each use</p>
2	Using the 8 ounce bottle with screw top pour _____mL of USP glycerin and _____ mL of NS into the bottle making sure the NS is at room temperature
3	Recap the bottle making sure the cap is secure; shake the bottle until the liquids are well combined
4	Attach the catheter tip of the 60 mL syringe to the access tubing
5	Hold the 60 mL syringe with liquid in one hand and put the end of the tubing in the
6	sink
7	Pour room temperature liquid from the 8 ounce mixing bottle into the 60 mL syringe
8	When the flush solution reaches the end of the tubing, pinch off the tubing to stop the flow of liquid
9	Position child comfortably on commode using a toilet seat insert and foot stool if needed
10	Give child toys/books for distraction
11	Attach and secure the access tubing to the low profile device
12	Unpinch the tubing to start the flow of the flushing solution and immediately start the stop watch
13	Hold the syringe at the child's shoulder height
14	If your child complains of cramping or discomfort, lower the height of the syringe to slow the flow of the flush
15	Raise or lower the syringe to adjust the flow of liquid. The higher the syringe the faster the liquid will go in
16	When the flush solution has infused, write the time from the stopwatch onto the record sheet under "Flow Time" but do not stop the stopwatch
17	Once the flush has infused, disconnect the tubing and rinse the syringe and tubing with a mixture of mild dish soap and tap water followed by tap water alone
	Once your child has passed a bowel motion and 5 minutes has gone by without any additional stool output stop the watch and record the time on the record sheet under column "Completion Time"

APPENDIX E
MICROBIOME DNA EXTRACTION AND PROCESSING FOR DOWNSTREAM
ANALYSIS

1	Ensure two heat blocks are available and set at 70°C and 95 °c
2	Obtain a cup of ice to store the weighed sample until InhibitEX buffer is added
3	Ensure Ethanol has been added to buffers AW1 & AW2
4	Mix all buffers before use
5	If a precipitate has formed in the InhibitEX buffer or AL buffer, dissolve by incubating at 37°C
6	All steps to be performed at room temperature, except otherwise noted
7	Ensure the sample is kept on ice until the addition of InhibitEX buffer
8	Weigh the stool sample in a 15 mL conical tube or homogenizer and place on ice
9	Add 10 volumes of InhibitEX buffer (e.g., add 10 mL buffer to 1 g stool)
10	Vortex vigorously for 1 minute or until stool sample is thoroughly homogenized.
11	If the stool sample is too viscous, use a homogenizer until no solid sample is visible
12	Transfer 2 mL of lysate into a 2 mL microcentrifuge tube
13	Incubate suspension at 95° C for 5 minutes, then vortex 15 seconds
14	Centrifuge sample at full speed for 1 minute to pellet stool particles
15	Pipette 15 µL proteinase K into a new 1.5 mL microcentrifuge tube
16	Transfer 200µL of the supernatant from step 13 into the 1.5ML microcentrifuge tube containing proteinase K
17	Add 200 µL Buffer AL and vortex 15 seconds
18	Incubate 70°C for 10 minutes. Then tap spine to bring condensate down
19	Add 200 µL 100% ethanol to lysate and mix by vortexing
20	Transfer the lysate from step 18 to a QIAamp spin column. Centrifuge full speed 1 min
21	Place the spin column in a new 2 mL collection tube. Discard THE tube containing the filtrate
22	Add 500 µL Buffer AW1. Centrifuge full speed for 1 minute
23	Place the spin column in a new 2 ML collection tube. Discard the tube containing the filtrate
24	Add 500 µL Buffer AW2. Centrifuge full speed for 3 minutes
25	Place the spin column in a new 2mL tube. Discard the tube containing the filtrate.
26	Centrifuge full speed for 3 minutes to remove any residual Buffer AW2
27	Place the spin column into a new 1.5 mL microcentrifuge tube
28	Pipette 200 µL Buffer ATE directly onto the membrane. Incubate for 5 minutes. Centrifuge full speed for 1 minute to elute DNA.
28	Freeze at -80° for downstream analysis

APPENDIX F
THREATS TO STATISTICAL CONCLUSION VALIDITY

Threat	Assessment of Potential Threat & Proposed Control
Insufficient Power (Increases probability of a Type I error)	<p>Within subject analysis - the strength of the ability to determine treatment effects requires a stable baseline, and is a function of the number of baseline and intervention data points (Cook & Campbell, 1979). This study design features a prolonged pre-operative baseline assessment and a minimum of 6 weeks of daily measurement for each treatment variable meeting the criteria for sufficient power analysis</p> <p>Cross –over analysis: The study will use 6 subjects randomized to 2 treatment orders. Although the design confers greater precision in estimating treatment difference and generally requires fewer subjects, the limited number of subjects will limit the power of the analysis increasing the probability of a Type II error (Piantadosi, 2005).</p>
Reliability of Measures (Impacts the relationship between variables)	All biological samples will be run in a single accredited laboratory. All fecal samples will be collected and stored for downstream analysis by the procedure detailed in the protocol (Shadish et al., 2002).
Reliability of Treatment Implementation (May result in underestimation in treatment effect.	Procedural fidelity will be ascertained each procedural variable at specified times throughout the study. Written instruction forms detailing interventions will be given to the family (Gast, 2010).
Random Heterogeneity of Respondents (Increases variability in outcome, increasing error variance)	<p>Within subject analysis – should not pose a problem if baseline is stable as the baseline reflects background variable effects (Shadish et al., 2002)</p> <p>Cross-over analysis: Period confounds should be accounted for using the two sample cross over <i>t</i>-test (Shuster, 2007)</p> <p>Both designs use subjects as their own control substantially decreasing variance due to subject heterogeneity (Cook & Campbell, 1979).</p>
Violated assumptions	<p>Within Subject analysis – violates statistical assumptions that error residuals are independent, normally distributed, and homoscedastic. Serial dependency can be modeled and removed.</p> <p>Cross-over analysis – treatment sequences considered independent. Carry-over effects should not be a factor due to design considerations but are accounted for using the two sample <i>t</i> test (Shuster, 2007)</p>

Shadish et al. (2002) defined threats to statistical conclusion validity as “reasons why inferences about covariation between two variables may be incorrect” (p. 45) violations of which, result in over or underestimation of the magnitude and statistical significance of treatment effects

**APPENDIX G
THREATS TO INTERNAL VALIDITY**

Threat	Assessment of Potential Threat & Proposed Control
Ambiguous Temporal Precedence (cause precedes effect)	This is a prospective study. Ascertainment of temporal precedence and ambiguity regarding direction of causal inference is not a risk
Attrition (subject mortality)	Attrition will have a significant effect on data analysis. Careful follow-up and provision of supplies may discourage subject fatigue and drop outs. A second randomization to treatment has been instituted after the dose response phase just before the treatment phase to insure randomization scheme if attrition occurs due to ineffective treatment or side effects (Portney & Watkins, 2009)
Maturation (confounding due to passage of time)	This study is several months in duration which should preclude maturational confounding. In addition, randomization to treatment sequence should minimize maturation as a potential bias (Shadish et al., 2002)
History (Confounding events other than the independent variable)	Effects of significant diet change or infection causing diarrhea will confound treatment results. A diary will be kept documenting any obvious historical confounds. In those instances, treatment length will be extended if necessary (Gast, 2010). Use of a match control that did not receive treatment would strengthen the design (Cook & Campbell, 1979) but would be unethical. In addition, disease instability may confound treatment effects. This population generally has a chronic but stable disease and should provide a stable sample to compare treatment effects (Piantadosi, 2005).
Order Effects (Position in sequence) & Carry Over Effects (Effects from previous phase impacting current phase)	Order effects which will be controlled by randomization to one of two treatment sequences (Kazdin, 2011). Carry over effects should not confound continence. There is a prolonged active washout period between serum and stool sampling
Regression (Tendency of extreme scores to regress toward the mean) Testing	Should not pose a threat in this study Should not pose a threat in this study
Instrumentation (reliability of measurement)	IOA will be checked at key points throughout the study and calculated using gross method. If IOA is <80%, additional observer training will be implemented. The family will have report forms to help standardize and quantify responses. (Gast, 2010; Kazdin, 2011)

Internal validity questions if the evidence supports a causal relationship between treatment and outcome variables (Portney & Watkins, 2009).

APPENDIX H
SOCIAL THREATS TO INTERNAL VALIDITY

Threat	Assessment of Potential Threat & Proposed Control
Diffusion and Imitation of Treatment	Many of the families who will be enrolled in this study interact socially, attend a support group together, and attend a multidisciplinary clinic in which they share a waiting room. Subjects are not blinded to treatment. Parents and children enrolled in the study may be influenced by parent or child preference of individuals already on ACE therapy. Blinding is not possible due to obvious differences in flush volume (Portney & Watkins, 2009)
Compensatory Equalization of Treatments	Subject or parental preference for one treatment over another based on intangibles not measured in the study may influence treatment implementation and/or evaluation of dependent measures. Blinding is not possible due to obvious differences in flush volume (Portney & Watkins, 2009)
Compensatory Rivalry	All subjects will receive all treatments so no subject should receive what they view as a less than desirable treatment, negating the threat of compensatory rivalry (Portney & Watkins, 2009)
Resentful Demoralization	All subjects will receive all treatments so no subject should receive what they view as a less than desirable treatment, negating the threat of resentful demoralization (Portney & Watkins, 2009)

Social Validity questions if there is evidence to support a causal relationship between treatment and outcome variables that are influenced by social interactions or the subject's awareness regarding treatment specifics (Portney & Watkins, 2009).

APPENDIX I
THREATS POSED TO CONSTRUCT VALIDITY OF CAUSE AND EFFECT

Threat	Assessment of Potential Threat & Proposed Control
Operational definitions	Efforts were made to ensure the construct of fecal incontinence was clearly defined. All measurement of continence, side effects, and physiologic outcomes are measured at an interval scale or higher minimizing the risk of miss-inference (Portney & Watkins, 2009)
Levels of Construct	Dosing regimen adjustment is built into the design, allowing dose-response and level of effect determination mitigating threat of levels of construct (Cook & Campbell, 1979)
Delayed Causation & Interaction	Slow diffusion of treatment causing a delay in treatment effect should not be problematic (Shadish et al., 2002). Carry-over and order effects have been adequately controlled for in both design and analysis (Jones & Kenward, 2003; Shuster, 2007)
Length of Follow-Up	Repeated measures over time negates concerns regarding potential bias due to misleading short term data (Portney & Watkins, 2009)
Experimenter Bias	Treatment or problems solving will be standardized across subjects. Inability to blind to investigator to treatment may increase risk of this threat (Portney & Watkins, 2009)
Psychometric Soundness of Instruments	Instrument validity is “the degree to which an instrument measures the construct it is intended to measure” (DeVon et al., 2007, p. 162). The Fecal Incontinence and Constipation on Quality of Life instrument has established validity and reliability for children with constipation and fecal incontinence due to neurogenic etiology and their caregivers (Ok & Kurzrock, 2011). The Wong-Baker FACES pain Rating Scale has well established validity and reliability in children (Tomlinson, Von Baeyer, Stinson, & Sung, 2010; Wong & Baker, 1988)

APPENDIX J
NEMOURS CLINICAL RESEARCH COMMITTEE APPLICATION AND REVIEW



Nemours Clinical Research Review Committee (CRRC)
807 Children's Way
Jacksonville, FL 32207
Phone: 904-697-3483 Fax: 904-697-3425

July 10, 2014

Kimberly Jarczyk, CPNP
Nemours Children's Clinic
807 Children's Way
Jacksonville, FL 32207

RE: A prospective pilot within subjects comparison of two antegrade flushing regimens in children.

Dear Ms. Jarczyk:

The Clinical Research Review Committee has carefully looked at the above-mentioned grant application. There were considerable comments that we would like you to review (reviewers' comments attached). Most of the concern had to do with a more narrow selection of patients given your small sample size. The Committee suggested perhaps restricting your cohort to subjects with spina bifida in order to homogenize the disease process you are studying. In addition, the Committee felt that with such a small sample size, the number of specific aims listed was overly ambitious, particularly when you wanted to sort out not just the best frequency and volume of these enemas, but also acceptability of the intervention, quality of life, and even changes in gut microbiota. We thought the latter were unrealistic expectations even for a pilot study with an N=6. The Committee recommended that you more carefully limit your aims to achievable goals within the timeframe of this proposal and with this limited number of patients, perhaps concentrating on the best way to deliver the treatment and its acceptability.

We realize that this is part of a PhD thesis for you and that you may have limited control over the design of these experiments. Hence, we will not mandate that you make these changes; however, we strongly recommend that you review these comments in order to improve the overall scientific merit of your study. Regardless, the study is now considered approved as is and you can submit this to the IRB.

I hope you can take these comments in the spirit of constructive criticism in which they are offered. Please do not hesitate to contact me if you have any questions or concerns.

Sincerely,

A handwritten signature in black ink that reads "Nelly Mauras".

Nelly Mauras, MD
Chair
Nemours Clinical Research Review Committee

NM:bt

Nelly Mauras, MD
Chair

Scott Penfil, MD
Vice-Chair

David Schaeffer, MD
Vice-Chair

Barbara Tyler, CCRC
Administrative Coordinator

Reviewer #1:

1. The overall goal and plan is in general very clear. Since this is a pilot study, the cross over plan with a small number of subjects seems like a good plan.
2. Patient recruitment: From the Nemours Children's Clinics and Spinal Defects clinic, however in the initial summary they only mention the spinal defect clinic. They have a long list of exclusions including electrolyte imbalance, high rectal tone quadriplegia, cardiac problems, requiring prophylactic antibiotics, cognitive delay ECT. They never include any inclusion criteria except having the surgery. They give no evidence that they can find 6 children in 18 months if they apply their exclusion criteria.
3. Recommendation 1: they need to provide evidence of the past two or three years that with all their exclusion criteria there is a sufficiently high volume of these procedures that it is reasonable to recruit 6 children in 18 months. I would expect no more than 50% of parents would agree or be able to follow through therefore they need to demonstrate that they are doing approximately 2 cases every 3 months who would in every other way meet the criteria.
4. Recommendation 2: they should have an inclusion criteria since it is small pilot study it will have little meaning if there are six different diagnoses included: Since it sounds like their primary focus is the Spinal Defect Clinic they should limit the diagnosis to children with spinal cord dysfunction. Also one of their outcome measures is FICQOL (constipation Measure in Spina Bifida) therefore if they include other diagnosis, I presume this measure would not be validated in that population and they need to provide justification that it is validated to use in the specific population. If they want to include other diagnosis, they should list those diagnosis that would be included and justification why all could be considered in one group for this study with many measures.
5. One aside Note: I would recommend they change the name of the clinic since most facilities who have SDC clinics use this abbreviation for Spinal Dysfunction Clinic. The term Defect has pejorative connotations of a child being defective.
6. Outcome Measure: Since this is a pilot study it is reasonable to measure a number of things to see if there are any trends, however, there are so many variables that it is hard to get a focus on outcome goals in the Hypotheses there are 3 Aims with a total of 8 measures. Unless the diagnosis are very tightly controlled, some of these measures will likely be different based on the underlying diagnosis. An additional complication is that the study includes a complicated dosing routine with increases to compensate for rectal incontinence, which make it further difficult to evaluate. However, the expectation is that this would be a real life type of treatment therefore family acceptance would be the primary measure, however this makes assessment of Microbial flora and immune response difficult to assess.
7. Recommendation: They might consider just focusing on the clinical aspects and not try to evaluate the bacterial flora and immune responses due to the high number of variables.
8. Treatments: Based on the literature review the two planned flushes seem appropriate. This study suggests that these solutions are commonly used, but there is no clear description of the current practice at the SDC where they work, and how the current proposal differs from current standard practice at this SDC.

9. Recommendation: Clearly define what the current practice is and how this proposal will alter that current practice, i.e. for those patients choosing not to enter the protocol, what will be their instruction and recommended flushing material and routine.
10. Budget: Included items - Flush solutions: if these are or one is the standard flush solution recommended regardless if you are in the protocol or not, then the solution cost cannot be billed to the protocol. If these are solutions not in general use and will have to be separately prepared for this study then this is a research cost, but justification for this needs to be given. The same is true for the lab studies. Are electrolytes usually checked after this procedure as a routine? If yes, then this is not research cost. If not, then it is OK. Please justify. I also feel the cost for the calprotectin and microflora testing seems hard to justify considering the multiple data points of this study. Further justification that this is likely to yield useful data would be helpful. As an example, the reviews lists many impacts on these measures including function of the appendix which some of these children have an others don't, the impact of flush volume which is very complex in the protocol seems like it would likely make the data impossible to correlate to any specific other measure.

Reviewer #2:

1. This is a very well written proposal. The Research plan is clearly outlined.
2. The potential benefit and significance is noteworthy.
3. The inclusion criteria are fairly vague. The investigators intend to include six children, ages 4 to 12 years, recruited from subspecialty clinics at Nemours Children's Clinic and the Pediatric Spinal Defects Clinic in Jacksonville, Florida. Children will be selected by purposive sampling and will include those who are scheduled to have an ACE stoma and will require regular antegrade enema administration to maintain continence. The variability in this population may be considered a weakness.

APPENDIX K
FDA IND APPLICATION

April 30, 2015

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Gastroenterology and Inborn Error Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

IND 126011 Corrected submission: USP Glycerin and Normal Saline

LCDR James B. Carr,

Enclosed you will find three corrected and bound copies our IND application for use of USP glycerin and normal saline as antegrade flushing solutions to be administered through a cecostomy or appendicotomy in treating children with intractable fecal incontinence IND 126011 that was submitted on February 27, 2015. The intent of this application is to obtain an IND waiver.

I have read, will comply with, and implement reporting requirements detailed in FDCA (21 U.S.C. §§ 301 et seq.) as well Title 21 of the Code of Federal Regulations (CFR). I will not charge for the investigational drug. I will comply with the regulations for “Good Laboratory Practice for Non clinical Laboratory Studies” (21CFR 58).

Upon review of 21 CFR 25 and the guidance provided in “Environmental Assessment of Human Drugs and Biologics”, both glycerin and saline qualify for categorical exclusion because they occur naturally in the environment, the volume and frequency of administration in this proposal does not substantially increase the active moiety and does not substantially alter or increase the concentration or distribution of the substance, metabolites, or products of degradation in the environment. To the best of the investigator’s knowledge, no extraordinary circumstances exist with the proposed used of glycerin and saline that would adversely affect the quality of the human environment.

In closing, I have resubmitted three bound copies of the corrected application in keeping with the submission requirements outlined in your letter in the event any portion of the initial submission was lost.

Sincerely,

Kimberly S. Jarczyk, M.S.N., C.P.N.P.

Table of Contents

Introductory Statement and General Investigational Plan	1
Chemistry, Manufacturing and Control Information	2
Pharmacology Toxicology Information	3
Investigator’s Brochure.....	9
Clinical Protocol.....	9
Summary of Previous Human Experience with the Investigational Drug.....	19
References.....	21
Additional Information	
Appendix A – Dr. Pam Pieper – Curriculum Vitae	
Appendix B – Dr. Donald George – Curriculum Vitae	
Appendix C – Kimberly S. Jarczyk, MSN, CPNP – Curriculum Vitae	
Appendix D – Dr. Mark Barraza – Curriculum Vitae	
Appendix E – Dr. Jonathan Shuster – Curriculum Vitae	
Appendix F – Letters of Acceptance from Data and Safety Monitoring Board Members	

Introductory Statement and General Investigational Plan

Broad Objectives and Planned Duration of the Proposed Clinical Investigation

Fecal incontinence in children past the expected time of toilet training has been associated with increased anxiety and depression, more social problems, worse school performance, and an increased incidence of abuse and bullying (Kaugars et al., 2010; Youssef, Langseder, Verga, Mones, & Rosh, 2005). It is particularly difficult to manage fecal incontinence using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumor, megarectum, or slow transit constipation. The Malone, or antegrade continence enema (ACE), procedure was popularized over 20 years ago as a means of helping children with intractable fecal incontinence attain stool continence. A catheterizable stoma from the abdominal wall into the cecum is constructed using the appendix, a tubularized portion of the bowel, or a low profile button device. The stoma allows for antegrade administration of enema solution into the colon. Many different prescribed ACE flushing regimens are used in practice, including licorice root, mineral oil, treacle/milk mix, tap water, normal saline, polyethylene glycol solutions with and without added electrolytes, phosphate soda solution, bisacodyl, and USP liquid glycerin (Bani-Hani et al., 2008; Marshall et al., 2001; Youssef, Barksdale, Griffiths, Flores, & Di Lorenzo, 2002).

A large body of literature demonstrates ACE therapy can be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in quality of life (Aksnes et al., 2002; Kaugars, Silverman, Kinservik, Heinze, Sander & Sood, 2010; Mousa et al., 2006; Tiryaki, Ergun, Celik, Ulman, & Avanoğlu, 2010). However, findings regarding effectiveness are highly variable. Short term reported success rates range from 79 to 98%, with long term outcomes ranging from 41% abandonment rate at 5 years to a mean time to first relapse lasting as long as 121.9 +/- 29.7 months (Aspirot, Fernandez, Di Lorenzo, Skaggs, & Mousa, 2009; Bani-Hani, Cain, King, & Rink, 2008; Becmeur et al., 2008; Curry, Osborne, & Malone, 1999; Dey et al., 2003; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Marshall, Hutson, Anticich, & Stanton, 2001; Mousa et al., 2006; Ok & Kurzrock, 2011; Siddiqui, Fishman, Bauer, & Nurko, 2011; Thomas et al., 2006; Yardley et al., 2009). This variability may be due to what is used to flush. Identifying a successful flushing regimen is determined by individual clinician preference and often requires multiple attempts before success is achieved. There are no prospective studies comparing the side effects or effectiveness of type, dose/volume, or frequency of different flushing regimens in preventing incontinence to inform practice.

The catheterizable stoma used for antegrade administration of enema solution is frequently made by bringing the appendix out through the abdominal wall or by placing a button into the cecum. The appendix and cecum have significant amounts of gut-associated lymphoid tissue (GALT), have high concentrations of microbiota, and serve an essential immune function (Anderson, Olaison, Tysk, & Ekborn, 2003; Andreu-Ballester et al., 2007; Barker, 2012; Dasso & Howell, 1997; Forchielli & Walker, 2005; Hooper, Littman & Macpherson, 2012; Janszky, Mukamal, Dalman, Jammal & Ahnve, 2011; Nicholson et al., 2012; Noverr & Huffnagle, 2004; Penders, Stobberingh, Van den Brandt, & Thijs, 2007). The appendix has higher concentrations of microbial biofilms compared to other areas of the colon, serves as a safe-house for symbiotic gut flora, and functions to preserve gut microbiota through re-inoculation with normal flora following gastrointestinal infections (Bazar, Lee, & Yun, 2004; Bollinger, Barbas, Bush, Lin, & Parker, 2007; Gebbers & Laissue, 2004; Kawanishi, 1987; Smith et al., 2009). The human gut contains 10^{14} bacteria (Jia, Li, Zhao & Nicholson, 2008). Bacterial composition varies along the bowel axis, with further differentiation of luminal or adherent microcolonies that lead to development of biofilms. Factors that influence microbial composition include pH, transit time, bile acids, pancreatic enzymes, mucus composition, nutrient consumption, medication, environment, bacterial adhesion capacity, and metabolic capacity. The most important function of the gut microbiome is colonization resistance, which is accomplished through competition for nutrients and secretion of bacteriocins (Penders et al., 2007). Microbiota function to degrade dietary substances and enhance digestive efficiency

while providing nutrients to the microbes themselves. These microbes are essential for host physiology, but in turn pose a threat of opportunistic invasion by resident bacteria with resulting pathologies.

ACE therapy administration through the appendix or into the cecum has the potential to disrupt the gut microbial ecosystem, causing dysbiosis and immune dysfunction. No studies evaluate the effects of appendicostomy/cecostomy flush on gut mucosal immunity and microbiota. The proposed research is significant in that it is the first prospective study to compare the effectiveness and tolerability of two commonly used ACE flushing regimens, and the first study to explore ACE flushing impact on the gut microbiome. Findings from this study have the potential to provide both clinical and biological insights into ACE administration safety and start to build a foundation of scientific evidence that could increase ACE effectiveness rates from 78% toward 98% or better, and provide a foundation for additional prospective, randomized, controlled trials.

We seek approval from the Food and Drug Administration to investigate and compare two distinct flushing regimens, one high volume saline flush and one low volume USP glycerin flush, in the immediate postoperative period in children requiring ACE therapy. We have chosen normal saline and USP glycerin because both have been widely used, have a proven safety record over a long time frame, and are available over the counter for retrograde enema administration in infants and children. Both have been widely used clinically for antegrade enema administration with no case reports detailing any adverse outcome. We could not find any evidence that either USP glycerin or normal saline have been withdrawn from investigation or marketing in any country for any reason related to safety and effectiveness.

The proposed pilot study is prospective and utilizes a cross over design embedded in a single subjects design employing six subjects randomized to two treatment sequences. The primary aims of this study are to 1) identify the minimal administration frequency and titration time to reaching effective dose; 2) compare which solution at an optimum dose is delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence and quality of life; and 3) determine if administration of antegrade enema solution through an appendicostomy/cecostomy affects gut microbiota and immune function. This study will involve children ages 3 to 12 years recruited from subspecialty clinics at Nemours entities in Florida and the Pediatric Community Spinal Defects Clinic in Jacksonville, Florida. Children will be selected by purposive sampling and will include those who are scheduled to have an ACE stoma and will require regular antegrade enema administration to maintain continence. Excluded will be children with preexisting electrolyte imbalance, chronic high rectal tone, quadraplegia, renal or cardiac disease, and those who cannot communicate or have significant cognitive delay that would interfere with their ability to fully participate in the study. Parents must have English language competency and be willing and able to participate in administration or oversight of the flushing regimen and data collection for a minimum of 20 consecutive weeks. Single subjects within and between subjects data will be analyzed using visual analysis. The cross over data will be analyzed using inferential statistics employing two-tailed hypothesis testing. Findings from this study will serve to provide a comparative analysis of two different regimens that will serve as a starting point to guide practice and provide a foundation for additional prospective, randomized, controlled trials.

Chemistry, Manufacturing, and Control Information

Drug/Active Ingredients, Pharmaceutical Class, Structural formula, Formulation, Route of Administration

Chemical name: Glycerol or 1,2,3-propanetriol

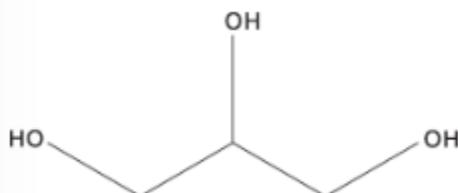
Common name: Glycerin

Empirical formula: C₃H₈O₃

Glycerol is the backbone common to all triglycerides. Glycerol is a water miscible trihydroxy sugar alcohol used in pharmaceuticals, foods, and personal care products that has over 1500 known uses and is virtually non toxic to human health and the environment. It is available over the counter and widely used for retrograde

enema administration in infants and children. Orally, after hydrolysis of glycerol esters in the intestine, it is readily absorbed in the intestine. It enters the glycolic pathway after conversion in the liver to glycerol-3-phosphate eventually yielding pyruvic acid WHO, 2002). As a suppository it is poorly absorbed, acts to hydrate stool in the rectum by osmotic absorption of water from the mucosa, acts as a lubricant to soften stool, and generally causes mechanical stimulation of the anocolonic reflex, promoting passage of retained stool. It is unclear the extent to which the hygroscopic or local irritant action is responsible for the laxative effect (Pharmacists, 2011; Rossoff, 1974, & WHO, 2002).

Structural formula of Synthetic USP Glycerin:



Manufacturing Information

HM Glycerin USP 6OZ. The NDC# is 62011-0115-01 and the brand is Healthmart.

Normal Saline - The NDC# is 00338-0049-04 and the brand is Baxter.

Environment

Upon review of 21 CFR 25 and the guidance provided in “Environmental Assessment of Human Drugs and Biologics”, both glycerin and saline qualify for categorical exclusion because they occur naturally in the environment, the volume and frequency of administration in this proposal does not substantially increase the active moiety and does not substantially alter or increase the concentration or distribution of the substance, metabolites, or products of degradation in the environment. To the best of the investigator’s knowledge, no extraordinary circumstances exist with the proposed used of glycerin and saline that would adversely affect the quality of the human environment.

Pharmacology and Toxicology Information

Animal Studies Using Glycerin

There are no animal studies evaluating instillation of glycerin into the cecum

<u>Sample</u>	<u>Dose</u>	<u>Route</u>	<u>Outcome</u>	<u>Reference</u>
6 rabbits	18,700 mg/kg bw	Occlusive dermal application for 8 hours	No deaths	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html</i>
12 female rats	27,260 mg/kg bw	Gavage	Muscle spasm, convulsions, lung congestion & death (3)	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i>

			Survivors normal within 2.5 hrs of dosing	http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
Mice & Guinea pigs	Not reported	Gavage	Tremor and convulsions with hyperemia of pylorus, small intestine and cerebral meninges	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
Rabbits	4 mL over 30% BSA 8 hr/day for 90 days	Topical	No irritation	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
6 rabbits	0.1 mL	Ocular instillation	Very low potential to irritate eyes	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
24 male Guinea pigs	0.1 mL of 0.1% in NS qod x 20 days	Injection	No indication of sensitization after 2 week exposure free period	<i>Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
Rats	1.75 mL of 50%/100 g bw	SQ injection	Severe hemolysis followed by necrosis of tubular portions of nephrons – reversible within 6 – 12 wks	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rats	1 mL 100%/100 g bw	Intra-peritoneal instillation	Severe convulsions, hemoglobinuria, renal damage died within 2 hrs of injection	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015 http://esis.jrc.ec.europa.eu/</i>

Rats	1 mL 100% or 50%/100 g bw	SQ injection	Hemoglobinuria, renal tubular necrosis, some convulsions with 100%	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD- ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rats	100% & 50% at 1 mL/100 g bw	IV	Severe Convulsions and death in all animals	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD- ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rabbit	Aq 100%	Anterior chamber of eye	Inflammation and edema of cornea & damage of endothelial cells	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbits	Aq 50%	Anterior chamber of eye	Significantly less reaction but visibly dehydrates lens can capsule wrinkling	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbit	Aq 30% for 20 min, 50% for 10 min or 92% for 4 min	Anterior chamber of ete	Normal deturgescence after 30% and 50% exposure but endothelium destruction with 92% > 30 min	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbit with corneal traume	Aq 43% dilution for 30 min x 20 d	Ophthalmic	Edema of conjunctiva lasting for several hrs	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rats	14 d 5d/wk 6hr/d Mean conc 1000, 1930 3910 mg/cu m	Respirable aerosol	Minimal to mild squamous metaplasia of epiglottis – greatest at highest test dose No systemic effects	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: <a href="http://www.chem.unep.ch/irptc/sids/OECD
SIDS/sidspub.html">http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html</i>

Rats	13 wk 6hr/d 5d/wk 0, 33, 165, & 662 mg/cu m	Respirable aerosol	Minimal to mild squamous metaplasia of epiglottis – considered local irritant effect	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html</i>
Rats	10% for 9 d	IV	Degeneration in renal tubular epithelium return to normal after 19 d	<i>NOWAK H ET AL; PATOL POL 30 (1): 61 (1979)</i>
Grow- ing pigs	84.51% added to feed for 138 d	Feeding trial	Pigs can be fed up to 10% crude glycerin with no effects on performance, carcass composition, or meat quality	<i>Lammers PJ et al; J Anim Sci 86 (11): 2962-2970 (2008)</i>
Rats	0 up to 60,000 mg/kg bw /d, for 20 wks	oral	At 5000 mg/kg bw marked hydropic and fatty degeneration of liver parenchymal cells	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>
Mice 6 to 8 wks	Given carcinogen followed by 0, 0.5 or 1% glycerol or water until 1 yr of age	oral	Lower incidence of liver and lung tumor after glycerin – no adverse treatment effects	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>
Rats	0 to 20,000 mg/kg bw /d	oral	No significant - treatment related effects	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>

Rats	4,000 to 10000 mg/kg bw for 2 years	Oral	No adverse effects up to 10,000 mg/kg bw	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002)</i> Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html
Rats	5%, 10%, & 20 % for 12 to 24 months	Oral	Glycerol does not initiate tumor development in rats	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002)</i> Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html
Mice	5% in drinking water for 1 – 20 weeks after sq injection of 4-NQO	Oral	Enhances lung tumor development - mainly adenomas. Tumor development independent from pulmonary cell kinetics	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002)</i> Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html
Rats	7 generations 15000 mg/kg bw d	Oral	Pups of treated dams mean weight 20% less than controls	<i>Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002).</i> Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html
Rats Mice Rabbits	Levels up to 1310, 1280, 1180 mg/kg bw daily during part of gestational period	oral	No maternal or tetragenic effects seen at highest dose level tested	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002)</i> Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD SIDS/sidspub.html

Summary of Previous Human Studies

Clinical Trials Involving USP Glycerin

<u>Dose</u>	<u>Route</u>	<u>Effects</u>	<u>Reference</u>
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Not reported	Not reported	Very slight diuresis in healthy individuals receiving a single dose May produce tissue dehydration and decreases in CSF pressure	<i>McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773</i>
Not reported. Employees engaged in glycerol manufacturing	Environmental exposure	No significant difference in sperm count or sperm quality parameters when compared with controls	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
0.05 mL of 10% solution for 21 days	Dermal – patch test	Slight irritation at 48 hrs and maximum rating of 4 on a 9 point scale at day 14 of a 21 day application	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Acute and chronic (42 d) ingestion	Oral	Increase in plasma glycerides in males only following acute ingestion and in both males and females with chronic ingestion (significantly greater increase in males)	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Orange juice mixed with 30 mL of 95% glycerol after each of 3 daily meals	Oral	No overt signs of toxicity or change in food consumption	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Repeated application of 100% solution	Ocular	Extensive changes to appearance of endothelium that disappeared within 90 minutes of application	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
24,000 mg/kg bw d for 50 days	Oral	Slight tendency toward increase in weight	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002).</i>

			Available from, as of February 18,2015,: http://www.inchem.org/pages/jecfa.html
Not reported. Workers in foam rubber factory	Dermal – patch testing	No sensitizing effects	United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: echa.europa.eu/

Case Reports

<u>Subject</u>	<u>Dose , Route & Purpose</u>	<u>Effects</u>	<u>Reference</u>
46 y.o. male	500 mL of 10% solution	Altered sensorium, generalized seizures, focal neurologic signs Managed conservatively and recovered within 48 hrs – case represents rare presentation of overdose with an otherwise safe drug used in neurology	<i>Singh R et al; Neurol India 49 (3): 320-1 (2001)</i>
Male	Adjunctive glycerin test used in the diagnosis of Meniere's disease	20 – 40dB hearing loss in uninvolved ear during standard testing that resolved within 3 days	<i>Mattox DE, Goode RL; Arch Otolaryngol 104 (6): 359-61 (1978)</i>
73 y.o. male	Oral solution used to treat elevated IOP	Developed severe pulmonary edema 45 minutes after administration	<i>Almog Y et al; Ann Ophthalmol 18 (1): 38-9 (1986)</i>
72 y.o. male	Dose should not exceed 1.5 g/kg bw in Klockhoff test for diagnosis of suspected Meniere's disease - Patient received 3.88-3.95 g/kg bw	Progressive neurological signs and pathologically elevated serum concentration of triglycerides (3,465 mg/dl)	<i>Andresen H et al; Clin Toxicol (Phila) 47 (4): 312-6 (2009)</i>
3 y.o. male	0.5 – 1.0 g/kg	Unique intolerance including mental changes, N&V, hypoglycemia and loss of consciousness following IV	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of</i>

		administration with spontaneous recovery after 30 min	February 18, 2015: <i>echa.europa.eu/</i>
Not reported	Rectal administration prior to coronary artery bypass	Acute colonic ischemia	European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: <i>echa.europa.eu/</i>
82 y.o. hypertensive and senile female	200 mL 50% solution for primary angle closure glaucoma	Headache, shaking of arm, quivering of eyes, and nausea	European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: <i>echa.europa.eu/</i>
68 y.o. female diabetic	280 mL of 50% solution	Severe daibetic acidosis within 3 days of ingestion	European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: <i>echa.europa.eu//</i>

The route of administration influences toxicity of glycerol in humans. Toxic effects from oral administration include nausea and vomiting. Glycerol has a CNS dehydration effect. Intraocular pressure begins to fall at plasma concentrations of 10 mmoles per liter. Concentration and dilutant used also influence toxicity. Use of saline as dilutant diminishes the toxic effects of glycerol. Toxic effects following intraperitoneal and subcutaneous administration are albuminuria, hemoglobinuria, anemia and renal damage. *European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/>*

Investigator's Brochure

No information is included in this section under 312.55

Clinical Protocol/General Investigational Plan

Specific Aims

The purpose of this prospective pilot study is to compare two distinct flushing regimens, one high volume saline flush and one low volume USP glycerin flush, in the immediate postoperative period in children requiring ACE therapy. Findings from this study will provide a comparative analysis of these two regimens that will serve as a starting point to guide practice and serve as a foundation for additional prospective, randomized, controlled trials. The aims of this study are to 1) identify the minimal administration frequency and titration time to reaching effective dose; 2) compare which solution at an optimum dose is delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence and

quality of life; and 3) determine if administration of antegrade enema solution through an appendicostomy/cecostomy affects gut microbiota and immune function.

Null Hypotheses for Aim 1:

- 1. There will be no differences in frequency of administration necessary to gain and maintain continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (**primary aim**).
- 2. There will be no differences in titration time to reaching effective dose between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypotheses for Aim 2:

- 1. There will be no difference in continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (**primary aim**).
- 2. There will be no difference in procedural time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
- 3. There will be no difference in side effects between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
- 4. There will be no difference in parent/patient satisfaction as measured by the Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida (FIC QOL) between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypotheses for Aim 3:

- 1. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on colonic microbiota in children requiring antegrade continence therapy.
- 2. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on gut mucosal health in children requiring antegrade continence therapy

Design

This prospective study will utilize a repeated measures, single subjects alternating treatments A-B-C-B'-C'-B1' withdrawal design in which all subjects are tested under all conditions and each subject acts as his or her own control. A within subjects cross-over design is embedded in the B'-C'-B1' treatment comparison phase of the study. Subjects will be randomly assigned to one of two treatment sequences to control for the possibility of order effects. The treatment will be replicated across 6 subjects randomized to 3 subjects per group. The patient and investigator cannot be blinded to flushing regimens contents due to dosing considerations. The high volume flushing regimen will be comprised of normal saline alone. The low volume regimen will be comprised of USP glycerin with a small volume of normal saline used as diluent. Volume and frequency of administration will be structured to find the lowest dosing of each regimen sufficient to maintain continence. Baseline data A will serve as the control and will be obtained pre-operatively. Ethics prohibit return to a no-treatment baseline phase as this would result in multiple daily episodes of fecal incontinence. The first B - C phase of the study will evaluate dose-response relationship and will be used to identify the optimal dose and frequency of ACE administration for normal saline and normal saline with USP glycerin. When the optimal dose has been identified, the child will continue on that dose for two weeks to insure treatment stability and

effectiveness. If continence cannot be achieved within the dosing guidelines, the child will be trialed on the alternative therapy but will not progress to the maintenance phase of the study. To prevent statistical bias from subject loss due to treatment failure, each child will be randomized to a second treatment sequence once they have achieved continence on optimal dosing with minimal side effects. The second phase B'-C'-B1' of the study will compare the effectiveness of the two regimens at optimal dose and administration frequency.

Rationale for Utilization of Within Subjects Designs

Any research design is a tool used to answer a question. Strategies, design choice, and use of design elements should be based on how best to answer the question at hand (Kazdin, 2011). The purpose of experimental design is to control the effects of random error and bias (Piantadosi, 2005). Both single subject and between group research make and test predictions about treatment effects, the first by evaluating treatment effects on an individual, the second by addressing group mean and variance (Kazdin, 2011). Single subjects and group designs, in their most rigorous form, rule out or make implausible rival hypotheses for the experimental outcome, improving quality of inference. (Cook & Campbell, 1979; Kazdin, 2011; Shadish, Cook, & Campbell, 2002). A randomized controlled trial (RCT) is considered the gold standard for intervention research (Piantadosi, 2005). However, a RCT is not the only standard for causal inference (Kazdin, 2011). Reliance on large numbers makes application of a RCT with small groups or rare disease problematic (Janosky et al., 2009).

The focus of this research involves instillation of a solution through an appendiceal stoma, a procedure used for over a century and widely popularized over 20 years ago. Case reports and retrospective studies detail widely divergent effectiveness rates (Bani-Hani, Cain, King, & Rink, 2008; Dey et al., 2003; Mousa et al., 2006; Siddiqui, Fishman, Bauer, & Nurko, 2011; Yardley et al., 2009). The literature and involved clinicians have identified the need for prospective trials comparing ACE flushing regimens. None have been undertaken to date. This is in large part because the small size and heterogeneity of this population does not lend itself to a large N study. Many clinical questions go unanswered due to over reliance on RCT large N methodology (Kazdin, 2011). This population is an exemplar of that problem.

The proposed study comparing two flushing regimens utilizes a cross-over design embedded in a single subject A-B-C-B'-C'-B1' design. Both methods are experimental and lay a foundation for causal inference (Chow & Liu, 2014; Elder, 1997; Portney & Watkins, 2009). In both designs, the subject acts as his or her own control minimizing within subject variability and ensuring the highest possible degree of equivalence across treatment conditions, thereby allowing greater precision and efficient estimates of treatment effects increasing internal validity and causal inference (Janosky et al., 2009; Piantadosi, 2005; Portney & Watkins, 2009). In both methods, subjects are randomized to treatment sequence. Randomization will decrease the threat of order effects in both methods, increase group equivalency in the cross-over design, and minimize variability in measurement due to subject or period differences, increasing internal validity and causal inference (Chow & Liu, 2014; Jones & Kenward, 2003).

Single subjects design is an inductive, experimental methodology with controlled introduction and manipulation of an independent variable. Single subjects design promotes exploration of inter-subject variability without the introduction of error inherent in group methodology in the absence of subject homogeneity. It allows for isolation of individual response to interventions and identification of valuable information from outliers that would be obscured or lost in group methodology. Single subjects design also allows for time series observations of response, providing continuous, and often a more accurate, representation of the dependent variable of interest that may be compromised when the data is collected as an isolated snapshot in group methods (Elder, 1997). This study is ideally suited to single-subject repeated measures design, because children requiring an ACE procedure comprise a very small population with widely disparate anatomic and physiologic causative factors making sample homogeneity difficult. Inclusion of heterogeneous subjects will allow differentiation of subject characteristics that impact response to treatment. The design allows for frequency, volume, and dose adjustment of each flushing regimen when indicated. The ability to adjust the treatment regimen facilitates dose response comparison and will aid in identifying which flushing regimen requires the minimal dose and administration frequency, is accomplished in the least amount

of time, and has the fewest side effects while achieving continence. Because this design allows for repeated measurement over time, it is particularly helpful when studying comparisons between several treatments and is more sensitive to variations in treatment response that might otherwise be missed using group methodology (Gast, 2010; Janosky et al., 2009; Kazdin, 2011).

Subjects will be limited to children who are scheduled for a cecostomy or appendicostomy, ensuring their gut is naïve to the effects of a flushing regimen and allowing a true no-treatment baseline. Flush effects are reversible, making this intervention amenable to a withdrawal design. There are no known carry-over effects associated with either flushing regimen that would impact treatment effect on continence. Specimen collection facilitating comparison of treatment effects on gut microbiota, electrolytes, and stool calprotectin occurs after an active wash out period at the completion of each flushing regimen, negating any carry-over effects. Given the pragmatic issues involved in answering the research question at hand, the chosen methods and design elements strengthen demonstration of the counterfactual and make implausible potential threats to validity, lending credence to the assumption that intervention effects are due to the treatment and not random error or bias.

Subjects

This study will involve six children, ages 3 to 12 years, recruited from subspecialty clinics at Nemours entities in Florida and the Pediatric Spinal Defects Clinic in Jacksonville, Florida. Children will be selected by purposive sampling and will include those who are scheduled to have an ACE stoma and will require regular antegrade enema administration to maintain continence. Subjects will be limited to children who are scheduled for a cecostomy or appendicostomy, ensuring their gut is naïve to the effects of a flushing regimen and allowing a true no-treatment baseline. Excluded will be children with preexisting electrolyte imbalance, chronic high rectal tone, quadriplegia, renal or cardiac disease, or those who require prophylactic antibiotics, cannot communicate, or have significant cognitive delay that would interfere with their ability to fully participate in the study. Parents must have English language competency and be willing and able to participate in administration or oversight of the flushing regimen and data collection for a minimum of twenty +consecutive weeks.

Setting

Once parental consent and child assent have been obtained, baseline data will be collected daily for a minimum of 2 weeks prior to surgery, including frequency and volume of episodes of fecal soiling, and frequency and severity of abdominal pain. Blood samples for electrolyte and stool for calprotectin and microbiota will be obtained in the immediate preoperative period. The initial stool specimens for analysis will be collected prior to initiation of any pre-operative bowel prep.

Postoperatively, the child will be randomly assigned to either the saline or USP glycerin protocol. The process will be restricted random assignment to force equal sample size and will be accomplished using the SAS random number generator ensuring subject assignment results in equal group size. A member of the research team will meet with the parent and child in the immediate postoperative period after surgical clearance has been obtained for initiation of the first flush. During that time, the flush protocol, including materials and procedures, will be reviewed in detail. The child will receive the first antegrade infusion during that in-patient visit. A member of the research team will be available for each subsequent flush during the hospitalization. This will allow the family to gain competency in a controlled environment and familiarize themselves with the prescribed protocol and procedures prior to transition to the home setting. The reliability or accuracy and consistency of measurements will be verified using interobserver agreement (IOA) calculated by gross method. If there is a significant discrepancy in observational accuracy, as demonstrated by a calculated IOA below 80%, additional observer training will be provided until the calculated IOA is 80% or higher (Gast, 2010). Procedural reliability will be ascertained for each procedural variable to assure the intervention is being implemented as described in the methods section of the proposal.

Measures

Dependent variables at baseline will include: (a) number of episodes of fecal soiling. In addition, fecal soiling will be scored based on frequency and volume of accidents(0 = no soiling, 1 = smear, 2 = moderate volume accident not visible through clothing per week, 3 = any accident visible through clothing), (b) frequency and severity of abdominal pain recorded daily and measured using the Wong-Baker Faces Pain Rating Scale as the age-appropriate visual analog scale, (c) serum electrolytes, (d) stool for calprotectin, (e) quality of life measured by the FIQoL and (f) utilization of molecular techniques for identification of 16SrRNA gene sequence in stool samples obtained intra-operatively to identify and quantify phylogenetic groups (Penders et al., 2007).

Dependent variables obtained post-operatively following initiation of cecosotmy/appendicostomy flush will include: (a) administration time in minutes per flush, (b) total procedural time from start of flush to completion of colonic emptying in minutes per flush, (c) volume of solution in mL (d) number of episodes of fecal soiling. In addition, fecal soiling will be scored based on frequency and volume of accidents (0 = no soiling, 1 = smear, 2 = one moderate volume accident not visible through, 3 = any accident visible through clothing), (e) frequency and severity of abdominal pain recorded daily and measured using the Wong-Baker Faces Pain Rating Scale as the age-appropriate visual analog scale (f) number and frequency of side effects per week with severity of side effects measured using the Wong-Baker Faces Pain Rating Scale as the age-appropriate visual analog scale, (g) serum electrolytes, (h) stool for calprotectin, (i) quality of life measured by the FIQoL, and (j) utilization of molecular techniques for identification of 16SrRNA gene sequence in stool samples to identify and quantify phylogenetic groups (Penders et al., 2007). Fecal soiling score is detailed in Table 1.1. Dependent variables, including type of sample or instrument, sample characteristics, and measurement and data level, are explicated in Table 1.2. Study timetable is explicated in Table 1.3.

Table 1.1- Level of Soiling

0	No soiling
1*	Smear
2*	One moderate volume accident not visible through clothing
3*	Any accident visible through clothing

*Change dose with any soiling > Level 0

Table 1.2 - Dependent Variables

Sample/Instrument	Variable	Measurement	Data Level
Blood	BMP	Electrolyte Balance	Ratio
Stool	Calprotectin	Mucosal Inflammation	Ratio
Stool	Colonic microbiome	Metagenomic Profiling	Ratio
		16SrRNA	
FIC QOL	Parent/child quality of life	Symptoms Rating Scale	Ordinal
WBFPRS	Abdominal Pain	Symptom Rating Scale	Ordinal
WBFPRS	Procedural side effects	Symptom Rating Scale	Ordinal
Stop Watch	Infusion Time	Minutes	Ratio
Stop Watch	Procedural Time	Minutes	Ratio

Administration time in minutes per flush will be defined as the time at which the tubing connected to the bag or syringe holding the flush solution is unclamped and the cecosotmy fluid starts to infuse into the patient to the time the infusion is completed (no more fluid left in the bag/syringe or tubing). The total procedural time is defined as the time the flush starts to infuse into the subject and ends following passage of stool when the child has sat on the commode for 5 minutes with no additional stool passage. Both administration and total procedural times will be measured using duration per occurrence direct observational recording completed by the parent or child. Volume and dose will be calculated with each flush. Accidents will be defined as non-toilet elimination, which will be tracked and tallied as the number of pairs of underwear soiled with stool with documentation of accident severity and the estimated time of each accident using event recording. Dependent variables will be measured and recorded by the parent or child using a data collection sheet specifically

designed for this study and reviewed weekly with the study coordinator to encourage completion of all relevant data.

The investigator will keep a research log documenting and detailing any event that may cause a change in level, stability, or trend of the dependent variable not related to the intervention, for example, treatment with antibiotics or an intercurrent illness. The number of episodes of fecal soiling per week will exclude accidents caused by illness, medications, or dietary indiscretion. Side effects will be measured using the Wong-Baker FACES Pain Rating Scale (WBFPRS). The WBFPRS has undergone extensive testing, is preferred by children, and has well established psychometrics in the pediatric population (Tomlinson, von Baeyer, Stinson, & Sung, 2010; Wong & Baker, 1988). The scale ranges from 0 (very happy without pain) to 10 (the worse pain imaginable). Each pain level is associated with a facial expression. The child is asked to choose the face that best describes his/her level of discomfort. The WBFPRS will be used to evaluate the presence and severity of flush side effects, including abdominal cramping, nausea, and vomiting. The parent will call if the child is having accidents or discomfort greater than a 4 on the WBFPRS associated with the flushing regimen. Documentation of severity of side effects will be completed by the parent and child on a data-collection form by viral, bacterial, or drug-induced gastroenteritis; these will be recorded and analyzed as confounds (Portney & Watkins, 2009).

The Fecal Incontinence and Constipation Quality of Life Measure in Children with Spinal Bifida (FIC QOL) will be used to assess child and parental perception of social validity (Nanigian et al., 2008). The tool will be administered preoperatively during the baseline period and at the end of each flushing regimen in the comparative phase of the study. The FIC QOL is a 51 item questionnaire with established validity and reliability in families of children with spina bifida who are incontinent for stool. This instrument measures those aspects of daily living that are significantly impacted by fecal incontinence. Of the 51 items, four address subject and family demographics. The remaining 47 items are divided into seven groupings that include bowel program, diet, symptoms, travel and socialization, family relationships, caregiver support and emotional impact, and financial impact (Nanigian et al., 2008; Ok & Kurzrock, 2011). In addition to the FIC QOL, a simple qualitative question will be directed to the children at the end of the study to ascertain which flushing regimen they prefer and why.

Table 1.3 Timeline for Completion.

Overview of Timeline

	Baseline		Dose Response Phase							Flush Effectiveness Phase										
Weeks:	1	2	3	4	5	6	7	8	9	10+	11	12	13	14	15	16	17	18	19	20
Randomize		X								X										
<u>Order:</u>																				
A	NT	NT																		
B-C			B	B	B	B	C	C	C	C										
C-B			C	C	C	C	B	B	B	B										
B'-C'-B ₁ '											B'	B'	B'	B'	C'	C'	C'	C'	B ₁ '	B ₁ '
C'-B'-C ₁ '											C'	C'	C'	C'	B'	B'	B'	B'	C ₁ '	C ₁ '
<u>Visits:</u>																				
Hospital			X																	
Home				X																
Clinic						X				X				X				X		X
<u>Biomarker</u>																				
Stool	X					X				X				X				X		
BMP	X					X				X										
SIM	X					X				X										
<u>Instrument</u>																				
FIC QOL	X													X				X		
<u>Measures:</u>																				
Soiling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Abd Pain	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Admin T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Proc T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ISE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cost	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

- A - Baseline
- NT - No treatment
- B - Saline dose-response phase
- C - USP Glycerin dose-response phase
- B' - Initial trial of saline effectiveness phase
- C' - Initial trial of USP glycerin effectiveness phase
- B₁' - Second trial of saline effectiveness phase
- C₁' - Second Trial of USP glycerin effectiveness phase
- Stool - Metagenomic Profiling 16SrRNA (collected and batched for downstream analysis)
- BMP - Basic Metabolic Profile
- SIM – Stool Inflammatory Marker (Calprotectin)
- Visits - Procedural fidelity and inter-rater reliability will be measured at each visit
- FIC QOL - Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida
- Abd Pain - Abdominal Pain
- Admin T - Administration time
- Proc T - Procedural time
- ISE - Infusion side effects

Dosing

High Volume Regimen:

The high volume regimen (B) consists of a normal saline flush at a starting dose of 10mL/kg infused every other day and adjusted until stability of target outcomes is achieved. At any point the subject is having episodes of fecal soiling, the dosing strategy will be increased by 5 to 10 mL/kg-increments with a subsequent increase in frequency, if needed, so as not to exceed 20 mL/kg and a maximum dose of 500 mL in children 5 years of age and under and 1000 mL administered daily in children older than 5. If the child does not attain continence on the maximum dose, he/she will be trialed on the alternate flushing solution but will not progress to the maintenance phase of the study. If the child is having side effects greater than 4 on the WBFPRS at the starting dose of 10mL/kg, flush volume will be incrementally decreased as needed by 2.5 mL/kg to the lowest dose of 5 mL/kg daily. The goal is to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize effects results in episodes of fecal soiling or the child continues to have side effects greater than 4 on the WBFPRS at the lowest dose of administration, the child will be dropped from the study. The decision tree for dose adjustment of Normal Saline is detailed in Tables 1.4 and 1.5.

Low Volume Regimen:

The low volume regimen (C) will consist of USP glycerin diluted in normal saline prior to antegrade instillation through the low profile device. The child will start on an every other day dose of 20 mL of USP glycerin and >20 mL of saline (used as diluent at a dose sufficient to allow the solution to easily infuse through the ACE access tubing) and adjusted until stability of target outcome is achieved. At any point the child is having episodes of fecal soiling, the volume of USP glycerin will be increased in 10 mL increments with subsequent increase in frequency, if needed, so as not to exceed 40 mL of USP glycerin administered daily. If the child does not attain continence on the maximum dose of USP glycerin, he/she will be placed on the alternate flushing regimen but will not progress to the maintenance phase of the study. If the subject is having side effects greater than 4 on the WBFPRS at the starting dose of 20mL of USP glycerin, the volume of the USP glycerin will be decreased as needed by 5 mL increments until the child's symptoms are 4 or less on the WBFPRS or the lowest dose of 5 mL daily is reached. The goal is to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize effects results in episodes of fecal soiling greater than one smear per week or the child continues to have side effects greater than 4 on the WBFPRS at the lowest dose of administration, the child will be placed on the alternative flushing regimen but will not advance to the maintenance phase of the study. The decision tree for dose adjustment of USP glycerin is detailed in Tables 1.4 and 1.5.

Table 1.4 - Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Maintain Continence in the Absence of Side Effects

*Change dose with any soiling > Level 0 as defined in Table 1.5

Normal Saline (High volume regimen)	USP Glycerin + Normal Saline as Diluent (Low volume regimen - Max dose for children 5 and under is 30 mL + > 30 mL qd)
B1 = 10mL/kg maximum dose 1000 mL qod B2 = 10mL/kg maximum dose 1000 mL q3d B3 = 10mL/kg maximum dose 1000 mL qd B4 = 15mL/kg maximum dose 1000 mL qd B5 = 15mL/kg maximum dose 500 or 1000 mL q3d B6 = 15mL/kg maximum dose 500 or 1000 mL qd	C1 = 20 mL + > 20 mL qod C2 = 20 mL + > 20 mL q3d C3 = 20 mL + > 20 mL qd C4 = 25 mL + > 30 mL qd C5 = 25 mL + > 30 mL qod C6 = 25 mL + > 30 mL q3d C7 = 30 mL + > 40 mL qod C8 = 30 mL + > 40 mL q3d C9 = 30 mL + > 40 mL qd C10 = 40 mL + > 50 mL qod C11 = 40 mL + > 50 mL q3d C12 = 40 mL + > 50 mL qd

Table 1.5 - Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Minimize Side Effects and Maintain Continence

Normal Saline* (High volume regimen with maximum dose for less than 5 and over 5 yrs.)	USP Glycerin + NS as Diluent* (Low volume regimen)
B1 = 10 mL/kg maximum dose 500 or 1000 mL qod B7 = 7.5 mL/kg maximum dose 500 or 1000 mL qod B8 = 7.5 mL/kg maximum dose 500 or 1000 mL q3d B9 = 7.5 mL/kg maximum dose 500 or 1000 mL qd B10 = 5.0 mL/kg maximum dose 500 or 1000 mL qod B11 = 5.0 mL/kg maximum dose 500 or 1000 mL q3d B12 = 5.0 mL/kg maximum dose 500 or 1000 mL qd	C1 = 20 mL + > 20 mL qod C10 = 15 mL + > 15 mL qod C11 = 15 mL + > 15 mL q3d C12 = 15 mL + > 15 mL qd C13 = 10 mL + > 10 mL qod C14 = 10 mL + > 10 mL q3d C15 = 10 mL + > 10 mL qd C16 = 5 mL + > 5 mL qod. C17 = 5 mL + > 5 mL q3d C18 = 5 mL + > 5 mL qd

*Change dose with any side effects >4 on the WBFPRS or soiling ≥ Level 1 as defined in Table 1.5

Optimal Dose Regimen:

Once the optimal dose has been established, the child will be maintained on that dose and frequency for at least 2 weeks or until stability in dependent measures without significant variability or trend is achieved. The child will be scheduled to come into the clinic for a visit once the above criteria have been met, at which time labs will be drawn and a stool sample collected.

Regimen Comparative Phase:

Following completion of the dose-response phase, the comparative portion of the study will begin by administering either the established effective dose of normal saline (B') or the established effective dose of USP glycerin (C'). Patients will be randomized for a second time to either a B'-C'-B1' or a C'-B'-C1' sequence. The last flush in the dose response sequence will be withdrawn and the initial flush in the comparative treatment phase will be introduced the following day at the previously established minimum effective dose and frequency. Children will remain on treatment for 4 weeks, at which point the treatment will be withdrawn. Children will then be placed on the next treatment in the sequence at the pre-established effective dose and frequency for 4 weeks. The second flush will then be withdrawn and the initial flush in the sequence will be reintroduced for an additional two weeks (B1' or C1'). At the conclusion of the study, the child will be placed on the flushing regimen of his/her choice.

Data Evaluation

Data will be obtained and dose response adjustment will be made during the B-C or C-B phase when indicated. Once data has been collected, it will be graphed on an equal interval line graph with the proportion of ordinate and abscissa scaled at a 2:3 ratio to ensure consistency of data presentation and prevent data distortion during visual analysis. Dependent measures will be placed on the ordinate scale with time-by-day on the abscissa scale. Separation of dose-response and comparative phases of the study will be designated by a bold vertical line and high and low dose regimen changes by a thin vertical line. Each phase change will be labeled with solution name, dose, and frequency. Independent variable effect on target behaviors will be analyzed using visual analysis. Analysis within each condition will include: (a) condition length defined as the number of data points contained within each phase, (b) level stability with a stability envelope calculated using the median and a stability criterion of 80% of the data points falling within 15% of the calculated median for the phase, (c) relative change in level, (d) absolute change in level, (e) estimation of trend direction using split middle method, (f) trend stability with a stability envelope using the same criteria as level stability, and (g) identification of multiple paths within trends, if present. Analysis between conditions will include: (a) the number of variables that have changed between adjacent conditions, (b) change in trend direction between conditions, (c) assessment if trend change is in keeping with intervention goals, (d) assessment for change in trend stability, (e) assessment of immediacy of effect in change in level and trend, (f) calculation of absolute and median level change, (g) calculation of percentage of non-overlapping and overlapping data points and (h) percentage of data points exceeding the median (Gast, 2010; Hartmann et al., 1980; Ma, 2006; Kazdin, 2011; McDowall, McCleary, Meidinger, & Hay, 1980; Portney & Watkins, 2009). Testing in multiple subjects will allow for analysis of replication of treatment effects. Analysis will include between-series strategies comparing data points, including frequency, mean occurrence, and immediacy and magnitude of effect within and between treatment conditions. In addition to visual analysis of time series parameters, inferential procedures will be used to compare interventional effects and increase the reliability of visual methods analysis.

The independent variable is nominal and dichotomous. Dependent variables are comprised of either interval or ratio level measurements. The comparative phase of the study is a 2-treatment crossover design with each child receiving both treatments. Each child will be randomly assigned to either a C'-B'-C1' or B'-C'-B1' treatment sequence with half of the subjects allocated to each sequence (Portney & Watkins, 2009). The flushing regimen's effect on outcomes of interest, including number of soiling episodes, level of soiling, abdominal pain, procedural side effects, infusion time, procedural time, electrolyte balance, fecal calprotectin, and quality of life, will be analyzed using a two-tailed, two-sample pooled variance t test with a significance level set at 0.05. Confidence intervals will be calculated to provide precision of mean differences estimates (Polit, 2010). The two sample t test will be used to test treatment differences in the cross-over design. Because the design is comparing two treatment sequences, the groups are independent (Chow & Liu, 2014). Confounding by carry over and direct-by-period interaction is a potential with cross-over designs which, if present, can bias treatment effects (Jones & Kenward, 2003; Senn, 2002; Shuster, 2007). Jones et. al. (2003) and Sen (2002) suggest use of a one sample t test for analysis of cross-over designs (treatment one is subtracted from treatment two). Shuster (2007) advocates a two sample t test in the analysis of a randomized 2 treatment cross-over design (period two is subtracted from period one irrespective of treatment order). Analysis of a one sample t test in a cross-over design ignores treatment ordering. Two sample t test analysis compares ordering and yields potentially useful data on carry over. When μ is the main treatment effect, and τ is carry over, results from a one sample or two sample t test will yield unbiased estimates of μ and variance when the sample size is equal and $\tau = 0$. If $\tau \neq 0$, the expected value of μ should be similar using either the one sample or two sample method. However, the one sample method does not account for carry over effects increasing variance. If sample sizes are unequal and $\tau \neq 0$ (conditional on sample size), the point estimates in the one, but not the two, sample t test will be biased. Using the 2 sample method will lend precision in the presence of carry over effects, and precision and accuracy in the presence of unequal sample size when $\tau \neq 0$ (Shuster, 2009). Subject characteristics will be described, when appropriate, using frequency distribution and graphed using either histograms or pie-charts. Changes in gut microbiota will be analyzed using descriptive statistics (Polit, 2010).

Summary of Previous Human Experience with the Investigational Drug

Retrospective Studies and Case Reports Addressing Antegrade Colonic Flush Administration

<u>Pediatric Subjects</u>	<u>Solution & Dose</u>	<u>Side Effects from Flush</u>	<u>Reference</u>
40	Dose not reported 1) GoLYTLEY 2) Liquirice root 3) Oil/water mix 4) Treacle/milk mix 5) Water only 6) Oil only	None	Marshall, J., Hutson, J. M., Anticich, N., & Stanton, M. P. (2001). Antegrade continence enemas in the treatment of slow-transit constipation. <i>Journal of Pediatric Surgery</i> , 36, 1227-1230. doi:10.1053/jpsu.2001.25768
62	1) Polyethylene Glycol with electrolytes (50 – 1,000 mL) 2) Phosphate enema 3) NS alone (50 – 1000 mL)	None	Dey, R., Ferguson, C., Kenny, S. E., Shankar, K. R., Coldicutt, P., Baillie, C. T., ...Turnock, R. R. (2003). After the honeymoon- Medium-term outcome of antegrade continence enema procedure. <i>Journal of Pediatric Surgery</i> , 38, 65-68. doi:10.1053/jpsu.2003.50012
26	Flush solution not reported. Dosage (250 – 1000 mL)	None	Becmeur, F., Demarche, M., Lacreuse, I., Molinaro, F., Kauffmann, I., Moog, R., Rebeuh, J. (2008). Cecostomy button for antegrade enemas: Survey of 29 patients. <i>Journal of Pediatric Surgery</i> , 43, 1853-1857. doi:10.1016/j.pedsurg.2008.03.028
71	Tap water (300 – 1000 mL)	Minor deviations in serum sodium and serum chloride present in 18/71 patients Significant hypernatremi and hyperchloremia in	Yerkes, E. B., Rink, R. C., King, S., Cain, M. P., Kaefer, M., & Casale, A. J. (2001). Tap water and the Malone antegrade continence enema: A safe combination? <i>Journal of Urology</i> , 166, 1476-1478.

		1/71 used softened tap water	
236	<p>1) Tap water alone volume (100 to 1000)</p> <p>2) 60 mL of USP glycerin & 60 mL of NS</p> <p>3) GoLYTELY – 1 liter</p> <p>4) MiraLAX 17 grams mixed in 250 mL tap water</p> <p>5) Mineral oil 30 mL</p>	None	Bani-Hani, A. H., Cain, M. P., King, S., & Rink, R. C. (2008). Tap water irrigation and additives to optimize success with the Malone antegrade continence enema: The Indiana University algorithm. <i>Journal of Urology</i> , 180, 1757-1760. doi:10.1016/j.juro.2008.04.074
105	<p>1) Normal saline or GoLYTELY(23 +/- 0.7 mL/kg)</p> <p>2) USP glycerin – dose not reported</p> <p>3), bisacodyl – dose not reported</p> <p>4) magnesium citrate – dose not reported</p> <p>5) phosphosoda – dose not reported</p>	None	Siddiqui, A. A., Fishman, S. J., Bauer, S. B., & Nurko, S. (2011). Long-term follow-up of patients after antegrade continence enema procedure. <i>Journal of Pediatric Gastroenterology and Nutrition</i> , 52, 574-580
23	USP glycerin 10 – 60 mL & 15-775 mL tap water with a total volume of irrigation solution ranging from 30 – 800 mL	None	Chu, D., Balsara, Z.R., Routh, J.C., Ross, S.S., & Wiener, J.S. (2012). Experience with glycerin for antegrade continence enema in patients with neurogenic bowel. <i>The Journal of Urology</i> , 189,690-693.
1 – Case report	Hypertonic saline	Death	Schreiber, C. K., & Stone, A. R. (1999). Fatal hypernatremia associated with the antegrade continence enema procedure. <i>Journal of Urology</i> , 162, 1433-1434.

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APPENDIX L
IAA AGREEMENT BETWEEN NEMOURS AND UNIVERSITY OF FLORIDA

IRB Authorization Agreement

Name of Institution or Organization Providing IRB Review:

Nemours Foundation
10140 Centurion Parkway
Jacksonville, FL 32256

IRB Registration #: 00000998 (IRB #2)

Federal Wide Assurance (FWA) #: 00000293

Name of Institution Relying on the Designated IRB:

University of Florida College of Medicine – Jacksonville (the "UFCOM-Jax")
UF Health Science Center Jacksonville
Suite 9015, 9th Floor, Tower II
580 West Eighth Street
Jacksonville, FL 32209

Federal Wide Assurance (FWA) #: 00005790

The Officials signing below agree that the UFCOM-Jax may rely on the NEMOURS FOUNDATION IRB (NEMOURS IRB) for review and continuing oversight of research studies conducted by or in collaboration with University of Florida faculty, students, and/or personnel as described below.

NEMOURS and UF agree to the following:

- This agreement pertains only to the following specific protocol:
 - *A Prospective Pilot Within Subjects Comparison of Two Antegrade Flushing Regimens in Children*
 - Nemours Principal Investigator: Kimberly S. Jarczyk, MSN, CPNP
 - Relative to any subject risks associated with this project, Ms. Jarczyk's engagement in this project is as a Nemours employee.
 - Subject recruitment and study interventions will take place at Nemours and/or Wolfson hospitals
 - No subject recruitment or study interventions will occur at UF Health facilities.
 - At least one MD will serve as a co-investigator on this project at all times.
 - UF Co-investigators: Pam Pieper, PhD, ARNP, PPCNP-BC; Jonathan Shuster, PhD
 - Dr. Pieper and Dr. Shuster's engagement will be limited to analysis of de-identified data.
- Each institution shall only be responsible for the conduct of its own researchers.
 - In the event of regulatory noncompliance or risk to subjects, NEMOURS IRB may take appropriate actions relative to investigators engaged in the above

named protocol.

- NEMOURS will appropriately notify UFCOM-Jax in the event of any unanticipated problems or regulatory noncompliance.
- UFCOM-Jax remains responsible for ensuring compliance with the IRB's determinations and with the terms of its OHRP-approved Federalwide Assurance.
- This document must be kept on file at both institutions and provided to OHRP upon request.
- NEMOURS may only publicize this collaboration with written approval from the UF's Institutional Official or designee.
- Kimberly Jarczyk will provide UFCOM-Jax with copies of approved IRB protocols and modifications.

University of Florida



David P. Norton, PhD
Vice President for Research

7-2-2015
Date

Nemours Foundation



Paul E. Garfinkel, MSH, CIP
Director, Clinical Research Operations and
Human Subjects Protection
Institutional Official

7-1-2015
Date

APPENDIX M MANAGEMENT OF RESEARCH PHARMACEUTICAL PRODUCTS



Institutional-Review¶

Addendum for Management of Research Pharmaceutical Products¶

Version September 2014¶



PI Name: → Kimberly S. Jarczyk, MSN, ARNP, C-PNP and Pam Pieper, PhD, ARNP, PPCNP-BC¶
 Study Title: → A Within-Subjects Comparison of Two Antegrade Flushing Regimens in Children¶
 Sponsor: → Kimberly Jarczyk¶
 Product(s): → Normal Saline and USP Glycerin¶
 Inpatient → → Outpatient → ¶
 Location: → → Wilmington → → Jacksonville → → Orlando → → Pensacola¶
 → → → → Other: ¶

Background: When drugs, biologics, or other products are used in human research, a plan for research product management (approved by a Nemours Investigational Pharmacist or delegate) must be submitted to the IRB at the time of the initial application for approval of the study. Appropriate management of drugs, biologics and other pharmaceutical products used in research allows the IRB to determine that participants and others are adequately protected and that the scientific integrity of the study is assured.¶

Process: The investigator (or qualified delegate) must consult with the Investigational Pharmacist (DE) Director of Pharmacy (ORL / NCH) or a delegated site-authorized associate (JAX, PNS) on the development of the Research Product Management Plan. That plan must contain the following elements, as applicable.¶

This form, when completed, may be used as the plan document that is submitted with the IRB application.¶

X ^α	Element ^α	Comment / Description ^α
X ^α	Copy of protocol, manual of procedures, or pharmacy manual ^α	Copy of Protocol ^α
X ^α	Randomization Process (how and who) ^α	This prospective study will utilize a repeated measures, single subjects alternating treatments A-B-C-B'-C'-B1' withdrawal design in which all subjects are tested under all conditions and each subject acts as his or her own control. A within-subjects cross-over design is embedded in the B'-C'-B1' treatment comparison phase of the study. The process will be restricted random assignment to force equal sample size and will be accomplished using the SAS random number generator. Subjects will be randomly assigned by the investigator to one of two treatment sequences to control for the possibility of order effects. The treatment will be replicated across 12 subjects randomized to 6 subjects per group. Baseline data A will serve as the control and will be obtained pre-operatively. Randomization will occur during baseline.

Version September 2014¶

Page 1 of 3¶

		prior to surgery. The first B--C phase of the study will evaluate dose-response relationship and will be used to identify the optimal dose and frequency of ACE administration for normal saline and normal saline with USP glycerin. When the optimal dose has been identified, the child will continue on that dose for two weeks to insure treatment stability and effectiveness. If continence cannot be achieved within the dosing guidelines, the child will be dropped from the study. To prevent statistical bias from subject loss due to treatment failure, each child will be randomized to a second treatment sequence once they have achieved continence on optimal dosing with minimal side effects	ix
xix	Requirements for product accountability <ul style="list-style-type: none"> •→ Controlled substance? •→ Non-Controlled? 	Both normal saline and USP glycerin are non-controlled and available OTC	ix
xix	Requirements for product destruction <ul style="list-style-type: none"> •→ Hazardous? •→ Non-Hazardous? 	Both normal saline and USP glycerin are non-hazardous. The volume and frequency of administration in this proposal does not substantially alter or increase the concentration or distribution of the substance, metabolites, or products of degradation in the environment. To the best of the investigator's knowledge, no extraordinary circumstances exist with the proposed use of saline and glycerin that would adversely affect the quality of the human environment.	ix
xix	Participant charging requirements (if any) <ul style="list-style-type: none"> •→ Charging for <u>investigational</u> articles may only occur under very limited circumstances and must be compliant with FDA regulations 	Participants will not be charged for either solution	ix
xix	Are additional supplies required? <ul style="list-style-type: none"> •→ Labels (compliant with regulations) •→ Auxiliary "Investigational Drug" Label •→ Vials •→ Additional meds (including supportive medications) •→ Other 	The normal saline and USP glycerin will be dispensed in their original containers. Because the route of administration constitutes an off-label use of both medications, the containers will be labeled using templates for regulation-compliant medication labels provided by Marjorie Delucia.	ix
xix	Names of study personnel approved to handle research product	Kim Jarczyk, Tara Spruill, and Kaitlyn Smith	ix
xix	Training plan for study personnel approved to handle product	Kim Jarczyk is the Principal Investigator for the study Tara Spruill and Kaitlyn Smith will receive individual training	ix
xix	Where will the product be stored? Be specific —must meet the specific environmental and security requirements for the product <ul style="list-style-type: none"> •→ Locked storage (cabinet/refrigerator/freezer) •→ Temperature monitoring plan for ambient/refrigerator/freezer (includes notification system for variances) 	The study drug will be stored in a locked cabinet in the medication room in the Continence Clinic on the 3 rd floor in the Nemours Children's Specialty Care Building on 807 Children's Way in Jacksonville, Florida. The medication will be stored in a separate cabinet separated from other drugs that are stocked in the clinic for patient care use. The cabinet is locked with restricted key access. The room temperature in the cabinet in which the drug is stored will be monitored on a continuous basis using a MCC USB-	ix

Addendum for Management of Research Pharmaceutical Products--continued ¶

	<ul style="list-style-type: none"> •→ Access limited to study personnel ¶ •→ Investigational research pharmaceutical products stored separately from clinic supplies. ¶ 	501-LCD thermometer which allows for storage and downloading of temperature data. Staff will monitor the device daily. Following weekends or holidays, the stored temperature data will be reviewed. Any temperature excursions will be discussed with a pharmacist prior to dispensing the stored flush solution. ... ¶	¶
x ^α	<p>Temperature requirements: ¶</p> <ul style="list-style-type: none"> •→ Ambient ¶ •→ Refrigerator ¶ •→ Freezer ¶ 	Ambient ¶	¶
x ^α	<p>Temperature Monitoring ¶</p> <ul style="list-style-type: none"> •→ Log developed or provided ¶ •→ Dual Monitoring in place ¶ •→ Procedure for handling out of range variances ¶ 	A daily hard copy temperature log will be kept. The MCC-USB-501-LCD device continuously monitors and stores ambient temperatures. If a significant variance from the acceptable temperature range is found, the principal investigator will be notified, who will contact the pharmacist for instructions before any of the stored drug is dispensed. ¶	¶
x ^α	<p>Regulatory Binder forms include: ¶</p> <ul style="list-style-type: none"> •→ Shipment receipt and product inventory (includes lot # and expiration dates) ¶ •→ Participant product accountability record ¶ 	Shipment receipt and product inventory including lot # and expiration dates will be stored in the regulatory binder with a separate log maintained for participant product accountability. ¶	¶
x ^α	Final disposition procedure (compliant with applicable regulations) ¶	To be disposed of by the family through regular household plumbing ¶	¶

¶

¶

Approved by: (typed name): ····· ¶

¶

Date: ····· ¶

¶

Approver comments (if any): ····· ¶

Addendum¶

Research Involving Investigational Drugs or Biologics¶

Instructions:¶

Note: The word 'drug' is used for simplicity. Where applicable read 'biologic'. If needed, submit separate applications for multiple investigational drugs.¶

- ¶
- **Parental Permission and/or Informed Consent requirements:**¶

The investigator must assure that the information included in the parental permission/informed consent form includes:¶

- → A clear statement that the drug is investigational and has not been approved or, if studying an approved drug, that it is approved but not for the use being studied.¶
- → A brief description of the drug, e.g., purpose, and mechanism.¶
- → If studying an approved drug, a statement that the drug may be available without participating in the study. An exception to this may be granted if the off-label use of the drug is unrealistic or unsafe outside of a carefully controlled clinical study.¶
- → Information about the potential costs of the study drug.¶

Principal Investigator: **Kimberly S. Jarczyk, MSN, ARNP, CPNP and Pam Pieper, PhD, ARNP, PPCNP-BC**¶

Study Title: **A WITHIN SUBJECTS COMPARISON OF TWO ANTEGRADE FLUSHING REGIMENS IN CHILDREN**¶

A. → List the investigational drugs that will be used in this study.¶

Name of Drug ^α	Is this drug or biologic FDA approved? ^α	IND# ^α	IND HOLDER / SPONSOR ^α
Normal Saline ^α	<input type="checkbox"/> Yes... <input checked="" type="checkbox"/> No ^α	126011 ^α	Kimberly S. Jarczyk ^α
USP Glycerin ^α	<input type="checkbox"/> Yes... <input checked="" type="checkbox"/> No ^α	126011 ^α	Kimberly S. Jarczyk ^α
..... ^α	<input type="checkbox"/> Yes... <input type="checkbox"/> No ^α ^α ^α
..... ^α	<input type="checkbox"/> Yes... <input type="checkbox"/> No ^α ^α ^α

1. → The IND#(s) for the investigational drug(s) is verified by:¶

Sponsor protocol with the IND number. (Page: 1^α

The Investigator's Brochure may not be used to verify the → → IND#.) Reference: 126011¶

Communication from the sponsor. Attached.¶

Application to and communication from the FDA. (Required if the investigator holds, or is applying for, → → the IND.) Attached.¶

2. → If the Principal Investigator has applied for, or is the sponsor of an IND, are copies of all relevant correspondence with the FDA included with this application? Yes No N/A¶

3. → Is this drug commercially available? Yes No. If yes, is research being conducted:¶

a. → To support a new indication or support a change in advertising or labeling of the product (for example, a new age group or population, or new diagnosis) Explain:¶

b. → In a dosage level that might significantly increase the risk to the subject population Explain:¶

c. → Via a new route or for use in a different part of the body. Explain: Both normal saline and USP glycerin are available over the counter for retrograde enema administration. Both will be used for antegrade enema administration¶

d. → In a new patient population and may result in a significant increase in risk(s) to the subject → population. Explain:¶

IRB Application Addendum -- Research Involving Investigational Drugs or Biologics ¶

e. → → That may be deemed a significant risk when used for an unapproved purpose. Explain: " " " " ¶

f. → → None of the above apply. ¶

¶

An IND may be required if the research meets one of the criteria, above. The IRB will assess whether or not an IND is required for this research. If an IND is required and has not been applied for, IRB approval will be deferred pending documentation from the FDA. ¶

4. → Is the Investigator's Brochure(s) or a Package Insert for investigational drugs included with this application? Yes No If no, reference the section(s) in the protocol that includes the rationale for use, the safety and efficacy, and prior clinical data for this investigational drug. pp 2-3 and pp29-39 ¶

¶

5. → Will participants be charged for this drug? Yes No. If yes, ¶

a. → What is the cost? " " " " ¶

b. → Justify the charge: " " " " ¶

c. → If the drug is under an IND, attach or reference documentation of FDA approval for this charge. " " " " ¶

¶

6. → Is the investigational drug subject to the Controlled Substances Act? Yes No. If yes, the investigator must assure that adequate precautions will be taken to prevent theft or diversion of the substance into illegal channels of distribution. Describe: " " " " ¶

¶

7. → Explain how the investigator will assure that investigational drugs will be adequately controlled for the duration of the study. ¶

a. → The hospital or affiliated clinic pharmacy will control the investigational drug. Yes No. If yes, the investigator must assure that required pharmacy approvals have been obtained. ¶

b. → The investigational drug will be controlled within the investigator's office or a specific clinical research area according to sponsor and / or biomedical research standard operating procedures. Yes No. If yes, describe: The study drug will be stored in a locked cabinet in the medication room in the Continence Clinic on the 3rd floor in the Nemours Children's Specialty Care Building on 807 Children's Way in Jacksonville, Florida. The medication will be stored in a cabinet separated from the cabinets in which other drugs are stocked for patient care use. The cabinet is locked with restricted key access. The room temperature in the cabinet in which the drug is stored will be monitored on a continuous basis using a MCC USB-501-LCD thermometer which allows for storage and downloading of temperature data. Staff will monitor the device daily. Following weekends or holidays, the stored temperature data will be reviewed. Any temperature excursions will be discussed with a pharmacist prior to dispensing the stored flush solution. ¶

• → If an investigator at AIDHC will control the investigational drug, the IRB requires documentation of Pharmacy approval. Pharmacy documentation is attached: Yes No NA ¶

¶

8. → How will the investigator assure that the investigational drug will be used only in approved research protocols and only under the direction of approved investigators? Describe: The USP glycerin and normal saline will be stored in a locked cabinet in the medication room in the Continence Clinic specifically designated for research drug. The inside of the cabinet will be clearly labeled with a sign denoting the drugs are for research purposes only. The cabinet is separate from the cabinets in which other medications are stored in the clinic space. ¶

¶

9. → This is an investigator initiated study and the Nemours investigator holds the IND. Yes No. If yes: ¶

a. → The complete plan for control, dispensing, and storage of investigational drugs at Nemours should be contained in the protocol. Reference page or section: 15 ¶

b. → The investigator is prepared for an on-site review to assure that all necessary processes are in place to assure compliance with the FDA sponsor regulations listed below. Yes No ¶

c. → ¶

10. An "Addendum for Control of Research Pharmaceutical Products" must be completed, approved, and submitted with your IRBNet application to the IRB. The addendum is available in the IRBNet forms library. This is required for final approval of your study. ¶

¶

21-CFR-11 (Electronic records and electronic signature)¶
21-CFR-54 (Financial Disclosure by Clinical Investigators)¶
→ → → [FDA forms 3454 and 3455]¶
21-CFR-210 (Current Good Manufacturing Practice In Manufacturing, Processing, Packing, Or Holding of
→ → → · Drugs; General)¶
21-CFR-211 (Current Good Manufacturing Practice for Finished¶
→ → → · Pharmaceuticals)¶
21-CFR-312 (Investigational New Drug Application)¶
21-CFR-314 (Drugs for Human Use)¶
21-CFR-320 (Bioavailability and Bioequivalence Requirements)¶
21-CFR-330 (Over-The-Counter (OTC) Human Drugs Which are Generally Recognized as Safe and → →
→ → → · Effective and Not Misbranded)¶
21-CFR-601 (Biologics Licensing)¶

¶

APPENDIX N

IRB SUBMISSION, APPROVAL, AND APPLICATION FOR AND APPROVAL FOR AMMENDMENTS

Title: A Within Subjects Comparison of Two Antegrade Flushing Regimens in Children

Principal Investigator: Kimberly S. Jarczyk, MSN,CPNP, Pam Pieper, PhD, ARNP, PPCNP-BC

Co-Investigator(s): Donald George, MD, James Sylvester, PhD, Mark Barraza, MD, Tara Spruill, RN, BSN, Karl Mann BS, Kaitlyn Smith, RN, BSN

Abstract: FECAL INCONTINENCE PAST THE TIME OF TOILET TRAINING IS DEVASTATING TO AFFECTED CHILDREN. ANTEGRADE CONTINENCE ENEMA (ACE) THERAPY ADMINISTERED THROUGH A CATHETERIZABLE STOMA SURGICALLY PLACED IN THE CECUM HAS HELPED CHILDREN WITH INTRACTABLE FECAL INCONTINENCE ATTAIN CONTINENCE FOR STOOL. THERE ARE A NUMBER OF RETROSPECTIVE STUDIES DEMONSTRATING THE EFFECTIVENESS OF ACE THERAPY AND SEVERAL PROSPECTIVE STUDIES THAT DEMONSTRATE IMPROVEMENT IN QUALITY OF LIFE FOLLOWING AN ACE PROCEDURE. THERE ARE NO PROSPECTIVE TRIALS EVALUATING THE EFFECTIVENESS OF DIFFERENT FLUSHING REGIMENS. THE CATHETERIZABLE STOMA USED FOR THE ANTEGRADE ADMINISTRATION OF ENEMA SOLUTION IS FREQUENTLY MADE BY BRINGING THE APPENDIX OUT THROUGH THE ABDOMINAL WALL OR BY PLACING A SKIN-LEVEL DEVICE (BUTTON) INTO THE CECUM. ACE THERAPY ADMINISTRATION THROUGH THE APPENDIX OR INTO THE CECUM HAS THE POTENTIAL TO DISRUPT THE GUT MICROBIAL ECOSYSTEM, CAUSING DYSBIOSIS AND IMMUNE DYSFUNCTION. THE EFFECTS OF ACE ADMINISTRATION ON COLONIC MICROBIOME AND MUCOSAL IMMUNITY HAVE NOT BEEN INVESTIGATED. THIS STUDY WILL COMPARE A HIGH VOLUME NORMAL SALINE FLUSH AND A LOW VOLUME USP GLYCERIN FLUSH. THE PROPOSED PILOT STUDY IS PROSPECTIVE AND UTILIZES A CROSS - OVER DESIGN EMBEDDED IN A SINGLE SUBJECTS DESIGN EMPLOYING TWELVE SUBJECTS RANDOMIZED TO TWO TREATMENT SEQUENCES. THE PRIMARY AIMS OF THE STUDY ARE TO COMPARE WHICH SOLUTION, GIVEN AT AN OPTIMAL DOSE AND FREQUENCY, IS DELIVERED IN THE LEAST AMOUNT OF TIME, WITH FEWER SIDE EFFECTS, WHILE PROMOTING THE HIGHER DEGREE OF FECAL CONTINENCE AND QUALITY OF LIFE, AND TO DETERMINE IF ADMINISTRATION OF ANTEGRADE ENEMA SOLUTION THROUGH AN APPENDICOSTOMY/CECOSTOMY AFFECTS GUT MICROBIOTA AND GUT IMMUNE FUNCTION. THIS STUDY WILL INVOLVE CHILDREN AGES 3 TO 12 YEARS RECRUITED FROM SUBSPECIALTY CLINICS AT NEMOURS CHILDREN'S SPECIALTY CARE AND THE PEDIATRIC SPINAL DEFECTS CLINIC IN JACKSONVILLE, FLORIDA. CHILDREN WILL BE SELECTED BY PURPOSIVE SAMPLING AND WILL INCLUDE THOSE WHO ARE SCHEDULED TO HAVE AN ACE STOMA AND WILL REQUIRE REGULAR ANTEGRADE ENEMA ADMINISTRATION TO MAINTAIN CONTINENCE. EXCLUDED WILL BE CHILDREN WITH PREEXISTING ELECTROLYTE IMBALANCE, CHRONIC HIGH RECTAL TONE, QUADRIPLÉGIA, RENAL OR CARDIAC DISEASE, AND THOSE WHO CANNOT COMMUNICATE OR HAVE SIGNIFICANT COGNITIVE DELAY THAT WOULD INTERFERE WITH THEIR ABILITY TO FULLY PARTICIPATE IN THE STUDY. PARENTS MUST HAVE ENGLISH LANGUAGE COMPETENCY AND BE WILLING AND ABLE TO PARTICIPATE IN ADMINISTRATION OR OVERSIGHT OF THE FLUSHING REGIMEN AND DATA COLLECTION FOR A MINIMUM OF 20 CONSECUTIVE WEEKS. SINGLE SUBJECTS WITHIN AND BETWEEN SUBJECTS DATA WILL BE ANALYZED USING VISUAL ANALYSIS. THE CROSS- OVER DATA WILL BE ANALYZED USING INFERENTIAL STATISTICS EMPLOYING TWO-TAILED HYPOTHESIS TESTING. FINDINGS FROM THIS STUDY WILL SERVE TO PROVIDE A COMPARATIVE ANALYSIS OF TWO DIFFERENT REGIMENS THAT WILL SERVE AS A STARTING POINT TO GUIDE PRACTICE AND PROVIDE A FOUNDATION FOR ADDITIONAL PROSPECTIVE, RANDOMIZED, CONTROLLED TRIALS.

Background

State the Problem

Fecal incontinence in children past the expected time of toilet training has been associated with increased anxiety and depression, more social problems, worse school performance, and an increased incidence of abuse and bullying (Kaugars et al., 2010; Youssef, Langseder, Verga, Mones, & Rosh, 2005). It is particularly difficult to manage fecal incontinence using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumors, megarectum, or slow transit constipation. The Malone, or antegrade continence enema (ACE), procedure was popularized over 20 years ago as a means of helping children with intractable fecal incontinence attain stool continence. A catheterizable stoma from the abdominal wall into the cecum is constructed using the appendix, a tubularized portion of the bowel, or a low profile button device. The stoma allows for antegrade administration of enema solution into the colon.

A large body of literature demonstrates ACE therapy can be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in quality of life. However, findings regarding effectiveness are highly variable. This variability may be due to what is used to flush. No prospective trials compare the effectiveness and adverse effects of different flushing regimens. No studies evaluate the effects of appendicectomy or cecostomy flush on gut microbiota.

Justification for Conducting the Study

The proposed research is significant in that it is the first prospective study to compare the effectiveness and tolerability of two commonly used ACE flushing regimens and the first study to explore ACE flushing impact on the gut microbiome. Findings from this study have the potential to provide both clinical and biological insights into ACE administration safety and start to build a foundation of scientific evidence that could increase ACE effectiveness rates from 78% toward 98% or better and provide a foundation for additional prospective, randomized, controlled trials.

Synopsis of the Literature

Studies Addressing the Effectiveness of ACE Therapy in Promoting Continence

A number of retrospective studies evaluate overall continence rates in children following an ACE. Findings from these studies are highly variable. Short term reported success rates range from 79 to 98% with long term outcomes ranging from 41% abandonment rate at 5 years to a mean time to first relapse lasting as long as 121.9 +/- 29.7 months (Aspirot, Fernandez, Di Lorenzo, Skaggs, & Mousa, 2009; Bani-Hani, Cain, King, & Rink, 2008; Becmeur et al., 2008; Curry, Osborne, & Malone, 1999; Dey et al., 2003; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Marshall, Hutson, Anticich, & Stanton, 2001; Mousa et al., 2006; Ok & Kurzrock, 2011; Siddiqui, Fishman, Bauer, & Nurko, 2011; Thomas et al., 2006; Yardley et al., 2009).

A number of prospective trials demonstrate significant improvement in somatic functions, psychosocial functioning, and quality of life (QOL) after an ACE procedure (Aksnes et al., 2002; Mousa et al., 2006; Tiryaki, Ergun, Celik, Ulman, & Avanoğlu, 2010). Many different prescribed ACE flushing regimens are used in practice, including licorice root, mineral oil, treacle/milk mix, tap water, normal saline, polyethylene glycol solution, phosphate soda solution, bisacodyl, and USP liquid glycerin (Bani-Hani et al., 2008; Marshall et al., 2001; Youssef, Barksdale, Griffiths, Flores, & Di Lorenzo, 2002). A single prospective efficacy study utilized colonic manometry to compare the motor response of three stimuli (meal, antegrade saline

infusion, and antegrade bisacodyl administration) on the number of high-amplitude contractions and motility index in 13 pediatric patients. Findings in this study demonstrated that there was no significant difference in parameters after ingestion of a meal or saline infusion at 10 to 20 mg/kg with a maximum volume of 700 mL. Bisacodyl at a dose of 0.2 mg/kg with a maximal dose of 10 mg significantly increased the motility index and high-amplitude propagated contractions when compared to meal or saline (Gomez, Mousa, Liem, Hayes, & Di Lorenzo, 2010). Bani-Hani, Cain, King, and Rink (2008) identified timing of accidents as the most important factor in troubleshooting flushing regimen failure. Siddiqui et al. (2011) postulated an inverse relationship between increased commode time and long term ACE adherence.

Identifying a successful flushing regimen is determined by individual clinician preference and often requires multiple attempts before success is achieved. There are no prospective studies comparing the effectiveness of type, dose/volume, or frequency of different flushing regimens in preventing incontinence to inform practice.

Studies Addressing Side Effects Associated with ACE Therapy

Several case reports detail morbidity and mortality associated with a particular flushing solution, including hypocalcemia and hyperphosphatemia, following retrograde administration of phosphate enemas in children (Helikson, Parham, & Tobias, 1997; Ismail, Al-Mutairi, & Al-Anzy, 2000), hypernatremia following retrograde administration of hypertonic saline (Schreiber & Stone, 1999), and water intoxication following retrograde enema therapy using tap water (Chertow & Brady, 1994). Solution composition, volume, retention time, and underlying electrolyte imbalances are all factors that increase morbidity and mortality (Yerkes et al., 2001). Several case series describe a variety of side effects of flushing regimens, including pain with stomal intubation, nausea, vomiting, abdominal cramping, sweating, dizziness, and pallor (Dey et al., 2003; King et al., 2005; Bani-Hani et al., 2008).

A single retrospective study evaluated the safety of a specific flushing solution. Yerkes et al. (2001) evaluated the safety of tap water antegrade flush in 71 patients using serum electrolytes obtained pre- and post-operatively as the dependent variable. The timing and place of the laboratory evaluation varied widely due to the retrospective nature of their study. The ACE flush was administered at home every day or every other day at volumes ranging from 300 to 1,000 mL. Clinically insignificant electrolyte abnormalities attributed to the flush included minor deviations in serum sodium or serum chloride in 18 patients. More significant hyperchloremia (107 to 113 mmol/L, with upper limits set at 105 mmol/L) was noted in 12 patients. Significant hypernatremia and hyperchloremia was noted in a single subject who used softened water to flush, which corrected when the flush was changed to untreated water. There have been no prospective trials detailing and comparing adverse effects associated with different antegrade flushing regimens.

Studies Addressing Gut Microbiota

The catheterizable stoma used for antegrade administration of enema solution is frequently made by bringing the appendix out through the abdominal wall or by placing a button into the cecum. The appendix and cecum have significant amounts of gut-associated lymphoid tissue (GALT), have high concentrations of microbiota, and serve an essential immune function (Penders, Stobberingh, Van den Brandt, & Thijs, 2007). The appendix has higher concentrations of microbial biofilms compared to other areas of the colon, serves as a safe-house for symbiotic gut flora, and functions to preserve gut microbiota through re-inoculation with normal flora

following gastrointestinal infections (Bazar, Lee, & Yun, 2004; Bollinger, Barbas, Bush, Lin, & Parker, 2007; Gebbers & Laissue, 2004; Kawanishi, 1987; Smith et al., 2009).

The human gut contains 10^{14} bacteria (Jia, Li, Zhao & Nicholson, 2008). Bacterial composition varies along the bowel axis, with further differentiation of luminal or adherent microcolonies that lead to development of biofilms. Factors that influence microbial composition include pH, transit time, bile acids, pancreatic enzymes, mucus composition, nutrient consumption, medication, environment, bacterial adhesion capacity, and metabolic capacity. The most important function of the gut microbiome is colonization resistance, which is accomplished through competition for nutrients and secretion of bacteriocins (Penders et al., 2007). Microbiota function to degrade dietary substances and enhance digestive efficiency while providing nutrients to the microbes themselves. These microbes are essential for host physiology but, in turn, pose a threat of opportunistic invasion by resident bacteria with resulting pathologies.

The immune system maintains a delicate homeostatic and symbiotic balance protecting host-microbial ecosystem dualism through the mechanism of stratification and compartmentalization. Stratification minimizes direct contact between the gut microbiota and the intestinal epithelial surface. Compartmentalization utilizes anatomic adaptation to confine bacteria that breach the mucosal surface to limit systemic immune system exposure. Immune system response to microbiota plays an important role in host vulnerability to disease. Host-microbial symbiosis and dysbiosis are extraordinarily complex phenomena. The immune system controls the composition, diversity, and location of gut microbiota while the microbiota has a profound effect on lymphoid tissue formation and immune system development (Forchielli & Walker, 2005). Microbiota have been shown to have protective properties against autoimmune disease and, conversely, can cause inflammation and metabolic dysregulation in an immune compromised host (Hooper, Littman, & Macpherson, 2012; Nicholson et al., 2012).

Disruptions in the gut microbiota due to diet, including infant feeding regimens, microbial inoculations, and antibiotics, can alter mucosal immunity and mechanisms involved in regulating immune tolerance outside the GI tract (Noverr & Huffnagle, 2004). Microbiome composition imbalance has been associated with diverse disorders including cancer, inflammatory bowel disease (IBD), atopy, asthma, obesity, and autism (Barker, 2012). The appendix is a secondary lymphoid organ that is an important constituent of the mucosa-associated lymphoid tissue system; it has a pronounced function in children. Neonatal appendectomy in rabbits impaired mucosal immunity (Dasso & Howell, 1997). Long-term effects of appendectomy include moderate immune function changes in part due to a decrease in immunoglobulin production, particularly IgA; increased risk of Crohn's disease; and a moderately increased risk of acute myocardial infarction (Anderson, Olaison, Tysk, & Ekblom, 2003; Andreu-Ballester et al., 2007; Janszky, Mukamal, Dalman, Hammar, & Ahnve, 2011). ACE therapy administered through the appendix or into the cecum has the potential to disrupt the gut microbial ecosystem causing dysbiosis and immune dysfunction. Effects of appendiceal or cecal administration of ACE on colonic microbiome and mucosal immunity has not been investigated.

Synopsis of previous animal and human studies involving USP Glycerin

There are no animal studies evaluating instillation of glycerin into the cecum. A composite of animal studies involving alternative routes of glycerin administration are included in Appendix A. There are no prospective human trials evaluating instillation of glycerin into the cecum. A

composite of human studies involving alternative routes of glycerin administration is included in Appendix B.

The Proposed Research as a logical Progression Toward Solving the Problem

Attaining continence is a highly socially significant issue for any child but particularly difficult to attain using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumors, megarectum, or slow transit constipation. ACE therapy has been shown to be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in independence and quality of life. However, research findings regarding short and long-term effectiveness are variable (Aspirot, Fernandez, Di Lorenzo, Skaggs, & Mousa, 2009; Bani-Hani, Cain, King, & Rink, 2008; Becmeur et al., 2008; Curry, Osborne, & Malone, 1999; Dey et al., 2003; King, Sutcliffe, Southwell, Chait, & Hutson, 2005; Marshall, Hutson, Anticich, & Stanton, 2001; Mousa et al., 2006; Ok & Kurzrock, 2011; Siddiqui, Fishman, Bauer, & Nurko, 2011; Thomas et al., 2006; Yardley et al., 2009). This variability may be due to what is used to flush. There are no prospective comparative studies documenting effectiveness rate, frequency and severity of side effects, or the time necessary to complete the flushing procedure. Sitting on the commode for one hour versus 20 minutes represents a major difference in age-appropriate expectations for a 6-year-old child. In a child, procedural time and side effects may be inversely related to adherence and negatively impact effectiveness of the procedure. In addition, there is no information on the effects of ACE flush on bowel health and the microbiome. Currently, the flushing regimen for each child is based on clinician preference and achieving continence can be a lengthy process of trial and error. This study is the next step in helping children using ACE flushing regimens attain continence while minimizing side effects and procedural time. It has the potential to identify effective dose and frequency and compare side effects of commonly used flushing solutions. Promoting continence at an earlier stage in therapy will decrease costs associated with ineffective trials and additional time spent in protective garments. In addition, this study makes beginning attempts to evaluate the effects of ACE therapy on gut microbiota and colonic health

Specific Aims

Aims

The purpose of this prospective pilot study is to compare two distinct flushing regimens, one high volume saline flush and one low volume USP glycerin flush, in the immediate postoperative period in children requiring ACE therapy. Findings from this study will provide a comparative analysis of these two regimens that will serve as a starting point to guide practice and serve as a foundation for additional prospective, randomized, controlled trials. The aims of this study are to (1) identify the minimal administration frequency and titration time to reaching effective dose; (2) compare which solution at an optimum dose is delivered in the least amount of time, with fewer side effects, while promoting the higher degree of fecal continence and quality of life; and (3) determine if administration of antegrade enema solution through an appendicostomy/cecostomy affects gut microbiota and mucosal health.

Hypotheses

Null Hypotheses for Aim 1

- 1. There will be no differences in frequency of administration necessary to gain and maintain continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (**primary aim**).
- 2. There will be no differences in titration time to reach an effective dose between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypotheses for Aim 2

- 1. There will be no difference in degree of continence between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (**primary aim**).
- 2. There will be no difference in procedural time between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
- 3. There will be no difference in side effects between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).
- 4. There will be no difference in parent/patient satisfaction as measured by the Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida (FIC QOL) between two different ACE flushing regimens using normal saline and normal saline with USP glycerin in children requiring antegrade continence therapy (secondary aim).

Null Hypothesis for Aim 3

- 1. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on colonic microbiota in children requiring antegrade continence therapy.
- 2. There will be no difference in the effect of two different ACE flushing regimens using normal saline and normal saline with USP glycerin on gut mucosal health in children requiring antegrade continence therapy

Research Plan

This prospective study will utilize a repeated measures, single subjects alternating treatments A-B-C-B'-C'-B1' withdrawal design in which all subjects are tested under all conditions and each subject acts as his or her own control (Gast, 2010; Janosky, Leininger, Hoerger, & Libkuman, 2009; Kazdin, 2011; Portney & Watkins, 2009). A within subjects cross-over design is embedded in the B'-C'-B1' treatment comparison phase of the study (Chow & Brady, 1994; Janosky, Leininger, Hoerger, & Libkuman, 2009; Jones & Kenward, 2003). The treatment will be replicated across twelve subjects randomized to six subjects per group. Randomization will be open label. The patient and investigator cannot be blinded to flushing regimens contents due to dosing considerations. The high volume flushing regimen will be comprised of normal saline alone. The low volume regimen will be comprised of USP glycerin with a small volume of normal saline used as diluent. Volume and frequency of administration will be structured to find the lowest dosing of each regimen sufficient to maintain continence. Baseline data A will serve as the control and will be obtained pre-operatively. Ethics prohibit return to a no-treatment

baseline phase as this would result in multiple daily episodes of fecal incontinence. The first B - C phase of the study will evaluate dose-response relationship and will be used to identify the minimum dosing volume and frequency delivered in the least amount of time with fewest side effects while promoting fecal continence of ACE administration for normal saline and normal saline with USP glycerin. When the optimal dose has been identified, the child will continue on that dose for 2 weeks to insure treatment stability and effectiveness. If continence cannot be achieved within the dosing guidelines, the child will be placed on alternative therapy but will not progress to the maintenance phase of the study. To prevent statistical bias from subject loss due to treatment failure, each child will be randomized to a second treatment sequence once they have achieved continence on optimal dosing with minimal side effects. The second phase B'-C'-B1' of the study will compare the effectiveness of the two regimens at optimal dose and administration frequency (see Study Schedule, p. 18).

Inclusion and Exclusion Criteria

This study will involve twelve children ages 3 to 12 years recruited from subspecialty clinics at Nemours Children's Specialty Care and the Pediatric Spinal Defects Clinic in Jacksonville, Florida. Children will be selected by purposive sampling and will include those who are scheduled to have an ACE stoma and will require regular antegrade enema administration to maintain continence. Excluded will be children with preexisting electrolyte imbalance, chronic high rectal tone, quadriplegia, renal or cardiac disease, or those who require prophylactic antibiotics, cannot communicate, or have significant cognitive delay that would interfere with their ability to fully participate in the study. Parents must have English language competency and be willing and able to participate in administration or oversight of the flushing regimen and data collection for a minimum of 20 consecutive weeks.

Step by Step Procedure

Once parental consent and child assent have been obtained, the child will be randomly assigned to either the saline or USP glycerin protocol. The family will be provided with a notebook containing the procedural flushing guidelines, data collection sheets, a stop watch, and flushing solution. Baseline data A will be collected daily for a minimum of two weeks prior to surgery, including frequency and volume of episodes of fecal soiling, and frequency and severity of abdominal pain. Blood samples for electrolyte and stool for calprotectin and microbiota will be obtained in the immediate preoperative period. Initial stool specimens for gut microbiota analysis will be collected prior to initiation of any pre-operative bowel prep. Per standard of care, a nurse from Nemours Pediatric Surgery will meet with the parent and child in the immediate postoperative period after surgical clearance has been obtained for flush initiation. During that time, the nurse will review the flush protocol in detail, including materials and procedures. The child will receive the first antegrade infusion during that in-patient stay. The nurse will be available if needed for each subsequent flush during the hospitalization. This will allow the family to gain competency in a controlled environment and familiarize themselves with the prescribed protocol and procedures prior to transition to the home setting. Data will not be collected during the hospital admission since learning the flushing procedure and post-operative abdominal discomfort will pose confounds to measures of flush time and side effects. The investigator will make a home visit for assistance in administration of the first flush following discharge. During that visit, flushing and data collection procedures will be reviewed. To reiterate, the first B - C phase of the study will evaluate dose-response relationship and will be used to identify the optimal dose and frequency of ACE administration for normal saline and normal saline with USP glycerin. When the optimal dose has been identified for each flush

solution, the child will continue on that dose for 2 weeks to insure treatment stability and effectiveness. If continence cannot be achieved within the dosing guidelines, the child will be placed on the alternative regimen but will not advance to the maintenance phase of the study. After completion of the B-C phase, once they have achieved continence on optimal dosing with minimal side effects, the child will be randomized to a second treatment sequence. The second phase B'-C'-B1' of the study will compare the effectiveness of the two regimens at optimal dose and administration frequency. After the completion of each phase, the child will come in for a clinic visit. During that visit, the laboratory evaluation will be completed and the new flushing regimen will be reviewed. Documentation will be completed by the parent and child on a data-collection form and reviewed weekly with the study coordinator to encourage completion of all relevant data. The investigator will keep a research log documenting and detailing any event that may cause a change in level, stability, or trend of the dependent variable not related to the intervention, for example treatment with antibiotics or an intercurrent illness. The number of episodes of fecal soiling per week will exclude accidents caused by viral, bacterial, or drug-induced gastroenteritis; these will be recorded and analyzed as confounds (Portney & Watkins, 2009).

Interobserver Agreement and Procedural Fidelity

The reliability or accuracy and consistency of measurements will be verified using interobserver agreement (IOA). Gross method will be used to calculate IOA comparing investigator and parent/child concurrent observations including flush time, procedural time, and number/level of soiling. IOA will be calculated by dividing the smaller number by the larger number and multiplying by 100. If there is a significant discrepancy in observational accuracy, as demonstrated by a calculated IOA below 80%, additional observer training will be provided until the calculated IOA is 80% or higher (Gast, 2010). Procedural reliability will be ascertained for each procedural variable to assure the intervention is being implemented as described in the methods section of the proposal. Procedural fidelity will be calculated by dividing the number of observed behaviors by the number of planned behaviors and dividing by 100 (Gast, 2010). IOA and procedural fidelity for antegrade infusion and data collection techniques conducted by the parent will be checked by the investigator and documented at each in-patient visit. A home visit will be scheduled by the investigator for initial home infusion. IOA and procedural fidelity will be checked during the first home visit and subsequent clinic visits scheduled with each phase change.

Measures

Each subject's baseline data will be obtained preoperatively. Data at baseline (A) will include continence data, serum electrolytes, and immune markers. The initial stool sample for microbiota analysis will be obtained pre-operatively prior to initiation of any bowel prep. Dependent variables at baseline will include: (a) number of episodes of fecal soiling. In addition, fecal soiling will be scored based on frequency and volume of accidents (0 = no soiling, 1 = a smear, 2 = one moderate volume accident that would be insufficient in volume to be visible through clothing if the child wears regular underwear, 3 = any large volume accident that would be visible through clothing if the child wears regular underwear; fecal soiling score is detailed in Table 1, (b) frequency, and severity of abdominal pain recorded daily and measured using the Wong-Baker Faces Pain Rating Scale (WBFPRS) as the age-appropriate visual analog scale, (c) serum electrolytes, (d) stool for calprotectin, (e) quality of life measured by the Fecal Incontinence and Constipation Quality of Life Measure in Children with Spinal Bifida (FIC QOL), and (f) stool samples obtained and stored for later microbial DNA analysis using

16SrRNA molecular techniques to identify 16SrRNA gene sequence to identify and quantify phylogenetic groups (Penders et al., 2007).

Dependent variables obtained post-operatively following initiation of cecosotmy/ appendicostomy flush will include: (a) administration time in minutes per flush, (b) total procedural time from start of flush to completion of colonic emptying in minutes per flush, (c) volume of solution in mL/dose, (d) number of episodes of fecal soiling. In addition, fecal soiling will be scored based on frequency and volume of accidents (0 = no soiling, 1 = smear, 2 = moderate volume accident that would be insufficient in volume to be visible through clothing if the child wears regular underwear, 3 = any large volume accident that would be visible through clothing if the child wears regular underwear, (e) frequency and severity of abdominal pain recorded daily and measured using the WBFPRS as the age-appropriate visual analog scale, (f) number and frequency of side effects per week with severity of side effects measured using the WBFPRS as the age-appropriate visual analog scale, (g) serum electrolytes, (h) stool for calprotectin, (i) quality of life measured by the FIC QOL, and (j) stool samples obtained and stored for later microbial DNA analysis using 16SrRNA molecular techniques to identify 16SrRNA gene sequence to identify and quantify phylogenetic groups (Penders et al., 2007). Dependent variables, including type of sample or instrument, sample characteristics, and measurement and data level, are explicated in Table 2

Table 1- Level of Soiling

0	No soiling
1*	smear
2*	moderate volume accident per week that would be insufficient in volume to be visible through clothing if the child wears regular underwear
3*	any large volume accident that would be visible through clothing if the child wears regular underwear

*Change dose with any soiling \geq level 1

Table 2 - Dependent Variables

Sample/Instrument	Variable	Measurement	Data Level
Blood	BMP	Electrolyte balance	Ratio
Stool	Calprotectin	Mucosal inflammation	Ratio
Stool	Colonic microbiome	Metagenomic profiling 16SrRNA	Ratio
FIC QOL	Parent/child quality of life	Symptoms rating scale	Ordinal
WBFPRS	Abdominal pain	Symptom rating scale	Ordinal
WBFPRS	Procedural side effects	Symptom rating scale	Ordinal
Stop Watch	Infusion time	Minutes	Ratio
Stop Watch	Procedural time	Minutes	Ratio

Administration time in minutes per flush will be defined as the time at which the tubing connected to the bag or syringe holding the flush solution is unclamped and the cecosotmy fluid starts to infuse into the patient to the time the infusion is completed (no more fluid left in the

bag/syringe or tubing). The total procedural time is defined as the time the flush starts to infuse into the subject and ends following passage of stool when the child has sat on the commode for 5 minutes with no additional stool passage. Both administration and total procedural times will be measured using duration per occurrence direct observational recording completed by the parent or child. Volume and dose will be recorded with each flush in mL/dose. Accidents will be defined as non-toilet elimination, which will be tracked and tallied as the number of pairs of underwear soiled with stool with documentation the severity of the accident and the estimated time of each accident using event recording. Dependent variables will be measured and recorded by the parent using a data collection sheet specifically designed for this study.

Side effects will be measured using the Wong-Baker FACES Pain Rating Scale (WBFPRS); see Appendix C). The WBFPRS has undergone extensive testing, is preferred by children, and has well established psychometrics in the pediatric population (Tomlinson, von Baeyer, Stinson, & Sung, 2010; Wong & Baker, 1988). The scale ranges from 0 (very happy without pain) to 10 (the worse pain imaginable). Each pain level is associated with a facial expression. The child is asked to choose the face that best describes his/her level of discomfort. The WBFPRS will be used to evaluate the presence and severity of flush side effects, including abdominal cramping, nausea, vomiting, sweating, dizziness, and pallor. The parent will call if the child is having flushing regimen-associated accidents or discomfort greater than a 4 on the WBFPRS. Documentation of side effect severity will be completed by the parent and child on a data-collection form and reviewed weekly with the study coordinator to encourage completion of all relevant data.

The investigator will keep a research log documenting and detailing any event that may cause a change in level, stability, or trend of dependent variables not related to the intervention, for example treatment with antibiotics or an intercurrent illness. Those episodes of fecal soiling will exclude accidents caused by viral, bacterial, or drug-induced gastroenteritis; these will be recorded and analyzed as confounds (Portney & Watkins, 2009).

The Fecal Incontinence and Constipation Quality of Life Measure in Children with Spinal Bifida (FIC QOL; see Appendix D) will be used to assess child and parental perception of quality of life impact and as an indirect measure of social validity (Nanigian et al., 2008). The tool will be administered preoperatively during the baseline period and at the end of each flushing regimen in the comparative phase of the study. The FIC QOL is a 51 item questionnaire with established validity and reliability in families of children with spina bifida who are incontinent for stool. This instrument measures aspects of daily living significantly impacted by fecal incontinence. Of the 51 items, four address subject and family demographics. The remaining 47 items are divided into seven groupings that include bowel program, diet, symptoms, travel and socialization, family relationships, caregiver support and emotional impact, and financial impact (Nanigian et al., 2008; Ok & Kurzrock, 2011). In addition to the FIC QOL, a simple qualitative question will be directed to the children at the end of the study to ascertain which flushing regimen they prefer and why.

For the high volume flush, the normal saline will be infused using a 1,000 mL enteral feeding bag with drip chamber and roller clamp. During the infusion, the bag will be hung from a hook located 6 feet above the floor on a wall to the side of or behind the commode. Tubing from the enteral feeding bag will be hooked to the low profile device access tubing and primed with the high volume flush to remove all air in the tube prior to hooking access tubing to the low profile device and infusing the solution. Step by step procedural directions for the parents are located in Appendix E. The instructions will be used to reinforce parent teaching and will serve as a check-off list to document procedural integrity which will be evaluated during the initial training

session, each additional hospital visit, the initial home visit, and with each subsequent home visit made with every change in phase.

For the low volume flushing regimen, an 8 ounce plastic bottle with a screw top will be used to mix USP glycerin with normal saline. The mixed solution will then be poured into a 60 mL catheter-tipped syringe attached to the low profile device access tubing. The tubing will be primed prior to hooking it to the low profile device. The child or parent will initially hold the syringe containing flush solution at approximately the child's shoulder level while the flush infuses. All components of the flush will be at room temperature prior to mixing and infusing the solution. Step by step procedural directions for the parents are listed in Appendix F.

For both the high and low volume flush, a stop watch will be used to measure the time it takes to complete the procedure. The stop watch will be started at the beginning of the infusion, the time in minutes and seconds from start to completion of the infusion will be documented. The stop watch will be stopped when the child feels the flush has been effective and he/she has not passed any additional stool for at least 5 minutes. Total time will be documented on the log. Following the flush, the tubing and bag or syringe will be washed in warm soapy water, rinsed, and allowed to air dry.

Recruitment and Consent

Subjects will be recruited from the population of children who have failed to achieve continence for stool using conservative means; are being followed clinically in Nemours Gastroenterology, Urology, Continence, or Surgery clinics or the community-based Pediatric Spinal Defects clinic; and are scheduled for appendicostomy/cecosotomy. This study confers no greater than minimal risk as categorized by the National Institutes of Health (National Institutes of Health [NIH], 1998). However, it involves vulnerable subjects and will require full Institutional Review Board (IRB) approval with legal guardian informed consent by at least one parent and child assent for children age 7 and above (Knox & Burkhart, 2007; Pieper, 2008). The study has been approved by the Nemours CRRC, but will require approval of the Nemours IRB.

Targeted Study Population and Research Setting

This study will involve twelve children ages 3 to 12 years recruited from subspecialty clinics at Nemours Children's Subspecialty Care and the Pediatric Spinal Defects Clinic in Jacksonville, Florida. Children will be selected by purposive sampling and will include those who are scheduled to have an ACE stoma and will require regular antegrade enema administration to maintain continence.

Identification and Recruitment of Subjects

Subjects will be identified during routine clinic visits in pediatric gastroenterology, general surgery, continence, or spinal defects clinic. When the determination is made that a child is going to have an appendicostomy/cecosotomy and meets study inclusion criteria, the clinician seeing the family in clinic will apprise them of the study. If they are interested in learning more, Kim Jarczyk will be notified and will meet with the family or contact the family by phone to explain the study in detail.

Protection of PHI

Consents and patient-completed data sheets will be stored in a locked file box housed within a locked cabinet in an office that remains locked when it is not in use. The data will be entered in REDCap and identifiable data will be accessible only to Kimberly Jarczyk. Dr. Shuster, Dr. Pieper, and Safety and Monitoring Board members will have access de-identified data. A summary of the patient's response to treatment that requires dosing adjustment will be

documented as a staff note in the patient's electronic medical record. A summary of each child's progress on therapy will be included as a part of the notes associated with each clinic visit following each phase completion. Any data used in reporting results will be de-identified.

Consent Process

If the family would like to participate, Kimberly Jarczyk or another of the investigators will attend the family while they are in clinic to review the study protocol and consent. The family will be provided with a copy of the consent to review. All questions will be answered. If the family is still interested in participation, consent and assent for children seven years and older will be obtained prior to enrollment in the study.

Subject Number

In a single subjects repeated measures design, the strength of the ability to determine treatment effects requires a stable baseline and is a function of the number of baseline and intervention data points (Cook & Campbell, 1979). This study design features a prolonged pre-operative baseline assessment and a minimum of 6 weeks of daily measurement for each treatment variable at optimal dosing, meeting criteria for sufficient power analysis. For the cross over design, there is insufficient existing data to conduct a power analysis for sample size estimation. A purpose of this pilot study is to collect data that will allow for power analysis and optimal sample size determination.

Description of Drugs

Treatment, Intervention and Observation High Volume Regimen

The high volume regimen (B) consists of a normal saline flush at a starting dose of 10mL/kg infused every other day and be adjusted until stability of target outcomes is achieved. The overall goal is to maximize dose at minimum frequency with minimal side effects. If the child is continent on an every other day dose, the administration frequency will be dropped to every third day. If they have breakthrough incontinence on an every third day dosing schedule, the frequency will be increased back to every other day and if needed to every day. At any point the subject is having episodes of fecal, the dosing strategy will be increased by 5-10 mL/kg-increments starting at an administration frequency of every other day with a subsequent increase in frequency, if needed, so as not to exceed 20 mL/kg and a maximum dose of 500mL a day for children under 5 years of age and 1000 mL for children over 5 years of age administered daily. If the child does not attain continence on the maximum dose, he/she will be placed on the alternative flush but will not progress to the maintenance phase of the study. If the child is having side effects greater than WBFPRS level 4 at the starting dose of 10mL/kg, flush volume will be incrementally decreased as needed by 2.5 mL/kg to the lowest dose of 5 mL/kg daily. The goal is to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize effects results in episodes of fecal soiling greater than one smear per week or the child continues to have side effects greater than WBFPRS level 4 at the lowest dose of administration, the child will be placed on the alternate flushing regimen but will not progress to the maintenance phase of the study. The decision tree for dose adjustment of Normal Saline is detailed in Tables 3 and 4.

Treatment, Intervention and Observation Low Volume Regimen

The low volume regimen (C) will consist of USP glycerin diluted in normal saline prior to antegrade instillation through the low profile device. The child will start on an every other day dose of 20 mL of USP glycerin and >20 mL of saline (used as diluent at a dose sufficient to allow the solution to easily infuse through the ACE access tubing) and be adjusted until stability of target outcome is achieved. The families will record the amount of saline used as diluent. The

overall goal is to maximize dose at minimum frequency with minimal side effects. If the child is continent on an every other day dose, the administration frequency will be dropped to every third day. If they have breakthrough incontinence on an every third day dosing schedule, the frequency will be increased back to every other day and if needed to every day. At any point the child is having episodes of fecal soiling, the volume of USP glycerin will be increased in 5 mL increments starting at an administration frequency of every other day with subsequent increase in frequency, if needed, so as not to exceed 40 mL of USP glycerin administered daily. If the child does not attain continence on the maximum dose of USP glycerin, he/she will be placed on the alternative flushing regimen but will not advance to the maintenance phase of the study. If the subject is having side effects greater than WBFPRS level 4 at the starting dose of 20mL of USP glycerin, the volume of the USP glycerin will be decreased as needed by 5 mL increments until the child's symptoms are less than or equal to WBFPRS level 4 or the lowest dose of 5 mL daily is reached. The goal is to find the lowest effective dose and flushing frequency with minimal side effects. If the dose necessary to minimize effects results in episodes of fecal soiling greater than one smear per week or the child continues to have side effects greater than WBFPRS level 4 at the lowest dose of administration, the child will be placed on the alternative flushing regimen but will not advance to the maintenance phase of the study. The decision tree for dose adjustment of USP glycerin is detailed in Tables 3 and Table 4.

Table 3 - Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Maintain Continence in the Absence of Side Effects

*Change dose with any soiling \geq level 1 as defined in Table 1

Normal Saline (High volume regimen with maximum dose for less than 5 and over 5 yrs.)	USP Glycerin + Normal Saline as Diluent (Low volume regimen - Max dose for children 5 and under is 30 mL + > 30 mL qd)
B1 = 10mL/kg maximum dose 500 or 1000 mL qod	C1 = 20 mL + > 20 mL qod
B2 = 10mL/kg maximum dose 500 or 1000 mL q3d	C2 = 20 mL + > 20 mL q3d
B3 = 10mL/kg maximum dose 500 or 1000 mL qd	C3 = 20 mL + > 20 mL qd
B4 = 15mL/kg maximum dose 500 or 1000 mL qod	C4 = 25 mL + > 30 mL qod
B5 = 15mL/kg maximum dose 500 or 1000 mL q3d	C5 = 25 mL + > 30 mL q3d
B6 = 15mL/kg maximum dose 500 or 1000 mL qd	C6 = 25 mL + > 30 mL qd
	C7 = 30 mL + > 40 mL qod
	C8 = 30 mL + > 40 mL q3d
	C9 = 30 mL + > 40 mL qd
	C10 = 35 mL + > 50 mL qod
	C11 = 35 mL + > 50 mL q3d
	C12 = 35 mL + > 50 mL qd

Table 4 - Dosing Strategy to Determine Minimum Volume and Frequency Necessary to Minimize Side Effects and Maintain Continence

Normal Saline* (High volume regimen with maximum dose for less than 5 and over 5 yrs.)	USP Glycerin + NS as Diluent* (Low volume regimen)
B1 = 10 mL/kg maximum dose 500 or 1000 mL qod	C1 = 20 mL + > 20 mL qod
B7 = 7.5 mL/kg maximum dose 500 or 1000 mL qod	C10 = 15 mL + > 15 mL qod
B8 = 7.5 mL/kg maximum dose 500 or 1000 mL q3d	C11 = 15 mL + > 15 mL q3d
B9 = 7.5 mL/kg maximum dose 500 or 1000 mL qd	C12 = 15 mL + > 15 mL qd
B10 = 5.0 mL/kg maximum dose 500 or 1000 mL qod	C13 = 10 mL + > 10 mL qod
B11 = 5.0 mL/kg maximum dose 500 or 1000 mL q3d	C14 = 10 mL + > 10 mL q3d
B12 = 5.0 mL/kg maximum dose 500 or 1000 mL qd	C15 = 10 mL + > 10 mL qd
	C16 = 5 mL + > 5 mL qod.
	C17 = 5 mL + > 5 mL q3d
	C18 = 5 mL + > 5 mL qd

*Change dose with any side effects > 4 on the WBFPRS or soiling \geq Level 1 as defined in Table 1

Optimal Dose Regimen

Once the optimal dose has been established, the child will be maintained on that dose and frequency for at least 2 weeks or until stability in dependent measures without significant variability or trend is achieved. The child will be scheduled to come into the clinic for a visit once the above criteria have been met, at which time labs will be drawn and a stool sample collected. The volume of solution in mL/ dose will be recorded for each treatment phase.

Comparative Phase

Following completion of the dose-response phase, the comparative portion of the study will begin by administering either the established effective dose of normal saline (B') or the established effective dose of USP glycerin (C'). Patients will be randomized for a second time to either a B'-C'-B1' or a C'-B'-C1' sequence. The last flush in the dose response sequence will be withdrawn and the initial flush in the comparative treatment phase will be introduced the following day at the previously established minimum effective dose and frequency. Children will remain on treatment for 4 weeks, at which point the treatment will be withdrawn. Children will then be placed on the next treatment in the sequence at the pre-established effective dose and frequency for 4 weeks. The second flush will then be withdrawn and the initial flush in the sequence will be reintroduced for an additional 2 weeks (B1' or C1'). At the conclusion of the study, the child will be placed on the flushing regimen of his/her choice.

Procurement, Storage, and Management of Research Pharmaceutical Products

Both normal saline (NDC# 00338-0049-04, 0.9% Sodium Chloride Irrigation, USP 500mL, Baxter brand) and USP glycerin (HM Glycerin USP, 16 ounces, NDC# is 62011-0115-01, HealthMark brand) are non-controlled and are available over the counter. Both solutions will be purchased from the Pavilion Pharmacy in the Pavilion Building at Baptist Medical Center on 836 Prudential Drive, STE 110 in Jacksonville, Florida. Participants will not be charged for either solution. The normal saline and USP glycerin will be dispensed in their original containers. Because the route of administration constitutes an off-label use of both medications, the containers will be labeled using regulation compliant medication labels. Only a PI or Co-Investigator will handle study drug. The PI will train those Co-Investigators designated in the IRB proposal in the proper storage, labeling, and dispensing of study drug. The study drug will be stored at ambient temperature (15 to 30 degrees Centigrade or 59 to 86 degrees Fahrenheit

with protection from moisture and light). The drug will be stored in a locked cabinet in the medication room in the Continence Clinic on the 3rd floor in the Nemours Children's Specialty Care Building on 807 Children's Way in Jacksonville, Florida. The medication will be stored in a separate cabinet from other drugs that are stocked in the clinic for patient care use. The cabinet is locked with restricted key access. The temperature in the cabinet in which the drug is stored will be monitored on a continuous basis using a MCC USB-501-LCD thermometer which allows for continuous monitoring, storage, and downloading of temperature data. Staff will monitor the device daily. Following weekends or holidays, the stored temperature data will be reviewed. Any temperature excursions will be discussed with a pharmacist prior to dispensing the stored flush solution. A daily hard copy temperature log will be kept. If a significant variance from the acceptable temperature range is found, the principal investigator will be notified, who will contact the pharmacist for instructions before any of the stored drug is dispensed. Shipment receipt and product inventory including lot # and expiration dates will be stored in the regulatory binder with a separate log maintained for participant product accountability. Both normal saline and USP glycerin are non-hazardous. The volume and frequency of administration in this proposal does not substantially alter or increase the concentration or distribution of the substance, metabolites, or products of degradation in the environment. To the best of the investigator's knowledge, no extraordinary circumstances exist with the proposed use of saline and glycerin that would adversely affect the quality of the human environment. Excess solution will be disposed of by the family through regular household plumbing.

Nature of Experimental Control

Any research design is a tool used to answer a question. Strategies, design choice, and use of design elements should be based on how best to answer the question at hand (Kazdin, 2011). The purpose of experimental design is to control the effects of random error and bias (Piantadosi, 2005). However, no design completely eliminates either. Whatever the chosen research method, good design and procedures are necessary to prevent confounding and improve causal inference (Shadish et al., 2002). Skillful statistical analysis cannot salvage significant design faults or increase the validity of a poorly designed study (Janosky et al., 2009). Skillful utilization of design elements can increase internal validity and causal inference (Cook & Campbell, 1979). Both single subject and between group research make and test predictions about treatment effects, the first by evaluating treatment effects on an individual, the second by addressing group mean and variance (Kazdin, 2011). Single subjects and group designs, in their most rigorous form, rule out or make implausible rival hypotheses for the experimental outcome improving quality of inference. (Cook & Campbell, 1979; Kazdin, 2011; Shadish et al., 2002, Cook, & Campbell, 2002). A randomized controlled trial (RCT) is considered the gold standard for intervention research (Piantadosi, 2005). However, a RCT is not the only standard for causal inference (Kazdin, 2011). Reliance on large numbers makes application of a RCT with small groups or rare diseases problematic (Janosky et al., 2009).

The focus of my research involves instillation of a solution through an appendiceal stoma, a procedure used for over a century and widely popularized over 20 years ago. Case reports and retrospective studies detail widely divergent effectiveness rates (Bani-Hani, Cain, King, & Rink, 2008; Dey et al., 2003; Mousa et al., 2006; Siddiqui, Fishman, Bauer, & Nurko, 2011; Yardley et al., 2009). The literature and involved clinicians have identified the need for prospective trials comparing ACE flushing regimens. None have been undertaken to date. This is in large part because the small size and heterogeneity of this population does not lend itself to a large N study.

Many clinical questions go unanswered due to over reliance on RCT large N methodology (Kazdin, 2011). This population is an exemplar of that problem.

The proposed study comparing two flushing regimens utilizes a cross-over design embedded in a single subject A-B-C-B'-C'-B1' design. Both methods are experimental and lay a foundation for causal inference (Chow & Liu, 2014; Elder, 1997; Portney & Watkins, 2009). In both designs the subject acts as his or her own control, minimizing within subject variability and ensuring the highest possible degree of equivalence across treatment conditions, thereby, allowing greater precision and efficient estimates of treatment effects, increasing internal validity and causal inference (Janosky et al., 2009; Piantadosi, 2005; Portney & Watkins, 2009). In both methods, subjects are randomized to treatment sequence. Randomization will decrease the threat of order effects in both methods, increase group equivalency in the cross-over design, and minimize variability in measurement due to subject or period differences, increasing internal validity and causal inference (Chow & Liu, 2014; Jones & Kenward, 2003).

Single subjects design is an inductive, experimental methodology with controlled introduction and manipulation of an independent variable. Single subjects design promotes exploration of inter-subject variability without the introduction of error inherent in group methodology in the absence of subject homogeneity. It allows for isolation of individual response to interventions and identification of valuable information from outliers that would be obscured or lost in group methodology. Single subjects design also allows for time series observations of response, providing continuous and often a more accurate representation of the dependent variable of interest that may be compromised when the data is collected as an isolated snapshot in group methods (Elder, 1997). This study is ideally suited to single-subject repeated measures design because children requiring an ACE procedure comprise a very small population with widely disparate anatomic and physiologic causative factors, making sample homogeneity difficult. Inclusion of heterogeneous subjects will allow differentiation of subject characteristics that impact response to treatment. The design allows for frequency, volume, and dose adjustment of each flushing regimen when indicated. The ability to adjust the treatment regimen facilitates dose-response comparison and will aid in identifying which flushing regimen requires the minimal dose and administration frequency, is accomplished in the least amount of time, and has the fewest side effects while achieving continence. Because this design allows for repeated measurement over time, it is particularly helpful when studying comparisons between several treatments and is more sensitive to variations in treatment response that might otherwise be missed using group methodology (Gast, 2010; Janosky et al., 2009; Kazdin, 2011).

Subjects will be limited to children who are scheduled for a cecostomy or appendicostomy, ensuring their gut is naïve to the effects of a flushing regimen and allowing a true no-treatment baseline. Flush effects are reversible, making this intervention amenable to a withdrawal design. There are no known carry-over effects associated with either flushing regimen that would impact treatment effect on continence. With regard to continence, the subject's bowel should return to a physiologic baseline between treatments. Specimen collection facilitating comparison of treatment effects on gut microbiota, electrolytes, and stool calprotectin occurs at the completion of each flushing regimen, negating any carry-over effects. Given the pragmatic issues involved in answering the research question at hand, the chosen methods and design elements strengthen demonstration of the counterfactual and make implausible potential threats to validity, lending credence to the assumption that intervention effects are due to the treatment and not random error or bias.

Potential threats to statistical validity are detailed in Appendix G. Potential threats to internal validity are detailed in Appendix H. Potential social threats to internal validity are detailed in Appendix I.

Randomization Procedures

Subjects will be randomly assigned to one of two treatment sequences to control for the possibility of order effects. To prevent statistical bias from subject loss due to treatment failure, each child will be randomized to a second treatment sequence once they have achieved continence on optimal dosing with minimal side effects. The process will be restricted random assignment to force equal sample size and will be accomplished using the SAS random number generator.

Physical Examinations, Laboratory Evaluation, Radiographic Evaluation, Special Test Procedures, Surveys, Questionnaires

A history and physical examination will be completed at each clinic visit. Stool samples will be obtained during the baseline period prior to any pre-operative bowel prep; additional stool samples will be obtained at the completion of each phase. Blood samples for electrolytes and immune markers will be drawn prior to surgery and, subsequently, following surgery after completion of B and C phases. Blood samples and stool for calprotectin will be sent for analysis; stool samples for microbiota analysis will be labeled with the patient identifier, date, time, and flush composition. Each stool sample for microbiota analysis will be processed by the investigator with oversight by Karl Mann and Dr. Sylvester to purify and extract microbial DNA for sequencing, flash frozen to -80° C, and archived for downstream-batch analysis using 16SrRNA as a taxonomic marker (Barker, 2012).

Responsible Party and Setting for Obtaining Information

The families will be responsible for keeping a log both pre and post-operatively. Kim Jarczyk will be responsible for collecting and entering the data in to REDCap for future analysis. Data will be collected in the clinic and home settings.

How Will Activities Answer the Study Questions

The B-C/C-B phase of the study will evaluate the dose-response relationship and will be used to identify the optimal dose and frequency of ACE administration for normal saline and normal saline with USP glycerin.

The B'-C'-B1'/C'- B'- C1' phase of the study will compare the effectiveness of the two regimens at optimal dose and administration frequency.

The Basic Metabolic Panel will evaluate flush effects on electrolyte balance.

Stool calprotectin will be used as a gut inflammatory marker.

Molecular techniques for identification of 16SrRNA gene sequence in stool samples will be used to identify and quantify microbiome phylogenetic groups.

Severity of side effects will be measured using the WBFPRS as the age-appropriate visual analog scale.

The FIC QOL will be used to assess child and parental perception of quality of life impact and as an indirect measure of social validity.

A simple qualitative question will be addressed to the child upon study completion to determine the child's flush preference and why.

Study Schedule

Overview of Timeline

	Baseline		Dose Response Phase							Flush Effectiveness Phase											
Weeks:	1	2	3	4	5	6	7	8	9	10+	11	12	13	14	15	16	17	18	19	20	
Randomize		X								X											
<u>Order:</u>																					
A	NT	NT																			
B-C			B	B	B	B	C	C	C	C											
C-B			C	C	C	C	B	B	B	B											
B'-C'-B ₁ '											B'	B'	B'	B'	C'	C'	C'	C'	B ₁ '	B ₁ '	
C'-B'-C ₁ '											C'	C'	C'	C'	B'	B'	B'	B'	C ₁ '	C ₁ '	
<u>Visits:</u>																					
Hospital			X																		
Home			X																		
Clinic						X				X				X				X			X
<u>Biomarker</u>																					
Stool	X					X				X				X				X			
BMP	X					X				X											
SIM	X					X				X											
<u>Instrument</u>																					
FIC QOL	X													X				X			
QQ																					X
<u>Measures:</u>																					
Soiling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Abd Pain	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Admin T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Proc T			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ISE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cost	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

A - Baseline

NT - No treatment

B - Saline dose-response phase

C - USP Glycerin dose-response phase

B' - Initial trial of saline effectiveness phase

C' - Initial trial of USP glycerin effectiveness phase

B₁' - Second trial of saline effectiveness phase

C₁' - Second Trial of USP glycerin effectiveness phase

Stool - Metagenomic Profiling 16SrRNA (collected and batched for downstream analysis)

BMP - Basic Metabolic Profile

SIM - Stool Inflammatory Marker (Calprotectin)

Visits - Procedural fidelity and inter-rater reliability will be measured at each visit

FIC QOL - Fecal Incontinence and Constipation Quality of Life Measure in Children with Spina Bifida

QQ - Qualitative question asked of the child upon study completion

Abd Pain - Abdominal Pain

Admin T - Administration time

Proc T - Procedural time

ISE - Infusion side effects

Procedures to Protect Privacy

Consents and patient completed data sheets will be stored in a locked file box housed within a locked cabinet in an office that remains locked when it is not in use. The data will be entered in REDCap and accessible only to Kimberly Jarczyk, Pam Pieper and Jonathan Shuster will have access to deidentified data in REDCap. Safety and Monitoring Board members will have access only to de-identified data. Any data used in reporting results will be de-identified.

A summary of the patient's response to treatment that requires dosing adjustment will be documented as a staff note in the patient's password-protected electronic medical record. A summary will be included in the notes associated with each clinic visit following each phase completion. The data can only be accessed by clinicians directly involved in the child's care.

How research interventions differ from standard therapies and alternatives if they exist

This research study includes clinical treatments and procedures that would be required during normal clinical care for children who require an appendicostomy or cecostomy for treatment of fecal incontinence. The protocol will compare two common ACE flushing solutions that have been used clinically for many years, but have not been studied. A child undergoing appendicostomy/cecostomy would be placed on an ACE flush, most likely using one of the two solutions used in this study. Dose and frequency would be adjusted in much the same manner it would be in this study. Children who were not enrolled in the study would not have blood drawn for a Basic Metabolic Panel or stool samples collected for calprotectin and storage for later microbial DNA analysis using 16SrRNA. Families would not complete a quality of life survey and children would not be asked to answer an open-ended question regarding flushing preference.

Statistical Analysis

Describe Statistical Analysis

The strength of an interrupted time series design is that effects of the intervention under investigation can be repeatedly and reliably measured over time. Time series parameters include mean intercept level, slope, and additional non-linear changes in shape. If the treatment is effective, it will result in a change in parameters that reverse with treatment withdrawal. Independent variable impact on time series parameters is based on the degree of change elicited in level, slope, or cycle of the measures process. The ability to determine independent variable effects is a function of a stable baseline and the number of baseline, intervention, and post-intervention data points (Biglan, Ary, & Wagenaar, 2000; Janosky, Leininger, Hoerger, & Libkuman, 2009; Shadish, Cook, & Campbell, 2002). In this study, each subject will have a minimum of 2 weeks of data collection pre-operatively, allowing for establishment of a stable baseline. Postoperatively, after titration, each regimen will be administered at optimum dosing for a minimum of 2 weeks at the end of the titration phase and 4 weeks during the comparative phase, for a total of 6 weeks per flush regimen at optimal dosing, yielding a total of at least 12 weeks of observations at the therapeutic dose and frequency threshold per subject.

Data will be obtained and graphed at the time of each flush to facilitate analysis and dose response adjustment during the B-C or C-B phase, when indicated. Data will be graphed on an equal interval line graph with the proportion of ordinate and abscissa scaled at a 2:3 ratio to ensure consistency of data presentation and prevent data distortion during visual analysis. Dependent measures will be placed on the ordinate scale with time-by-day on the abscissa scale.

Separation of dose-response and comparative phases of the study will be designated by a bold vertical line and high and low dose regimen changes by a thin vertical line. Each phase change will be labeled with solution name, dose, and frequency. Independent variable effect on target behaviors will be analyzed using visual analysis.

The independent variable is nominal and dichotomous. Dependent variables are comprised of either interval or ratio level measurements. The comparative phase of the study is a two-treatment crossover design with each child receiving both treatments. Each child will be randomly assigned to either a C'-B'-C1' or B'-C'-B1' treatment sequence with half of the subjects allocated to each sequence (Portney & Watkins, 2009). The flushing regimen's effect on outcomes of interest, including number of soiling episodes, level of soiling, abdominal pain, procedural side effects, infusion time, procedural time, electrolyte balance, fecal calprotectin, and quality of life will be analyzed using inferential statistics.

Data Analyses

Analysis within each condition will include: (a) condition length, defined as the number of data points contained within each phase; (b) level stability with a stability envelope calculated using the median and a stability criterion of 80% of the data points falling within 15% of the calculated median for the phase; (c) relative change in level; (d) absolute change in level; (e) estimation of trend direction using split middle method; (f) trend stability with a stability envelope using the same criteria as level stability; and (g) identification of multiple paths within trends, if present. Analysis between conditions will include: (a) number of variables that have changed between adjacent conditions, (b) change in trend direction between conditions, (c) assessment if trend change is in keeping with intervention goals, (d) assessment for change in trend stability, (e) assessment of immediacy of effect in change in level and trend, (f) calculation of absolute and median level change, (g) calculation of percentage of non-overlapping and overlapping data points, and (h) percentage of data points exceeding the median (Gast, 2010; Hartmann et al., 1980; Ma, 2006; Kazdin, 2011; McDowall, McCleary, Meidinger, & Hay, 1980; Portney & Watkins, 2009). Testing in multiple subjects will allow for analysis of replication of treatment effects. Analysis will include between-series strategies comparing data points, including frequency, mean occurrence, and immediacy and magnitude of effect within and between treatment conditions. In addition to visual analysis of time series parameters, inferential procedures will be used to compare interventional effects and increase the reliability of visual methods analysis.

Inferential analysis will be accomplished using a two-tailed, two-sample pooled variance t-test with a significance level set at 0.05. Confidence intervals will be calculated to provide precision of mean differences estimates (Polit, 2010). The two sample t-test will be used to test treatment difference in the cross-over design. Because the design is comparing two treatment sequences, groups are independent (Chow & Liu, 2014). Confounding by carry over and direct-by-period interaction is a potential with cross-over designs, which if present, can bias treatment effects (Jones & Kenward, 2003; Senn, 2002; Shuster, 2007). Jones et. al. (2003) and Sen (2002) suggest use of a one sample t-test for analysis of cross-over designs (treatment one is subtracted from treatment two). Shuster (2007) advocates a two sample t-test in the analysis of a randomized two treatment cross-over design (period two is subtracted from period one irrespective of treatment order). Analysis of a one sample t-test in a cross-over design ignores treatment ordering. Two sample t-test analysis compares ordering and yields potentially useful data on carry over. When μ is the main treatment effect, and τ is carry over, results from a one sample or two sample t-test will yield unbiased estimates of μ and variance when the sample size

is equal and $\tau = 0$. If $\tau \neq 0$, the expected value of μ should be similar using either the one or two sample method. However, the one sample method does not account for carry over effects, increasing variance. If sample sizes are unequal and $\tau \neq 0$ (conditional on sample size), the point estimates in the one, but not the two, sample t-test will be biased. Using the two sample method will lend precision in the presence of carry over effects, and precision and accuracy in the presence of unequal sample size when $\tau \neq 0$ (Shuster, 2009). Subject characteristics will be described, when appropriate, using frequency distribution and graphed using either histograms or pie-charts. Changes in gut microbiota will be analyzed using descriptive statistics (Polit, 2010).

Neither visual analysis nor inferential statistics alone will infer causality. Strictly speaking, correlation does not infer causation (Polit, 2010). Causal inference is primarily influenced by the research design. Visual analysis and statistical procedures are helpful in measuring the effects of potential causes (Kazdin, 2011). Appropriate use of statistics aid in the inference of causality by quantifying the effect chance plays on conclusions (Hill, 1965). The proposed study is prospective allowing for the determination of temporal precedence. Statistical and visual analysis will be used to assess contiguity with respect to presumed cause and effect. Strategic use of design elements limits alternative explanations for findings. The first phase of the proposed study evaluates dose response, which will assess biological gradient. The design assesses treatment response across multiple subjects, which may provide support for consistency across contexts.

Stopping Rules

The parent will call if the child is having accidents or discomfort greater than WBFPRS level 4 associated with the flushing regimen and the regimen will be changed. The child will be put on the alternate flushing regimen if the child has discomfort greater than WBFPRS level 4, cannot achieve and maintain continence, or has a clinically significant electrolyte imbalance or calprotectin elevation.

Data and Safety Monitoring Plan

This study has a Data and Safety Monitoring Board (DSMB). Although this is a low risk study and, based on previous studies, we do not anticipate any serious adverse effects for USP glycerin or normal saline at the doses we plan to use, as suggested by the IRB, we have established an expert and independent three-member DSMB. Kathryn Blake, Pharm D, Principal Research Scientist, Center for Pharmacogenomics and Translational Research at Nemours Children's Specialty Care in Jacksonville, Florida; Laurie Duckworth, PhD, ARNP, Clinical Associate Professor, Director of Clinical Research, University of Florida College of Nursing; and Salik Taufiq, MD, Division of Pediatric Gastroenterology and Nutrition at Nemours Children's Specialty Care in Jacksonville, Florida have agreed to be members of this expert panel. All are Nemours or University of Florida employees, are exceptionally qualified to serve on this expert panel, and are not otherwise involved in this study. Letters from these members agreeing to be part of the DSMB expert panel are located in Appendices K, L, and M, respectively. The DSMB will assess the safety and efficacy of study procedures, and monitor the overall conduct of the study. De-identified generated data will be made available to the panel. We will report our progress to the DSMB every 6 months. Any adverse events, abnormal laboratory results, or problems that arise during the conduct of this study will be reported at the time of the occurrence. The study may be modified or stopped based on its assessment of the participant safety needs. The members of the DSMB will review the study adverse event experience, and provide written reports to us following each scheduled DSMB review. Periodic DSMB reports will be forwarded to the IRB as the need arises.

Possible Discomforts and Risks

Risks

This study does not entail any additional physical, physiological, or social risks over standard-of-care therapy.

Discomforts

ACE flush can be associated with electrolyte imbalance, abdominal cramping or nausea. Additional discomforts above those often experienced during standard care include the discomfort associated with venipunctures to obtain BMP levels. These will be collected three times over the course of the study.

Economic Risks

This study does not entail additional economic risks to the participant above what would be encountered during standard-of-care treatment. The participant or his/her insurer will pay for regularly scheduled clinic visits and supplies associated with the ACE flush. These are considered standard therapy.

Procedures to Minimize Discomforts and Risks

Electrolytes will be drawn to monitor for electrolyte imbalance. If a participant experiences any side effects from an ACE flushing solution, the dose will be adjusted. If side effects do not resolve on a lower dose, the participant will not continue in the study and will be placed on an alternative ACE flush solution.

Children will be supplied with topical Lidocaine+Prilocaine to decrease the discomfort associated with venipuncture.

Compensation to participants will include (a) costs of supplies for stool collection and calprotectin analysis, (b) laboratory and basic metabolic panel (BMP) analysis fees, (c) costs of topical Lidocaine+Prilocaine and tegaderm used to minimize discomfort during venipuncture, (d) and \$25 stipend for each scheduled study visit (maximum total of \$125). Adolescent participants will receive a \$5 stipend for each scheduled study visit (maximum total of \$25).

Risk Benefit Analysis

Fecal incontinence in children past the expected time of toilet training has been associated with poor outcomes including increased anxiety and depression, more social problems, worse school performance, and an increased incidence of abuse and bullying (Kaugars et al., 2010; Youssef, Langseder, Verga, Mones, & Rosh, 2005). Helping children achieve continence can be life altering. Fecal incontinence is particularly difficult to manage using conservative measures in children with neuromuscular disorders, anorectal malformations, spinal cord injuries, spinal cord trauma or tumors, megarectum, or slow transit constipation. The ACE procedure was popularized over 20 years ago as a means of helping children with intractable fecal incontinence attain stool continence. A large body of literature demonstrates ACE therapy can be effective in helping children with intractable fecal incontinence attain continence for stool with resulting significant improvement in quality of life. However, findings regarding effectiveness are highly variable. This variability may be due to the flushing regimen utilized. No prospective trials compare the effectiveness and adverse effects of different flushing regimens. No studies evaluate the effects of appendicostomy or cecostomy flush on gut microbiota. Currently, identifying a successful flushing regimen is determined by individual clinician preference and often requires multiple attempts before success is achieved. There are no prospective studies comparing the effectiveness of type, dose/volume, or frequency of different flushing regimens in preventing incontinence to inform practice. This study involves minimal risk above standard therapy and has

the potential to benefit participants as well as contribute to our understanding of the effectiveness of both flushing regimens, benefiting future children requiring ACE therapy.

Justification for Conducting the Study

The proposed research is significant in that it is the first prospective study to compare the effectiveness and tolerability of two commonly used ACE flushing regimens and the first study to explore ACE flushing impact on gut microbiome. Risks associated with this study are low and do not constitute a substantial threat above risk exposure in standard-of-care therapies. Findings from this study have the potential to provide both clinical and biological insights into ACE administration safety. They will provide a foundation of scientific evidence to serve as a starting point to guide practice and for additional prospective, randomized, controlled trials.

Benefits to Subjects

Participants may or may not personally benefit from taking part in this study. However, each child will be closely monitored throughout the study and his/her treatment will be adjusted to find the best possible volume of flush that works in the least amount of time with the fewest side effects.

Benefits to Future Populations

Information from this study will help us start to understand how the two ACE flushing regimens compare with regard to side effects and effectiveness. A better understanding will eventually lead to better ACE treatment outcomes for other children who require ACE flushing to gain and maintain continence for stool.

Conflict of Interest

There are no real or potential conflicts of interest for any investigator in this study. None of the investigators hold a patent or license pending for any of the study medications, materials, or processes utilized in this study nor is there an intent to file a patent application at a later date. None of the investigators hold stock, give presentations, or serve as a consultant for any company that produces the drugs or materials used in this study.

IRBNet Board Action - Approval



Jarczyk, Kim <Kimberly.Jarczyk@nemours.org>

Yesterday, 1:44 PM
JarczykKimberly S

-----Original Message-----

From: Caroline Schierle [<mailto:no-reply@irbnet.org>]

Sent: Thursday, December 08, 2016 3:25 PM

To: Jarczyk, Kim <Kimberly.Jarczyk@nemours.org>

Subject: IRBNet Board Action

Please note that Nemours IRB 2 has taken the following action on IRBNet:

Project Title: [778885-4] A Within Subjects Comparison of Two Antegrade Flushing Regimens in Children Principal Investigator: Kimberly Jarczyk, MSN

Submission Type: Amendment/Modification

Date Submitted: November 30, 2016

Action: APPROVED

Effective Date: December 8, 2016

Review Type: Expedited Review

Should you have any questions you may contact Caroline Schierle at cschierl@nemours.org.

Thank you,
The IRBNet Support Team

www.irbnet.org



Application for Amendment to Approved Protocol
Version April 2016



Changes may not be initiated prior to IRB approval except as necessary to eliminate apparent immediate hazard to subjects [45CFR46.103(b)(4)(iii) and 21CFR56.108(a)(4)]; therefore, amendments **must** be approved by the IRB prior to implementation.

Use this form to request IRB review and approval of changes to an existing, ongoing, approved study. These include changes to the protocol or consent / assent forms, changes to add or remove study staff, changes to increase accrual, to provide or remove recruitment materials, and any other changes from the protocol application as approved by the IRB.

Reference the Checklist for Review of Amendments located on IRBNet Forms and Templates and NOHSP TeamShare.

Date: 08/10/2016

IRBNet #: 778885-2

- 1. → Title of Protocol: A Within Subjects Comparison of Two Antegrade Flushing Regimens in Children
- 2. → Principal Investigator: Jarczyk, Kimberly
- 3. → Study Coordinator: None
- 4. → List the (non-staff related) changes proposed or describe the main changes and attach the Summary of Changes by the sponsor, if applicable. Submit electronic versions of these documents with this application.

Document (include page / location)	Description of change

Section Break (Continuous)

5. → What is the justification/rationale for the changes? Kaitlyn Smith is no longer working at Nemours and has moved out of the area

6. → How do the changes affect (adversely or positively) the risks or benefits of study participation? Explain: Will have no impact. Tara Spruill and Kim Jarczyk remain available to obtain informed consent

7. → How do the changes affect the scientific merit of the study? Explain: No impact

8. → Do you plan to inform enrolled study subjects of these changes? (Consider: Is it possible that the changes proposed would affect a subject's decision to continue participation?)

→ Yes No If Yes, how will this be accomplished? If No, why not? They have already consented to the study. The personnel change does not impact them

- a. → How many participants have been enrolled in this study since it started? 4
- b. → How many subjects are active? 4

If participants will be notified via a revised parental permission or informed consent, or in writing, the proposed form, with changes highlighted, or letter must be attached with this submission and must be approved by the IRB prior to use.

9. → If a new site is being added, list the site and explain the relationship of the new site to the approved Nemours study. →

10. → Are study staff changes involved? Yes No For each added staff member, answer the applicable questions in the table below.

End of Protected Section

<input type="checkbox"/> Add ¶ <input checked="" type="checkbox"/> Remove	Name, Credentials: Smith, Kaitlyn, Site, Department: NCSC, Position: Co-I ¶ Responsibilities: <input type="checkbox"/> Recruitment <input checked="" type="checkbox"/> Consent <input type="checkbox"/> Research procedures <input type="checkbox"/> Data Collection <input type="checkbox"/> Data Analysis <input type="checkbox"/> Other duties: ¶ Qualifications: HSP Training documented: <input type="checkbox"/> Related experience or training: ¶
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(If you need more rows, contact NOHSP at 302-651-7613 or 53-7613 for assistance.) ¶

¶
All staff must have completed the required human subjects protection training listed on NOHSP TeamShare. ¶
New staff must complete and attach an Investigator Agreement & Disclosure Form. If applicable, a revised PPF / ICF and assents must be submitted with this application. ¶
Advertisements and flyers must be approved by Nemours PR/Communication Office. ¶
New and departing staff if available, must sign the IRBNet application. ¶

¶ Departure of a PI ¶

If a Principal Investigator of an interventional study is leaving Nemours, review by the convened IRB is required. The amendment must include a transition plan, attached with this submission, which assures the continued protection of current participants. ¶

¶ Signature Requirements ¶

The following signatures are required before submission to the IRB. ¶

Principal Investigator: ¶

- → Required for changes that might affect the risk or benefit of the study and require review by the convened IRB. ¶
- → The PI should also sign when adding new staff if there is not an existing study coordinator delegated with this task. ¶
- → Not required for minor changes that can be signed by delegated staff. ¶

Study Coordinator or other delegated staff: ¶

- → Can sign alone for minor changes submitted for expedited review that do not affect risk or benefit. ¶

Added Staff: ¶

- → Required. Must sign for information included in Investigator Agreement & Disclosure. ¶

¶

FW: IRBNet Board Action



Jarczyk, Kim <Kimberly.Jarczyk@nemours.org>

Yesterday, 1:44 PM

Jarczyk, Kimberly S

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Thank you,

The IRBNet Support Team

www.irbnet.org

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Appendices

- Appendix A Compilation of Animal Studies Using Glycerin
- Appendix B Compilation of Human Studies Using Glycerin
- Appendix C Wong-Baker FACES Pain Rating Scale Parental Instructions for Saline Flushing Regimen
- Appendix D Fecal Incontinence & Quality of Life Measure in Children with Spina Bifida
- Appendix E Parental Instructions on Normal Saline Flush Administration
- Appendix F Parental Instructions on USP Glycerin Flush Administration
- Appendix G Threats to Statistical Conclusion Validity
- Appendix H Threats to Internal Validity
- Appendix I Social Threats to Internal Validity

Appendix A
Animal Studies Using Glycerin

(There are no animal studies evaluating instillation of glycerin into the cecum)

<u>Sample</u>	<u>Dose</u>	<u>Route</u>	<u>Outcome</u>	<u>Reference</u>
6 rabbits	18,700 mg/kg bw	Occlusive dermal application for 8 hours	No deaths	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
12 female rats	27,260 mg/kg bw	Gavage	Muscle spasm, convulsions, lung congestion & death (3) Survivors normal within 2.5 hrs of dosing	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
Mice & Guinea pigs	Not reported	Gavage	Tremor and convulsions with hyperemia of pylorus, small intestine and cerebral meninges	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
Rabbits	4 mL over 30% BSA 8 hr/day for 90 days	Topical	No irritation	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
6 rabbits	0.1 mL	Ocular instillation	Very low potential to irritate eyes	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
24 male Guinea pigs	0.1 mL of 0.1% in NS qod x 20 days	Injection	No indication of sensitization after 2 week exposure free period	<i>Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html

Rats	1.75 mL of 50%/100 g bw	SQ injection	Severe hemolysis followed by necrosis of tubular portions of nephrons – reversible within 6 – 12 wks	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rats	1 mL 100%/100 g bw	Intra-peritoneal instillation	Severe convulsions, hemoglobinuria, renal damage died within 2 hrs of injection	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015 http://esis.jrc.ec.europa.eu/</i>
Rats	1 mL 100% or 50%/100 g bw	SQ injection	Hemoglobinuria, renal tubular necrosis, some convulsions with 100%	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rats	100% & 50% at 1 mL/100 g bw	IV	Severe Convulsions and death in all animals	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: http://esis.jrc.ec.europa.eu/</i>
Rabbit	Aq 100%	Anterior chamber of eye	Inflammation and edema of cornea & damage of endothelial cells	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbits	Aq 50%	Anterior chamber of eye	Significantly less reaction but visibly dehydrates lens can capsule wrinkling	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbit	Aq 30% for 20 min, 50% for 10 min or 92% for 4 min	Anterior chamber of eye	Normal deturgescence after 30% and 50% exposure but endothelium destruction with 92% > 30 min	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rabbit with corneal trauma	Aq 43% dilution for 30 min x 20 d	Ophthalmic	Edema of conjunctiva lasting for several hrs	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
Rats	14 d 5d/wk 6hr/d Mean conc 1000, 1930 3910 mg/cu m	Respirable aerosol	Minimal to mild squamous metaplasia of epiglottis – greatest at highest test dose No systemic effects	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>

Rats	13 wk 6hr/d 5d/wk 0, 33, 165, & 662 mg/cu m	Respirable aerosol	Minimal to mild squamous metaplasia of epiglottis – considered local irritant effect	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
Rats	10% for 9 d	IV	Degeneration in renal tubular epithelium return to normal after 19 d	<i>NOWAK H ET AL; PATOL POL 30 (1): 61 (1979)</i>
Grow- ing pigs	84.51% added to feed for 138 d	Feeding trial	Pigs can be fed up to 10% crude glycerin with no effects on performance, carcass composition, or meat quality	<i>Lammers PJ et al; J Anim Sci 86 (11): 2962-2970 (2008)</i>
Rats	0 up to 60,000 mg/kg body weight/d for 20 wks	oral	At 5000 mg/kg bw marked hydropic and fatty degeneration of liver parenchymal cells	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>
Mice 6 to 8 wks	Given carcinogen followed by 0, 0.5 or 1% glycerol or water until 1 yr of age	oral	Lower incidence of liver and lung tumor after glycerin – no adverse treatment effects	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>
Rats	0 to 20,000 mg/kg body weight/d	oral	No significant - treatment related effects	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015: http://www.inchem.org/pages/jecfa.html</i>
Rats	4,000 to 10000 mg/kg bw for 2 years	Oral	No adverse effects up to 10,000 mg/kg bw	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html</i>
Rats	5%, 10%, & 20 % for	Oral	Glycerol does not initiate tumor development in rats	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18,</i>

	12 to 24 months			2015: http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
Mice	5% in drinking water for 1 – 20 weeks after sq injection of 4-NQO	Oral	Enhances lung tumor development - mainly adenomas. Tumor development independent from pulmonary cell kinetics	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html
Rats	7 generations 15000 mg/kg bw/d	Oral	Pups of treated dams mean weight 20% less than controls	<i>Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015:</i> http://www.inchem.org/pages/jecfa.html
Rats Mice Rabbits	Levels up to 1310, 1280, 1180 mg/kg bw daily during part of gestational period	oral	No maternal or tetragenic effects seen at highest dose level tested	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015:</i> http://www.chem.unep.ch/irptc/sids/OECD/SIDS/sidspub.html

Appendix B
Summary of Previous Human Studies Involving USP Glycerin

<u>Dose</u>	<u>Route</u>	<u>Effects</u>	<u>Reference</u>
Not reported	Not reported	Very slight diuresis in healthy individuals receiving a single dose May produce tissue dehydration and decreases in CSF pressure	<i>McEvoy, G.K. (ed.). American Hospital Formulary Service - Drug Information 93. Bethesda, MD: American Society of Hospital Pharmacists, Inc., 1993 (Plus Supplements, 1993)., p. 1773</i>
Not reported. Employees engaged in glycerol manufacturing	Environmental exposure	No significant difference in sperm count or sperm quality parameters when compared with controls	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
0.05 mL of 10% solution for 21 days	Dermal – patch test	Slight irritation at 48 hrs and maximum rating of 4 on a 9 point scale at day 14 of a 21 day application	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Acute and chronic (42 d) ingestion	Oral	Increase in plasma glycerides in males only following acute ingestion and in both males and females with chronic ingestion (significantly greater increase in males)	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Orange juice mixed with 30 mL of 95% glycerol after each of 3 daily meals	Oral	No overt signs of toxicity or change in food consumption	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: www.inchem.org/documents/sids/sids/56815.</i>
Repeated application of 100% solution	Ocular	Extensive changes to appearance of endothelium that disappeared within 90 minutes of application	<i>Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 463</i>
24,000 mg/kg bw d for 50 days	Oral	Slight tendency toward increase in weight	<i>WHO/FAO: Expert Committee on Food Additives. Summary of Toxicological Data of Certain Food Additives Series 48: Aliphatic acyclic diols, triols, and related substances (56-81-5) (2002). Available from, as of February 18, 2015,: http://www.inchem.org/pages/jecfa.html</i>

Not reported. Workers in foam rubber factory	Dermal – patch testing	No sensitizing effects	<i>United Nations Environment Programme: Screening Information Data Sheets on Glycerol (56-81-5) (March 2002) Available from, as of February 18, 2015: echa.europa.eu/</i>
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Case Reports

<u>Subject</u>	<u>Dose , Route & Purpose</u>	<u>Effects</u>	<u>Reference</u>
46 y.o. male	500 mL of 10% solution	Altered sensorium, generalized seizures, focal neurologic signs Managed conservatively and recovered within 48 hrs – case represents rare presentation of overdose with an otherwise safe drug used in neurology	<i>Singh R et al; Neurol India 49 (3): 320-1 (2001)</i>
Male	Adjunctive glycerin test used in the diagnosis of Meniere's disease	20 – 40dB hearing loss in uninvolved ear during standard testing that resolved within 3 days	<i>Mattox DE, Goode RL; Arch Otolaryngol 104 (6): 359-61 (1978)</i>
73 y.o. male	Oral solution used to treat elevated IOP	Developed severe pulmonary edema 45 minutes after administration	<i>Almog Y et al; Ann Ophthalmol 18 (1): 38-9 (1986)</i>
72 y.o. male	Dose should not exceed 1.5 g/kg bw in Klockhoff test for diagnosis of suspected Meniere's disease - Patient received 3.88-3.95 g/kg bw	Progressive neurological signs and pathologically elevated serum concentration of triglycerides (3,465 mg/dl)	<i>Andresen H et al; Clin Toxicol (Phila) 47 (4): 312-6 (2009)</i>
3 y.o. male	0.5 – 1.0 g/kg	Unique intolerance including mental changes, N&V, hypoglycemia and loss of consciousness following IV administration with spontaneous recovery after 30 min	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: echa.europa.eu/</i>
Not reported	Rectal administration prior to coronary artery bypass	Acute colonic ischemia	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: echa.europa.eu/</i>
82 y.o. hypertensive and senile female	200 mL 50% solution for primary angle closure glaucoma	Headache, shaking of arm, quivering of eyes, and nausea	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available from, as of February 18, 2015: echa.europa.eu/</i>
68 y.o. female diabetic	280 mL of 50% solution	Severe diabetic acidosis within 3 days of ingestion	<i>European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition). Available</i>

Retrospective Studies and Case Reports Addressing Antegrade Colonic Flush Administration

<u>Pediatric Subjects</u>	<u>Solution & Dose</u>	<u>Side Effects from Flush</u>	<u>Reference</u>
40	Dose not reported 1) GoLYTLEY 2) Liquirice root 3) Oil/water mix 4) Treacle/milk mix 5) Water only 6) Oil only	None	Marshall, J., Hutson, J. M., Anticich, N., & Stanton, M. P. (2001). Antegrade continence enemas in the treatment of slow-transit constipation. <i>Journal of Pediatric Surgery</i> , 36, 1227-1230. doi:10.1053/jpsu.2001.25768
62	1) Polyethylene Glycol with electrolytes (50 – 1,000 mL) 2) Phosphate enema 3) NS alone (50 – 1000 mL)	None	Dey, R., Ferguson, C., Kenny, S. E., Shankar, K. R., Coldicutt, P., Baillie, C. T., ... Turnock, R. R. (2003). After the honeymoon- Medium-term outcome of antegrade continence enema procedure. <i>Journal of Pediatric Surgery</i> , 38, 65-68. doi:10.1053/jpsu.2003.50012
26	Flush solution not reported. Dosage (250 – 1000 mL)	None	Becmeur, F., Demarche, M., Lacreuse, I., Molinaro, F., Kauffmann, I., Moog, R., Rebeuh, J. (2008). Cecostomy button for antegrade enemas: Survey of 29 patients. <i>Journal of Pediatric Surgery</i> , 43, 1853-1857. doi:10.1016/j.jpedsurg.2008.03.028
71	Tap water (300 – 1000 mL)	Minor deviations in serum sodium and serum chloride present in 18/71 patients Significant hypernatremia and hyperchloremia in 1/71; used softened tap water	Yerkes, E. B., Rink, R. C., King, S., Cain, M. P., Kaefer, M., & Casale, A. J. (2001). Tap water and the Malone antegrade continence enema: A safe combination? <i>Journal of Urology</i> , 166, 1476-1478.
236	1) Tap water alone volume (100 to 1000) 2) 60 mL of USP glycerin & 60 mL of NS 3) GoLYTELY – 1 liter	None	Bani-Hani, A. H., Cain, M. P., King, S., & Rink, R. C. (2008). Tap water irrigation and additives to optimize success with the Malone antegrade continence enema: The Indiana University algorithm. <i>Journal of</i>

	4) MiraLAX 17 grams mixed in 250 mL tap water 5) Mineral oil 30 mL		<i>Urology</i> , 180, 1757-1760. doi:10.1016/j.juro.2008.04.074
105	1) Normal saline or GoLYTELY (23 +/- 0.7 mL/kg) 2) USP glycerin – dose not reported 3) bisacodyl – dose not reported 4) magnesium citrate – dose not reported 5) phosphosoda – dose not reported	None	Siddiqui, A. A., Fishman, S. J., Bauer, S. B., & Nurko, S. (2011). Long-term follow-up of patients after antegrade continence enema procedure. <i>Journal of Pediatric Gastroenterology and Nutrition</i> , 52, 574-580
23	USP glycerin 10 – 60 mL & 15-775 mL tap water with a total volume of irrigation solution ranging from 30 – 800 mL	None	Chu, D., Balsara, Z.R., Routh, J.C., Ross, S.S., & Wiener, J.S. (2012). Experience with glycerin for antegrade continence enema in patients with neurogenic bowel. <i>Journal of Urology</i> , 189, 690-693.
1 – Case report	Hypertonic saline	Death	Schreiber, C. K., & Stone, A. R. (1999). Fatal hypernatremia associated with the antegrade continence enema procedure. <i>Journal of Urology</i> , 162, 1433-1434.

The route of administration influences toxicity of glycerol in humans. Toxic effects from oral administration include nausea and vomiting. Glycerol has a CNS dehydration effect. Intraocular pressure begins to fall at plasma concentrations of 10 mmoles per liter. Concentration and dilutant used also influence toxicity. Use of saline as dilutant diminishes toxic effects of glycerol. Toxic effects following intraperitoneal and subcutaneous administration are albuminuria, hemoglobinuria, anemia, and renal damage. *European Chemicals Bureau; IUCLID Dataset, Glycerol (56-81-5) (2000 CD-ROM edition)*. Available from, as of July 18, 2011: <http://esis.jrc.ec.europa.eu/>

Appendix C



Appendix D
Fecal Incontinence & Quality of Life Measure in Children with Spina Bifida

BOWEL CARE SURVEY ADULT

PT. CODE # _____

Bowel care is defined by

- ◆ the cleaning of the body after a bowel movement
- ◆ changing diapers and/or clothing
- ◆ administration of laxatives, suppositories or enemas
- ◆ digital extraction of stool.

Time and effort spent on Bladder Care should not be included in this survey. Performing bladder catheterization or changing of diapers for urine should not be included.

The person who has the primary role of tending to your child's bowel care should fill out this survey.

Please circle or write in appropriate answers.

Section A							
What is your relationship to the patient?	Self	Mother	Father	Foster Mom	Foster Dad		
Other:							
Does anyone else do your child's bowel care?					YES	NO	
If yes, what is their relationship to your child?	Self	Mother	Father	Foster Mom	Foster Dad		
Other:							
As the primary bowel care person, what percent of the bowel care do you do?							%
Section B							
Please describe your bowel program:							
Do you do this the same time everyday?					YES	NO	
If yes, what time?							
Does your child feel/sense the coming of a bowel movement?					YES	NO	
What percentage of the time does your child produce a bowel movement into the toilet?							%
How many bowel movements per day does your child have?	1	2	3	4	5	more	
How many diaper changes per day for bowel movements (not urine)?	1	2	3	4	5	more	
How many bowel accidents (not in toilet) per week does your child have?							
What techniques do you use to produce bowel movements?	None	Digital Extraction		Sitting on the toilet and pushing			
What oral medicines do you use to produce bowel movements?	None	Mineral Oil	Stool Softener	Fiber	Laxatives		
Other:							
What rectal medicines do you use to produce bowel movements?	None	Theravac enema	Fleet enema	Tap Water enema	suppository		
Other:							
How many minutes per day are needed for your child's bowel care? This includes everything-bowel movements, medicines, diaper changes and clean-up							minutes

BOWEL CARE SURVEY ADULT

PT. CODE # _____

Section C		
Do you notice any significant changes in bowel movements with diet?	YES	NO
Is your child sensitive to foods in relation to bowel movements?	YES	NO
Do some foods change your child's bowel movements?	YES	NO
What foods do you try to avoid?		
Have you changed your child's diet to achieve better bowel care?	YES	NO
If so, what have you done with the diet to improve bowel care?		
Does your child have abdominal pain from constipation?	YES	NO
If so, how many episodes per month?	<input type="text"/>	
Has your child ever been admitted to a hospital for constipation?	YES	NO
If so, how many times?	<input type="text"/>	

For the following questions, it is important to distinguish bowel care from urine care. We are particularly interested in bowel care for this survey. Please try to exclude your child's other health issues when answering the questions and please focus on your child's bowel care. Some of the questions pertain to your child's feelings and other questions to your feelings. Please circle the appropriate answer. If you do not have an answer, please leave it blank.

Section D.	Never	Almost Never	Sometimes	Often	Almost Always
How often does your child's bowel care prevent <u>him/her</u> from going out of the house?	0	1	2	3	4
How often does your child's bowel care prevent you from going out of the house?	0	1	2	3	4
I avoid traveling with my child.	0	1	2	3	4
My child is afraid to go out because of stool incontinence.	0	1	2	3	4

Section E	Not At All	Slightly	Moderately	Very Much (A Lot)
My child's <u>bowel</u> care bothers me.	0	1	2	3
My child's <u>bladder/urine</u> care bothers me.	0	1	2	3
My child's bowel problems make me feel depressed.	0	1	2	3
My child's bowel problems make me feel anxious.	0	1	2	3

BOWEL CARE SURVEY ADULT

PT. CODE # _____

Section F	Not At All	Slightly	Moderately	Very Much (A Lot)
My child's bowel problems affect his/her relationship with siblings.	0	1	2	3
My child's bowel problems affect my relationship with my other children.	0	1	2	3
My child's bowel incontinence affects his/her ability to socialize and meet friends.	0	1	2	3
My child's bowel problems affect my relationship with my partner.	0	1	2	3

Section G.	Never	Almost Never	Some-times	Often	Almost Always
I worry about the smell of my child's stool incontinence.	0	1	2	3	4
My child worries about the smell of stool incontinence.	0	1	2	3	4

Section H					
Are you employed?			YES	NO	
If no, does your child's bowel care prevent you from working?			YES	NO	
	Not At All	Slightly	Moderately	Very Much (A Lot)	
If you do work, how much does your child's bowel care affect your job?	0	1	2	3	
To what extent does your child's bowel care affect your household tasks?	0	1	2	3	
Excluding your child's other health issues, does his/her bowel incontinence affect his/her physical activities (walking, wheelchair sports, etc.)?	0	1	2	3	

Section I					
Do you feel that you have exhausted all options for reaching stool continence?			YES	NO	
	Not At All	Slightly	Moderately	Very Much (A Lot)	
If my child was continent of stool, but the <i>urine</i> continence was <i>unchanged</i> , this would change my life.	0	1	2	3	
If my child was continent of stool, but the <i>urine</i> continence was <i>unchanged</i> , this would change his/her life.	0	1	2	3	

Appendix E

Steps	Parental Instructions for Saline Flushing Regimen
1	Assemble equipment: One liter container of Normal Saline (NS) premixed to 0.09% Each container will be marked by the investigator with ACE flush contents and a lot number One 1,000 mL enteral feeding bag with drip chamber and roller clamp One Christmas tree adapter connected to the end of the enteral feeding bag tubing The bag will be hung from a hook located 6 feet above the floor on a wall to the side of or behind the commode One appropriate access tube for Chait or Mickey low profile device Stop watch Clip board with data collection sheets and pen attached with string to the clipboard
2	Warm tap water and mild liquid dish detergent to wash equipment after each use
3	Make sure flushing solution is at room temperature
4	Insert the Christmas tree adapter into the end of the feeding bag tubing, making sure the fit is secure
5	Make sure the roller clamp is closed on the feeding bag tubing
6	Unscrew cap from the top of the feeding bag by rotating in a counter clockwise direction
7	Pour room temperature liquid from the 2 liter mixing bottle into the feeding bag
8	Screw the cap onto the top of the feeding bag by turning in a clockwise direction until secure
9	Hold the feeding bag with liquid in one hand at shoulder height and put the end of the tubing in the sink
10	Using your other hand, slowly loosen the roller clamp on the feeding bag tubing until the liquid fills the tube and then reclamp by tightening the roller clamp to stop the flow of liquid
11	Hang the feeding bag on the wall hook above the commode
12	Position child comfortably on commode using a toilet seat insert and a foot stool, if needed
13	Give child toys/books for distraction
14	Hook the end of the Christmas tree adapter into the access tubing and secure the access tubing to the button
15	Unclamp the roller clamp to start the flow of the flushing solution and immediately start the stop watch
16	If your child complains of cramping or discomfort, slowly tighten the roller clamp to slow the flow of the flush
17	When the flush solution has infused, write the time from the stopwatch onto the record sheet under "Flow Time," but do not stop the stopwatch
18	Disconnect the tubing and rinse with a mixture of mild dish soap and tap water followed by tap water alone and hang back on the hook to air dry
19	Once your child has passed a bowel motion and 5 minutes has gone by without any additional stool output, stop the watch and record the time on the record sheet under column "Completion Time"

Appendix F

Parental Instructions for USP Glycerin and Normal Saline*

- 1 Assemble equipment:
 - One appropriate access tube for Chait or Mickey low profile device
 - 60 mL catheter tipped syringe
 - One 8 ounce bottle with screw top to mix glycerin and NS
 - USP Glycerin dispensed in a resealing container appropriate for liquids
 - Glycerin container will be marked by the investigator with ACE Flush and a lot number
 - One 30 mL syringe for measuring liquid glycerin
 - One liter container of NS premixed to 0.09%
 - Stop watch
 - Clip board with data collection sheets and pen attached with string to the clipboard
 - 2 Warm tap water and mild liquid dish detergent to wash equipment after each use
 - 3 Using the 8 ounce bottle with screw top pour _____mL of USP glycerin and _____mL of NS into the bottle making sure the NS is at room temperature
 - 4 Recap the bottle making sure the cap is secure; shake the bottle until the liquids are well combined
 - 5 Attach the catheter tip of the 60 mL syringe to the access tubing
 - 6 Hold the 60 mL syringe with liquid in one hand and put the end of the tubing in the sink
 - 7 Pour room temperature liquid from the 8 ounce mixing bottle into the 60 mL syringe
 - 8 When the flush solution reaches the end of the tubing, pinch off the tubing to stop the flow of liquid
 - 9 Position child comfortably on commode using a toilet seat insert and foot stool if needed
 - 10 Give child toys/books for distraction
 - 11 Attach and secure the access tubing to the low profile device
 - 12 Unpinch the tubing to start the flow of the flushing solution and immediately start the stop watch
 - 13 Hold the syringe at the child's shoulder height
 - 14 If your child complains of cramping or discomfort, lower the height of the syringe to slow the flow of the flush
 - 15 Raise or lower the syringe to adjust the flow of liquid. The higher the syringe the faster the liquid will go in
 - 16 When the flush solution has infused, write the time from the stopwatch onto the record sheet under "Flow Time" but do not stop the stopwatch
 - 17 Once the flush has infused, disconnect the tubing and rinse the syringe and tubing with a mixture of mild dish soap and tap water followed by tap water alone
 - 18 Once your child has passed a bowel motion and 5 minutes has gone by without any additional stool output stop the watch and record the time on the record sheet under column "Completion Time"
-

Appendix G
Threats to Statistical Conclusion Validity

Threat	Assessment of Potential Threat & Proposed Control
Insufficient Power (Increases probability of a Type I error)	Within subject analysis - the strength of the ability to determine treatment effects requires a stable baseline, and is a function of the number of baseline and intervention data points (Cook & Campbell, 1979). This study design features a prolonged pre-operative baseline assessment and a minimum of 6 weeks of daily measurement for each treatment variable meeting the criteria for sufficient power analysis Cross-over analysis: The study will use 6 subjects randomized to 2 treatment orders. Although the design confers greater precision in estimating treatment difference and generally requires fewer subjects, the limited number of subjects will limit the power of the analysis increasing the probability of a Type II error (Piantadosi, 2005).
Reliability of Measures (Impacts the relationship between variables)	All biological samples will be run in a single accredited laboratory. All fecal samples will be collected and stored for downstream analysis by the procedure detailed in the protocol (Shadish et al., 2002).
Reliability of Treatment Implementation (May result in underestimation in treatment effect.	Procedural fidelity will be ascertained each procedural variable at specified times throughout the study. Written instruction forms detailing interventions will be given to the family (Gast, 2010).
Random Irrelevancies in the Experimental Setting (May inflate error)	Within subject analysis – should not pose a problem if baseline is stable as the baseline reflects background variable effects (Shadish et al., 2002) Cross-over analysis: Period confounds should be accounted for using the two sample cross over <i>t</i> -test (Shuster, 2007)
Random Heterogeneity of Respondents (Increases variability in outcome, increasing error variance)	Both designs use subjects as their own control substantially decreasing variance due to subject heterogeneity (Cook & Campbell, 1979).
Violated assumptions	Within Subject analysis – violates statistical assumptions that error residuals are independent, normally distributed, and homoscedastic. Serial dependency can be modeled and removed. Cross-over analysis – treatment sequences considered independent. Carry-over effects should not be a factor due to design considerations but are accounted for using the two sample <i>t</i> -test (Shuster, 2007)

Shadish et al. (2002) defined threats to statistical conclusion validity as “reasons why inferences about covariation between two variables may be incorrect” (p. 45) violations of which result in over- or underestimation of the magnitude and statistical significance of treatment effects.

Appendix H

Threats to Internal Validity

Threat	Assessment of Potential Threat & Proposed Control
Ambiguous Temporal Precedence (cause precedes effect)	This is a prospective study. Ascertainment of temporal precedence and ambiguity regarding direction of causal inference is not a risk
Attrition (subject mortality)	Attrition will have a significant effect on data analysis. Careful follow-up and provision of supplies may discourage subject fatigue and drop outs. A second randomization to treatment has been instituted after the dose response phase just before the treatment phase to insure randomization scheme if attrition occurs due to ineffective treatment or side effects (Portney & Watkins, 2009)
Maturation (confounding due to passage of time)	This study is several months in duration which should preclude maturational confounding. In addition, randomization to treatment sequence should minimize maturation as a potential bias (Shadish et al., 2002)
History (Confounding events other than the independent variable)	Effects of significant diet change or infection causing diarrhea will confound treatment results. A diary will be kept documenting any obvious historical confounds. In those instances, treatment length will be extended if necessary (Gast, 2010). Use of a match control that did not receive treatment would strengthen the design (Cook & Campbell, 1979) but would be unethical. In addition, disease instability may confound treatment effects. This population generally has a chronic but stable disease and should provide a stable sample to compare treatment effects (Piantadosi, 2005).
Order Effects (Position in sequence) & Carry Over Effects (Effects from previous phase impacting current phase)	Order effects which will be controlled by randomization to one of two treatment sequences (Kazdin, 2011). Carry over effects should not confound continence. There is a prolonged active washout period between serum and stool sampling
Regression (Tendency of extreme scores to regress toward the mean)	Should not pose a threat in this study
Instrumentation (reliability of measurement)	IOA will be checked at key points throughout the study and calculated using gross method. If IOA is <80%, additional observer training will be implemented. The family will have report forms to help standardize and quantify responses. (Gast, 2010; Kazdin, 2011)
Testing	Should not pose a threat in this study

Internal validity questions if there evidence to support a causal relationship between the treatment and outcome variable (Portney & Watkins, 2009).

Appendix I

Social Threats to Internal Validity

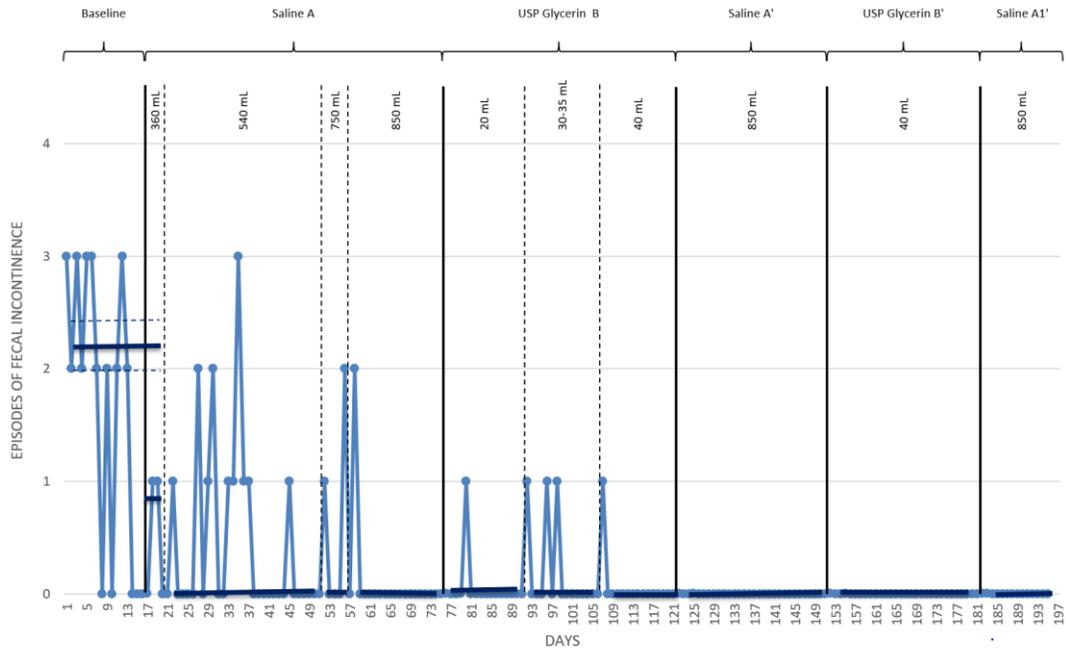
Threat	Assessment of Potential Threat & Proposed Control
Diffusion and Imitation of Treatment	Many of the families who will be enrolled in this study interact socially. Attend a support group together, and attend a clinic multidisciplinary clinic in which they share a waiting room. Subjects are not blinded to treatment. Parents and children enrolled in the study may be influenced by parent or child preference of individuals who are already on ACE therapy. Blinding is not possible due to obvious differences in flush volume (Portney & Watkins, 2009)
Compensatory Equalization of Treatments	Subject or parental preference for one treatment over another based on intangibles not measured in the study may influence treatment implementation and/or evaluation of dependent measures. Blinding is not possible due to obvious differences in flush volume (Portney & Watkins, 2009)
Compensatory Rivalry	All subjects will receive all treatments so no subject should receive what they view as a less than desirable treatment negating the threat of compensatory rivalry (Portney & Watkins, 2009)
Resentful Demoralization	All subjects will receive all treatments so no subject should receive what they view as a less than desirable treatment negating the threat of resentful demoralization (Portney & Watkins, 2009)

Social Validity questions if there is evidence to support a causal relationship between the treatment and outcome variable are influenced by social interactions or subject's awareness regarding treatment specifics (Portney & Watkins, 2009).

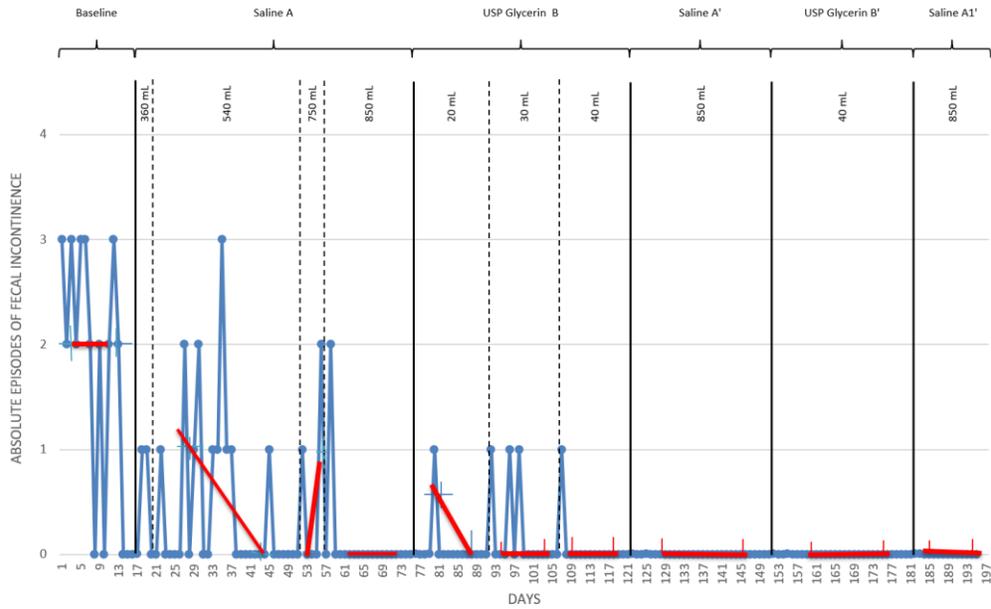
APPENDIX O

KJ001 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS DETAILING STABILITY AND TREND

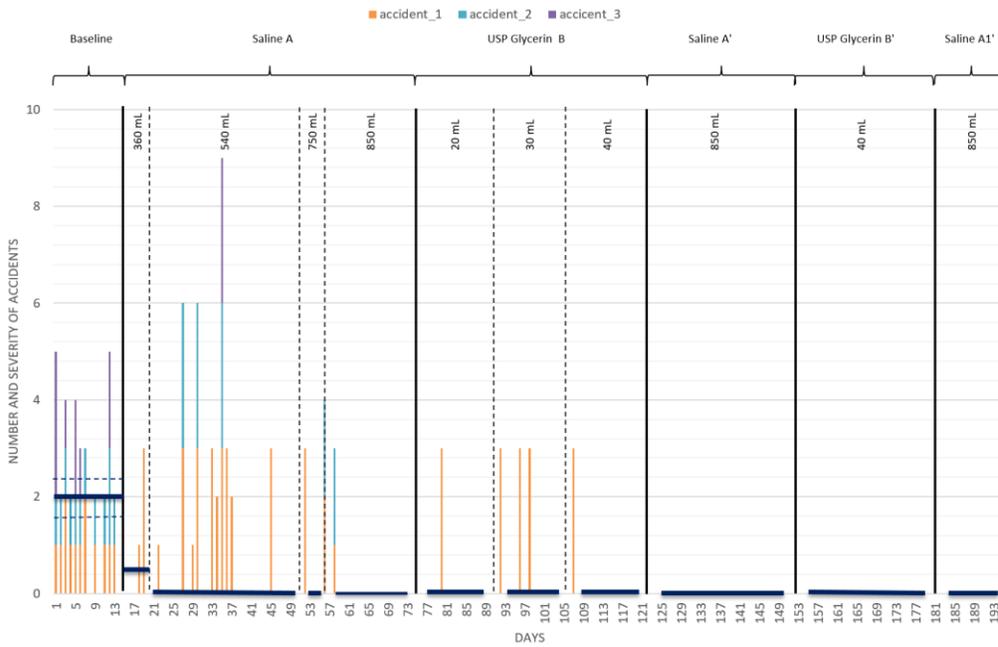
KJ001 STABILITY OF ABSOLUTE EPISODES OF INCONTINENCE DATA



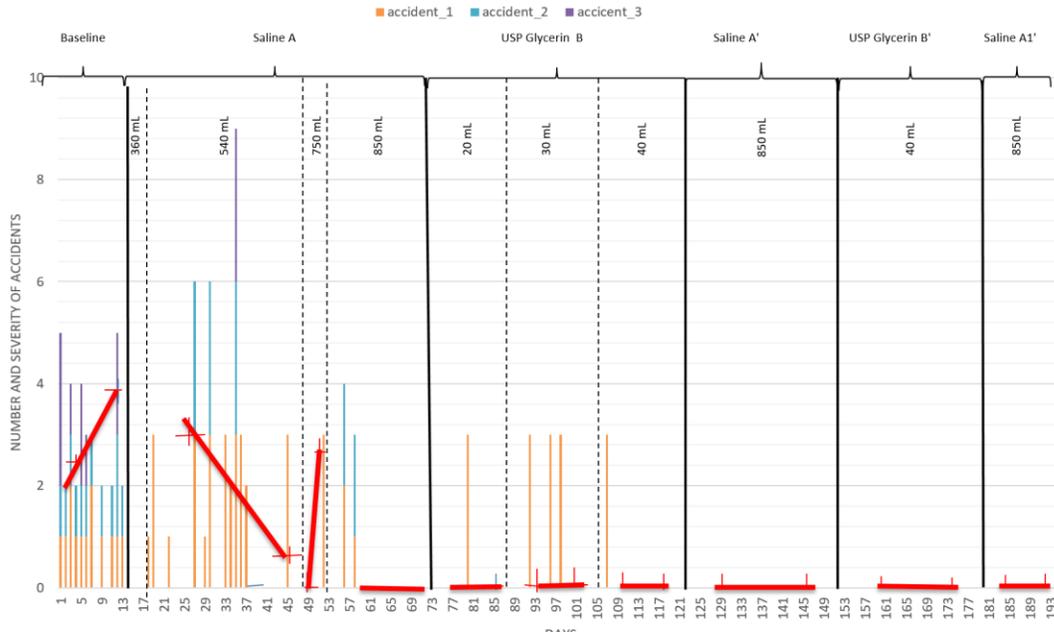
KJ001 TREND FOR ABSOLUTE EPISODES OF INCONTINENCE DATA



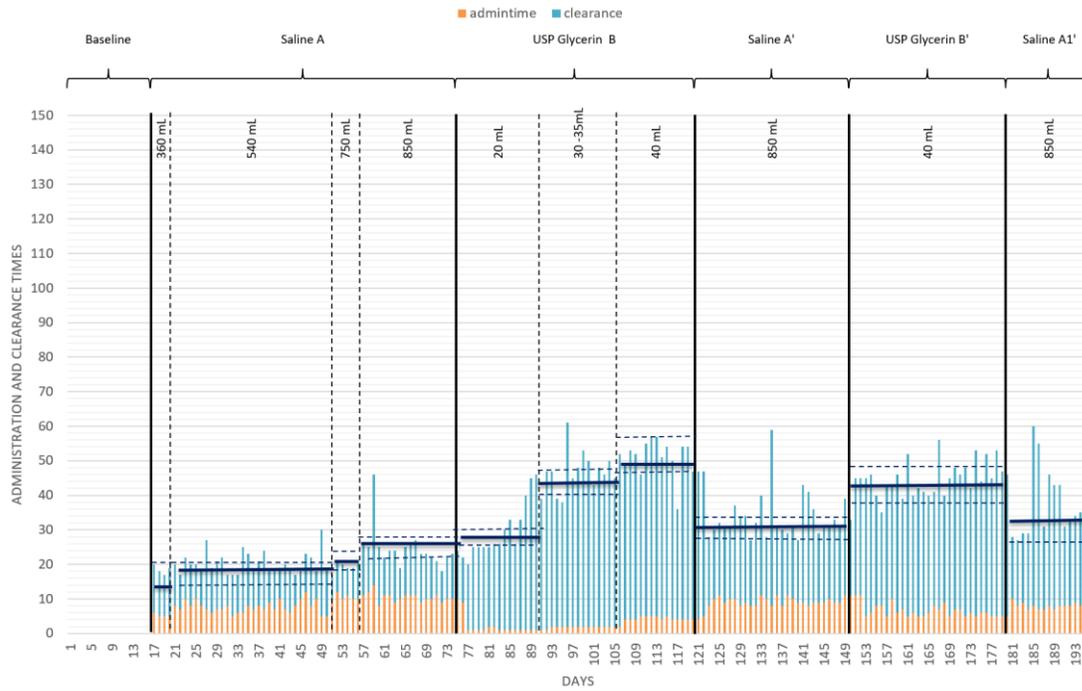
KJ001 STABILITY OF SEVERITY OF INCONTINENCE DATA



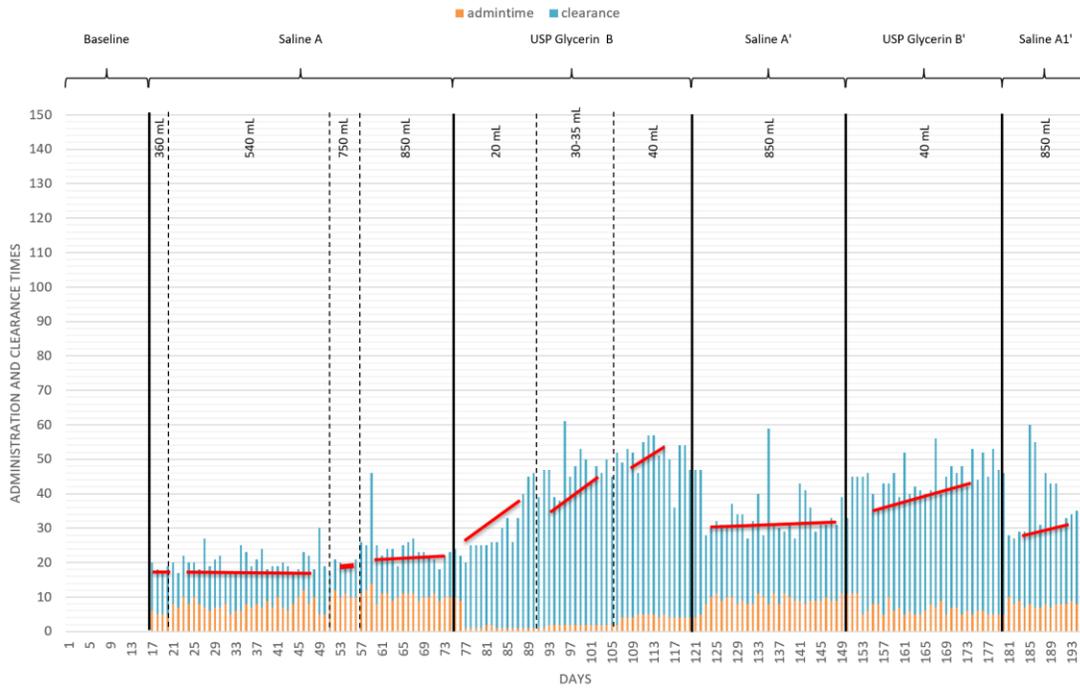
KJ001 TRENDS IN SEVERITY OF INCONTINENCE DATA



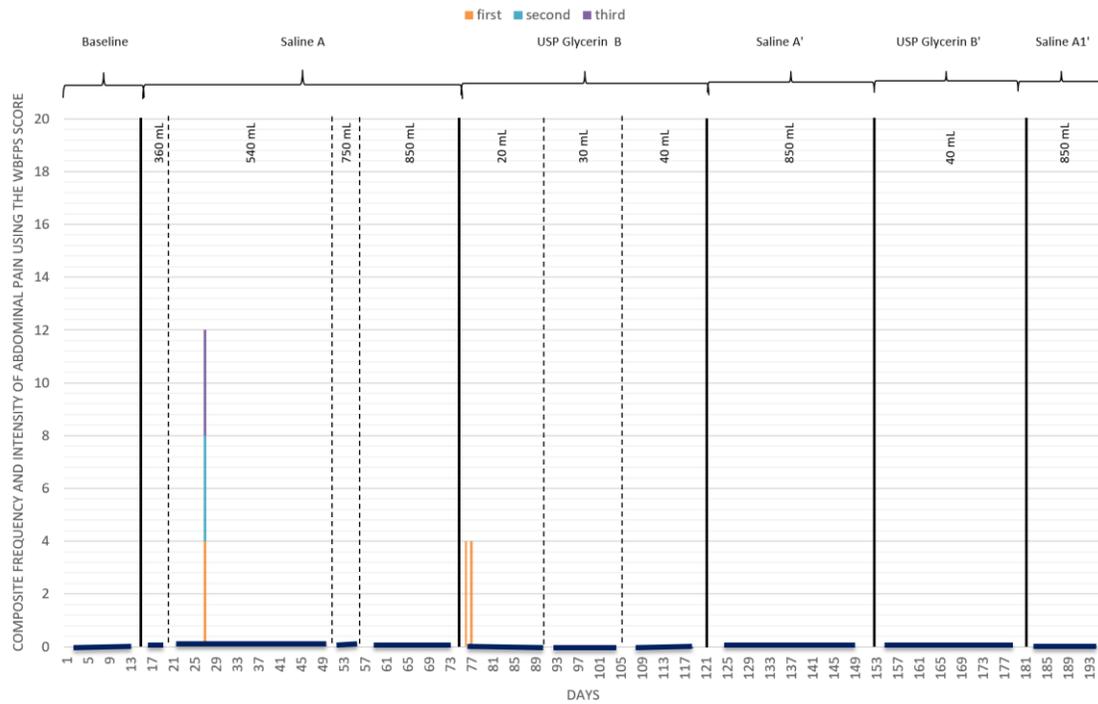
KJ001 STABILITY OF PROCEDURAL TIME DATA



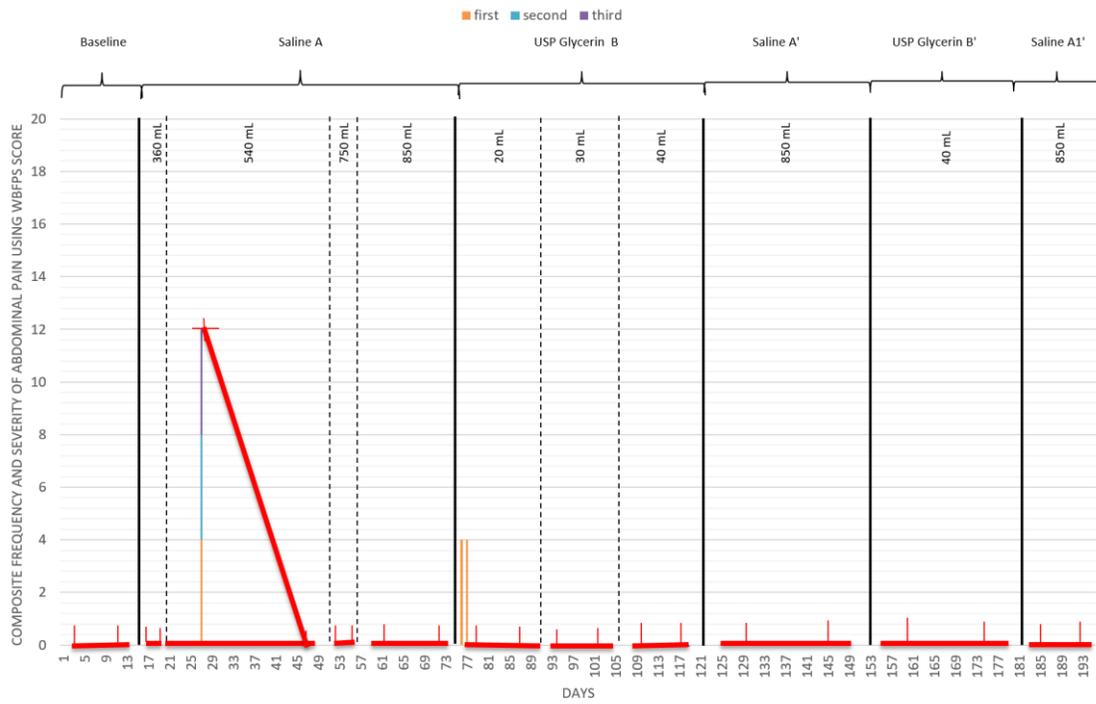
KJ001 TREND FOR PROCEDURAL TIME DATA



KJ001 STABILITY OF ABDOMINAL PAIN DATA



KJ001 TRENDS IN ABDOMINAL PAIN DATA



KJ001 Absolute Episodes and Severity of Fecal Incontinence in Baseline - Results of Single Subjects Data Analysis

Condition Sequence	Pre-Operative Data Absolute Count	Pre-Operative Data Severity
Median	2	2
Mean	1.929	2.429
Range	0-3	0-5
Stability Envelope	0.4	0.4
# data points inside envelope	6 of 14	5 of 14
% data points inside envelope	43%	36%
Stability	Unstable	Unstable
Trend direction	↔	↔
Multiple Paths Within Trend		
Absolute Level Change	3	5
Assessment	Improving	Improving
Relative Level Change	1.5	2
Assessment	Improving	Improving

KJ001 Episodes of Fecal Incontinence on Saline in Dosing Phase - Results of Single Subjects Analysis

	Baseline Absolute Count	Dosing Saline 360mL x 4d Absolute	Dosing Saline 540mL x 31d Absolute	Dosing Saline 740mL x 5d Absolute	Dosing Saline 850mL x 20d Absolute
Median	2	0.5	0	0	0
Mean	1.929	0.5	0.452	0.4	0.1
Range	0-3	0-1	0-3	0-2	0-2
Stability Envelope	0.4	0.1	0	0	0
# data points inside envelope	6 of 14	0 of 4	20 of 31	3 of 5	19 of 20
% data points inside envelope	43%	0%	64.52%	60%	95%
Stability	Unstable	Unstable	Unstable	Unstable	Stable
Trend	↔	↔	↓	↑	↔
Multiple Paths Within Trend					
Direction					
Absolute Level Change	3	0	1	0	2
Assessment	Improving		Deteriorating	Deteriorating	Deteriorating
Relative Level Change	1.5	0	3	2	0
Assessment	Improving		Deteriorating	Deteriorating	

KJ001 Episodes of Fecal Incontinence on Glycerin During Dosing Phase

	Dosing Glycerin 20 - 25mL x 15d Absolute	Dosing Glycerin 30mL x 4d Absolute	Dosing Glycerin 35mL x 11d Absolute	Dosing Glycerin 40mL x 16 d Absolute
Median	0	0	0	0
Mean	0.067	0.25	0.182	0.063
Range	0-1	0-1	0-1	0-1
Stability Envelope	0	0	0	0
# data points inside envelope	14 of 15	3 of 4	9 of 11	15 of 16
% data points inside envelope	93%	75%	82%	94%
Stability	Stable	Unstable	Stable	Stable
Trend	↔	↔	↔	↔
Multiple Paths Within Trend				
Direction				
Absolute Level Change	0	1	1	1
Assessment		Improving	Improving	Improving
Relative Level Change	0	0.5	0	0
Assessment		Improving		

KJ001 Severity of Fecal Incontinence on Saline in Dosing Phase - Results of Single Subjects Analysis

	Baseline Severity	Dosing Saline 360mL x 4d Severity	Dosing Saline 540mL x 31d Severity	Dosing Saline 740mL x 5d Severity	Dosing Saline 850mL x 20d Severity
Median	2	0.5	0	0	0
Mean	2.429	1	1.161	0.8	0.5
Range	0-5	0-3	0-9	0-4	0-3
Stability Envelope	0.4	0.1	0	0	0
# data points inside envelope	5 of 14	0 of 4	20 of 31	3 of 5	19 of 20
% data points inside envelope	36%	0%	64.52%	60%	95%
Stability	Unstable	Unstable	Unstable	Unstable	Stable
Trend	↔	↔	↓	↑	↔
Multiple Paths Within Trend					
Direction					
Absolute Level Change	5	0	0	0	3
Assessment	Improving			Deteriorating	Deteriorating
Relative Level Change	2	0	0	4	0
Assessment	Improving			Deteriorating	

KJ001 Severity of Fecal Incontinence on Glycerin During Dosing Phase - Results of Single Subjects Analysis

	Dosing Glycerin 20 - 25mL Severity	Dosing Glycerin 30mL Severity	Dosing Glycerin 35mL Severity	Dosing Glycerin 40mL Severity
Median	0	0	0	0
Mean	0.02	0.75	0.545	0.188
Range	0-3	0-3	0-3	0-3
Stability Envelope	0	0	0	0
# data points inside envelope	14 of 15	3 of 4	9 of 11	15 of 16
% data points inside envelope	93%	75%	82%	94%
Stability	Stable	Unstable	Stable	Stable
Trend	↔	↔	↔	↔
Multiple Paths Within Trend				
Direction				
Absolute Level Change	0	3	3	3
Assessment		Improving	Improving	Improving
Relative Level Change	0	1.5	0	0
Assessment		Improving		

KJ001 Procedural Time Chart on Saline in Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 360mL x 4d	Dosing Saline 540mL x 31d	Dosing Saline 740mL x 5d	Dosing Saline 850mL x 20d
Median	18.5	19	20	23.5
Mean	18.5	20.258	20	24.35
Range	18 -20	17-30	19-21	18-46
Stability Envelope	3.7	3.8	4	4.7
# data points inside envelope	4 of 4	20 of 31	5 of 5	15 of 20
% data points inside envelope	100%	65.00%	100%	75%
Stability	Stable	Unstable	Stable	Unstable
Trend Direction	↔	↔	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	1	3	0	4
Assessment	Improving	Improving		Improving
Relative Level Change	2	1	0.5	4
Assessment	Deteriorating	Improving	Improving	Improving

KJ001 Procedural Time Chart on Glycerin in Dosing Phase - Results of Single Subjects Analysis

	Dosing USP Glycerin 20 - 25mL x 15d	Dosing USP Glycerin 30 - 35mL x 15d	Dosing USP Glycerin 40mL x 16d
Median	26	47	51.5
Mean	30.933	47.467	50.563
Range	20-46	38-61	36-57
Stability Envelope	5.2	9.4	10.3
# data points inside envelope	8 of 15	11 of 15	14 of 16
% data points inside envelope	53%	73%	88%
Stability	Unstable	Unstable	Stable
Trend Direction	↑	↑	↑
Multiple Paths Within Trend			
Absolute Level Change	19	5	2
Assessment	Deteriorating	Deteriorating	Improving
Relative Level Change	14	2	2.5
Assessment	Deteriorating	Improving	Improving

KJ001 Procedural Time Chart in Maintenance Phase - Results of Single Subjects Analysis

	Maintenance Saline 850mL x 30d	Maintenance Glycerin 40mL x 28d	Maintenance Saline 850mL x 15d
Median	31.5	45	33
Mean	34.267	44.929	36.933
Range	27-59	35-56	27-60
Stability Envelope	6.3	9	6.6
# data points inside envelope	19 of 30	22 of 28	8 of 15
% data points inside envelope	63%	79%	53%
Stability	Unstable	Unstable	Unstable
Trend Direction	↔	↑	↑
Multiple Paths Within Trend			
Absolute Level Change	17	1	2
Assessment	Deteriorating	Deteriorating	Deteriorating
Relative Level Change	2	5	5
Assessment	Deteriorating	Deteriorating	Deteriorating

KJ001 Severity of Cramping With Saline Flush During Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 360mL x 4d Severity	Dosing Saline 540mL x 31d Severity	Dosing Saline 740mL x 5d Severity	Dosing Saline 850mL x 20d Severity
Median	0	0	0	0
Mean	0	0.387	0	0.2
Range	0-0	0-12	0-0	0-4
Stability Envelope	0	0	0	0
# data points inside envelope	4 of 4	30 of 31	5 of 5	19 of 20
% data points inside envelope	100%	97%	100%	95%
Stability	Stable	Stable	Stable	Stable
Trend direction	↔	↑	↔	↔
Multiple Paths Within Trend				
Direction				
Absolute Level Change	0	0	0	0
Assessment				
Relative Level Change	0	0	0	0

KJ001 Severity of Cramping With Glycerin Flush During Dosing Phase - Results of Single Subjects Analysis

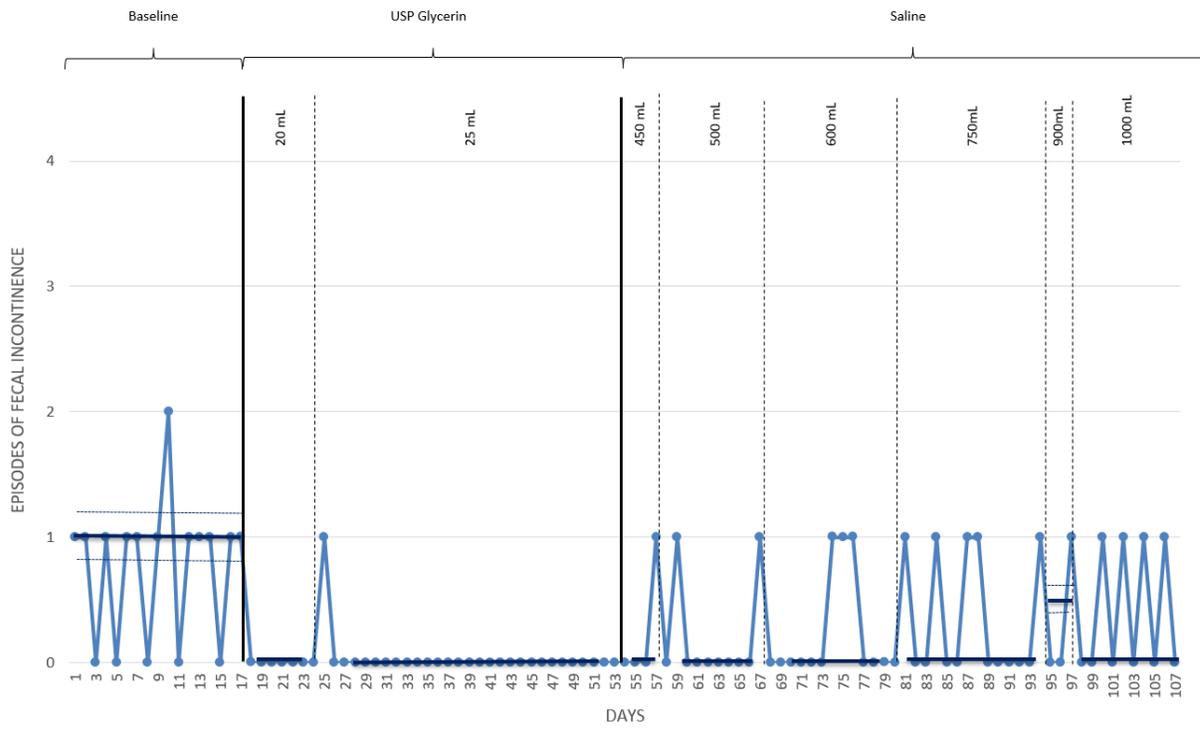
	Dosing Glycerin 20 - 25mL x 15d Severity	Dosing Glycerin 30 - 35mL x 15d Severity	Dosing Glycerin 40mL x 16d Severity
Median	0	0	0
Mean	0	0	0
Range	0-0	0-0	0-0
Stability Envelope	0	0	0
# data points inside envelope	15 of 15	15 of 15	16 of 16
% data points inside envelope	100%	100%	100%
Stability	Stable	Stable	Stable
Trend direction	↔	↔	↔
Multiple Paths Within Trend Direction			
Absolute Level Change Assessment	0	0	0
Relative Level Change Assessment	0	0	0

KJ001 Episodes and Severity of Cramping With Flush During Maintenance Phase - Results of Single Subjects Analysis

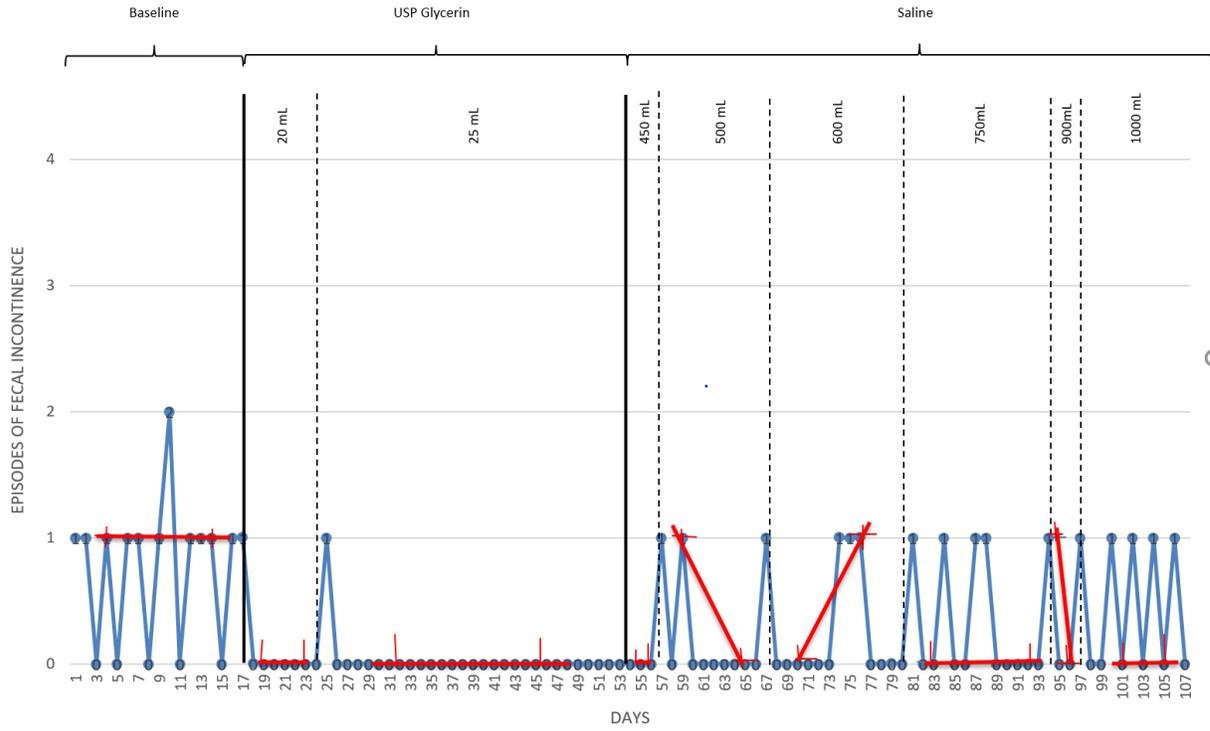
	Maintenance Saline 850mL x 30d Absolute	Maintenance Saline 850mL x 30d Severity	Maintenance Glycerin 40mL x 28d Absolute	Maintenance Glycerin 40mL x 28d Severity	Maintenance Saline 850mL x 15d Absolute	Maintenance Saline 850mL x 15d Severity
Median	0	0	0	0	0	0
Mean	0	0	0	0	0	0
Range	0-0	0-0	0-0	0-0	0-0	0-0
Stability Envelope	0	0	0	0	0	0
# data points inside envelope	30 of 30	30 of 30	28 of 28	28 of 28	15 of 15	15 of 15
% data points inside envelope	100%	100%	100%	100%	100%	100%
Stability	Stable	Stable	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔	↔	↔
Multiple Paths Within Trend Direction						
Absolute Level Change Assessment	0	0	0	0	0	0
Relative Level Change Assessment	0	0	0	0	0	0

APPENDIX P
 KJ002 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS
 DETAILING STABILITY AND TREND

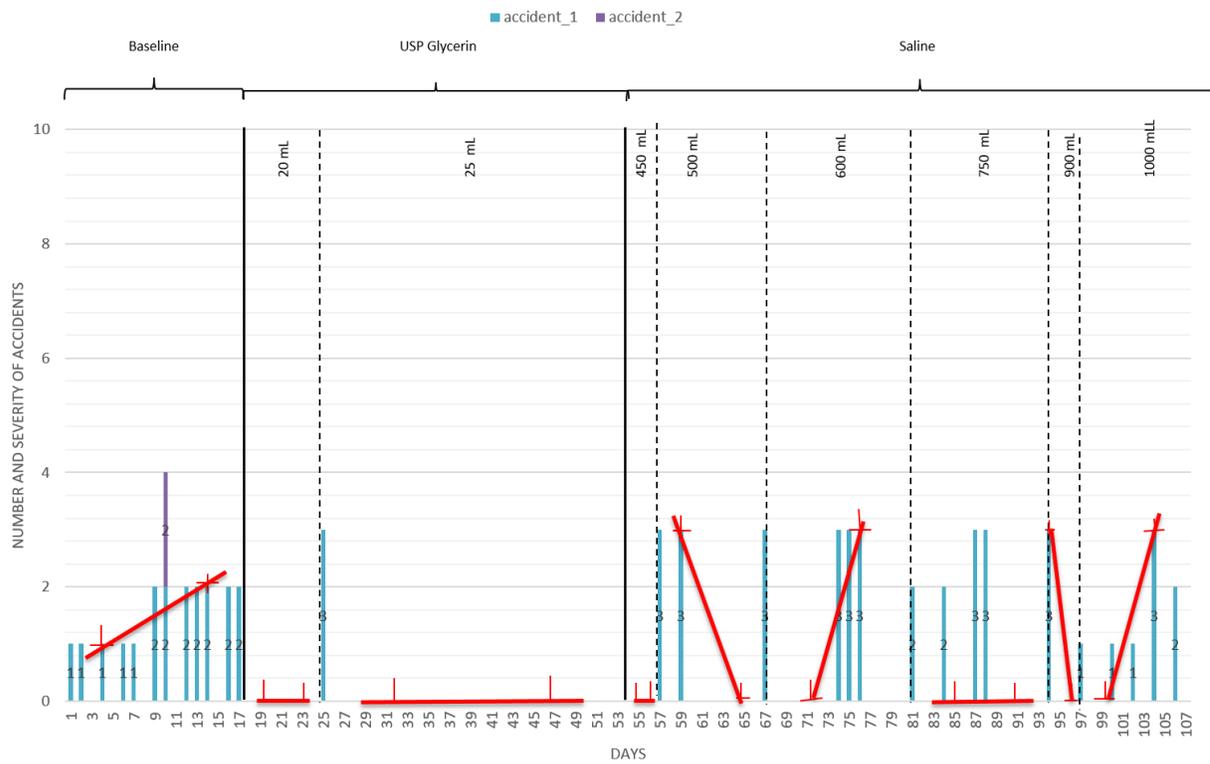
KJ002 STABILITY OF ABSOLUTE EPISODES OF INCONTINENCE DATA



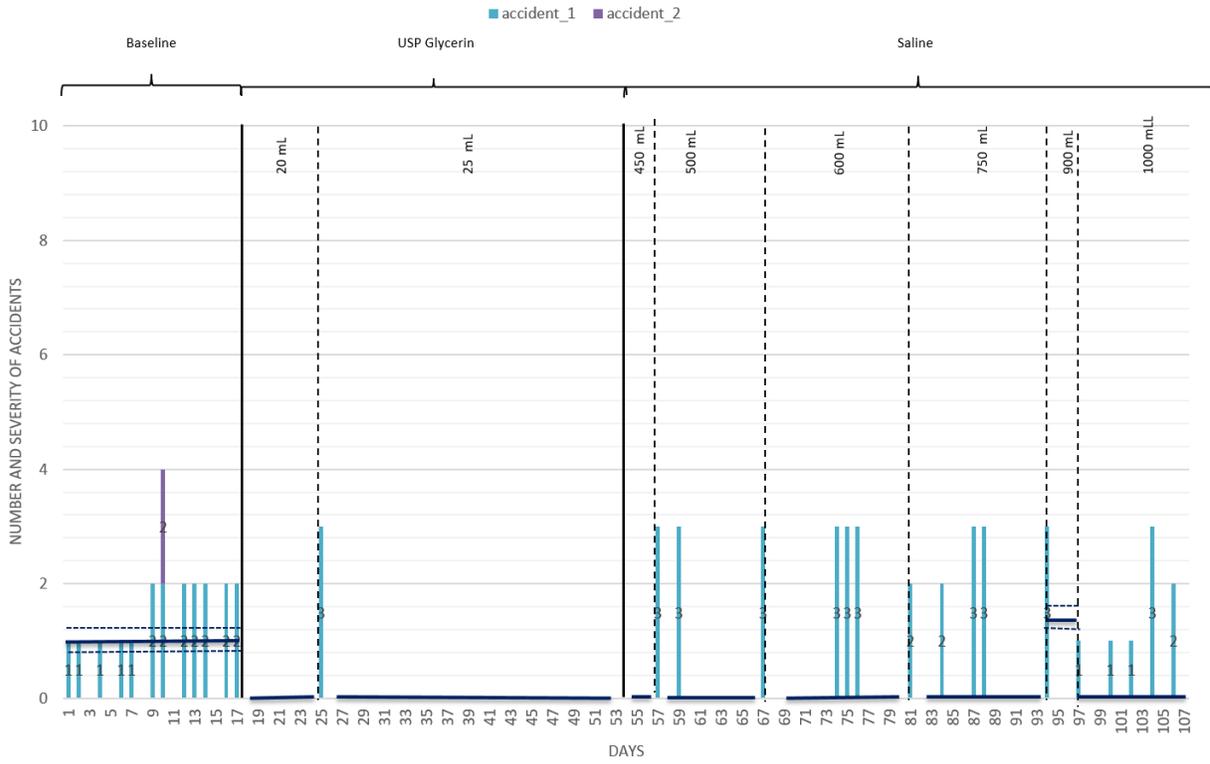
KJ002 TREND FOR ABSOLUTE FREQUENCY OF INCONTINENCE DATA



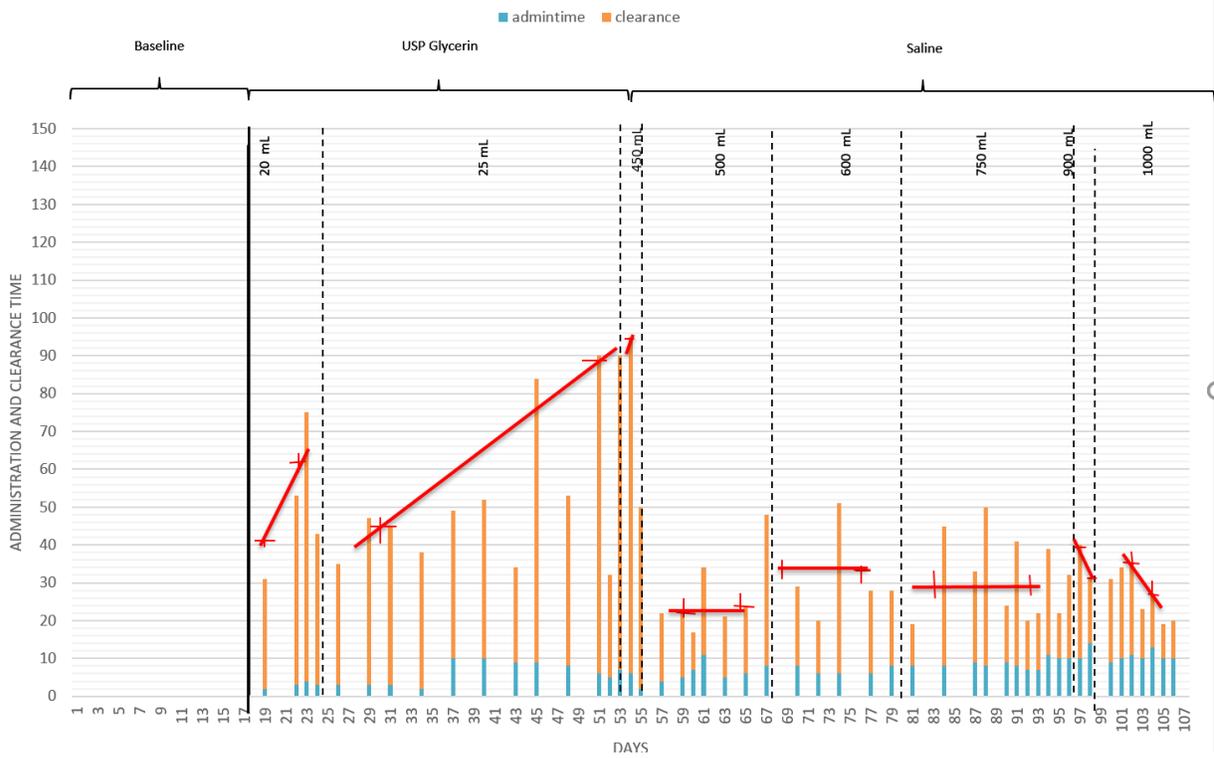
KJ002 TREND FOR SEVERITY OF INCONTINENCE DATA



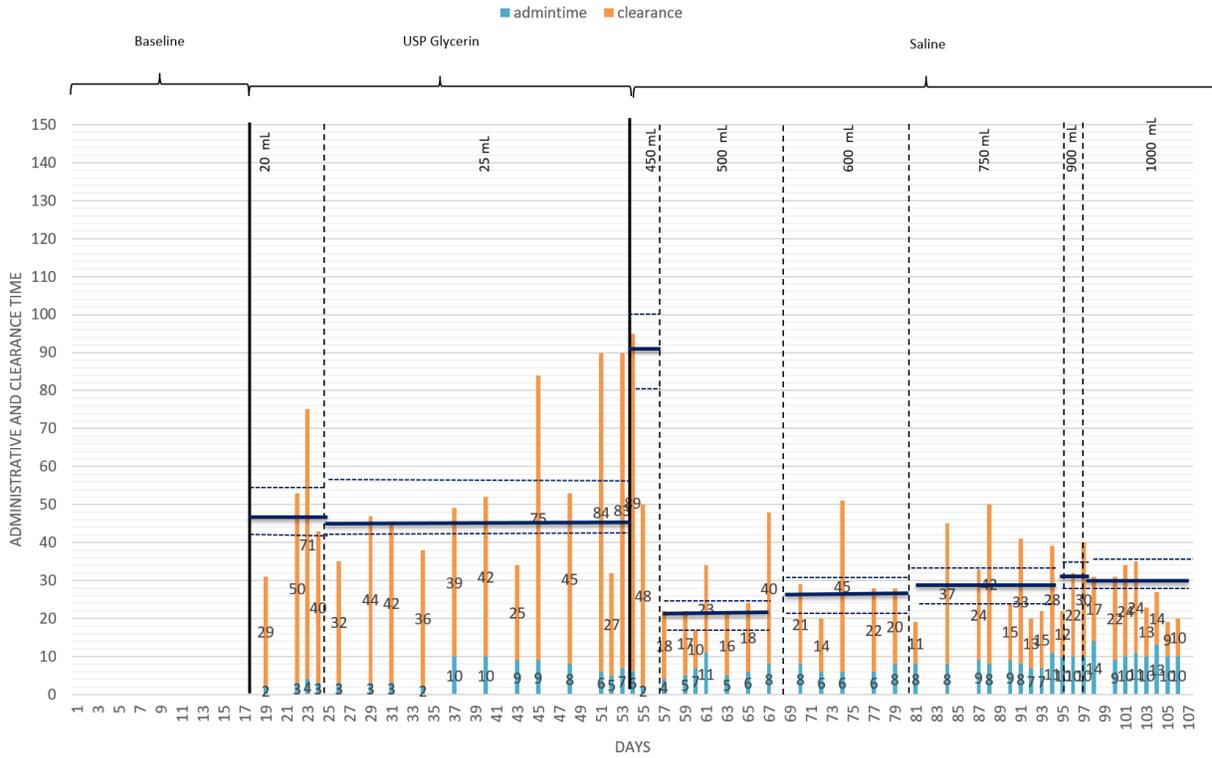
KJ002 STABILITY FOR SEVERITY OF INCONTINENCE DATA



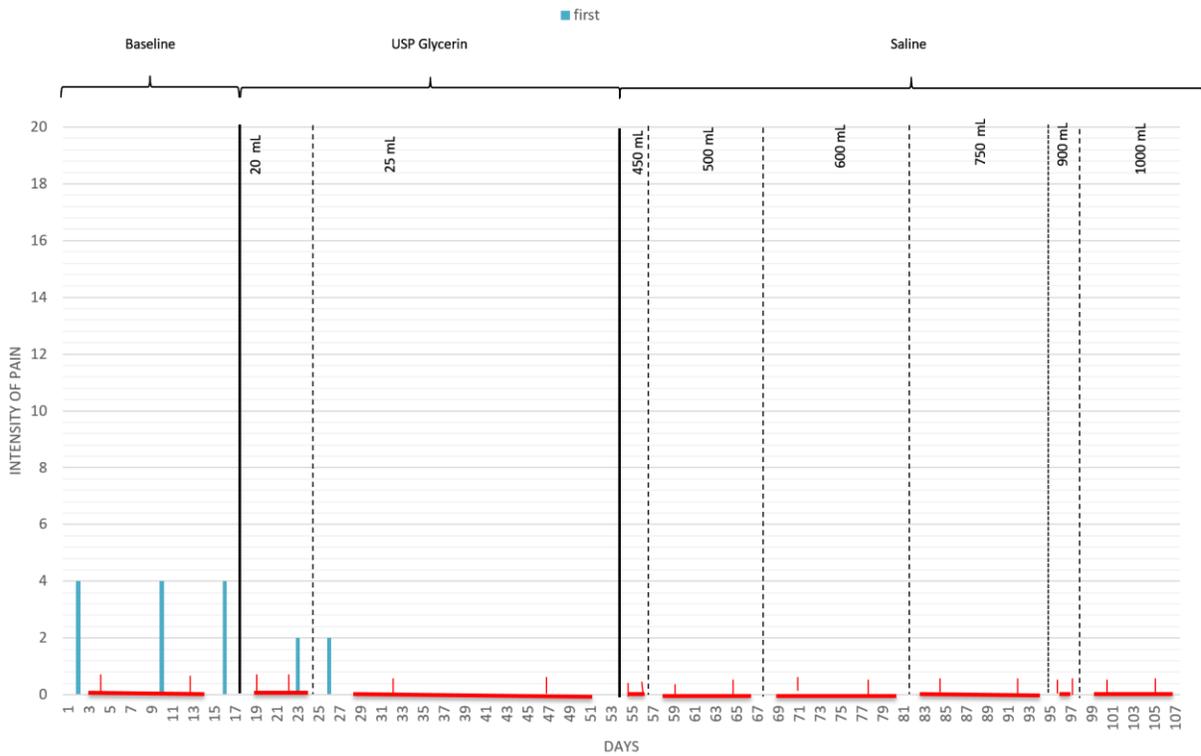
KJ002 TREND FOR PROCEDURAL TIME DATA



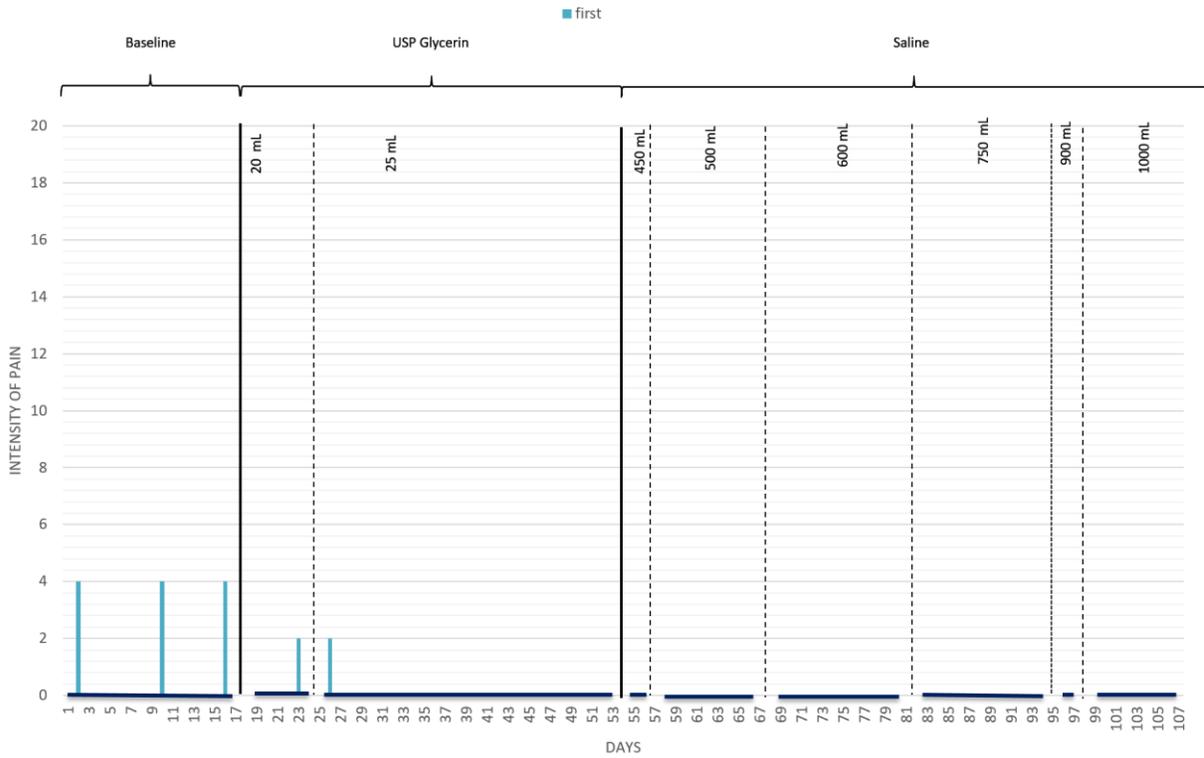
KJ002 STABILITY FOR PROCEDURAL TIME DATA



KJ002 TREND FOR ABDOMINAL PAIN DATA



KJ002 STABILITY OF ABDOMINAL PAIN DATA



KJ002 Episodes and Severity of Fecal Incontinence in Baseline Phase - Results of Single Subjects Analysis

	Pre-Operative Data 17 days duration Absolute	Pre-Operative Data 17 days duration Severity
Median	1	1
Mean	0.765	1.235
Range	0-2	0-4
Stability Envelope	0.2	0.2
# data points inside envelope	11 of 17	5 of 17
% data points inside envelope	65%	29%
Stability	Unstable	Unstable
Trend direction	↔	↑
Multiple Paths Within Trend		
Absolute Level Change	0	1
Assessment	Zero Celerating	Deteriorating
Relative Level Change	0	1
Assessment	Zero Celerating	Deteriorating

KJ002 Episodes of Fecal Incontinence on Saline Flush in Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 450mL 2doses x 2d Absolute	Dosing Saline 500mL 7 doses x 12 Absolute	Dosing Saline 600 5 doses x 11d Absolute	Dosing Saline 750mL 9 doses x 15d Absolute	Dosing Saline 900mL 2 doses x 2d Absolute	Dosing Saline 1000mL 10 doses x 11d Absolute
Median	0	0	0	0	0.5	0
Mean	0	0.167	0.333	0.267	0.5	0.455
Range	0-0	0-1	0-1	0-1	0-1	0-1
Stability Envelope	0	0	0	0	0.1	0
# data points inside envelope	2 of 2	10 of 12	8 of 12	11 of 15	1 of 2	6 of 11
% data points inside envelope	100%	83%	67%	73%	50%	55%
Stability	Stable	Stable	Unstable	Unstable	Unstable	Unstable
Trend direction	↔	↓	↑	↔	↓	↔
Multiple Paths Within Trend						
Absolute Level Change	0	0	1	0	1	1
Assessment	Zero Celerating	Zero Celerating	Improving	Zero Celerating	Improving	Deteriorating
Relative Level Change	0	0	0.5	0	Insufficient #	1
Assessment	Zero Celerating	Zero Celerating	Deteriorating	Zero Celerating	Insufficient #	Deteriorating

KJ002 Severity of Fecal Incontinence on Saline Flush in Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 450mL 2 doses x 2d Severity	Dosing Saline 500mL 7 doses x 12d Severity	Dosing Saline 600mL 5 doses x 11d Severity	Dosing Saline 750mL 9 doses x 15d Severity	Dosing Saline 900mL 12 doses x 2d Severity	Dosing Saline 1000mL 10 doses x 11d Severity
Median	0	0	0	0	1.5	0
Mean	0	0.5	1	0.667	1.5	0.727
Range	0-0	0-3	0-12	0-3	0-3	0-3
Stability Envelope	0	0	0	0	0.6	0
# data points inside envelope	2 of 2	10 of 12	8 of 2	11 of 15	1 of 2	6 of 11
% data points inside envelope	100%	83%	67%	73%	50%	55%
Stability	Stable	Stable	Unstable	Unstable	Unstable	Unstable
Trend direction	↔	↓	↑	↔	↓	↔
Multiple Paths Within Trend						
Absolute Level Change	0	0	3	0	3	2
Assessment	Zero Celerating	Zero Celerating	Improving	Zero Celerating	Improving	Deteriorating
Relative Level Change	0	0	1.5	0	Insufficient #	1
Assessment	Zero Celerating	Zero Celerating	Deteriorating	Zero Celerating	Insufficient #	Deteriorating

KJ002 Episodes and Severity of Fecal Incontinence on Glycerin Flush in Dosing Phase - Results of Single Subjects Analysis

	Dosing Glycerin 20 mL 7 doses 7d Absolute	Dosing Glycerin 20 mL 7 doses 7d Severity	Dosing Glycerin 25mL 11 doses in 30d Absolute	Dosing Glycerin 25mL 11 doses in 30d Severity
Median	0	0	0	0
Mean	0.143	0.429	0	0
Range	0-1	0-3	0-0	0-0
Stability Envelope	0	0	0	0
# data points inside envelope	6 of 7	6 of 7	30 of 30	30 of 30
% data points inside envelope	86%	86%	100%	100%
Stability	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	1	0	0	0
Assessment	Deteriorating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	2	0	0	0
Assessment	Deteriorating	Zero Celerating	Zero Celerating	Zero Celerating

KJ002 Procedural Time Chart in Glycerin and Saline in Dosing Phase - Results of Single Subjects Analysis

	Dosing Glycerin 20mL 4 doses/7 d	Dosing Glycerin 25mL 11 doses/30 d	Dosing Saline 450mL 2 doses/2d	Dosing Saline 500mL 7 doses/12d	Dosing Saline 600 5 doses/11d	Dosing Saline 750mL 9 doses/15d	Dosing Saline 900mL 2doses/2d	Dosing Saline 1000mL 10 doses/11d
Median	48	47	92.5	22	29	28	30.5	31
Mean	50.5	50.818	92.5	27.1	35.2	31.33	30.5	29.2
Range	31-75	32-90	90-95	17-50	20-51	19-50	22-39	19-40
Stability Envelope	9.6	9.4	18.5	4.4	5.8	5.6	6.1	6.2
# data points inside envelope	1 of 4	5 of 11	2 of 2	4 of 7	2 of 5	2 of 9	0 of 2	5 of 10
% data points inside envelope	25%	45%	100%	57%	40%	22%	0%	50%
Stability	Unstable	Unstable	Stable	Unstable	Unstable	Unstable	Unstable	Unstable
Trend direction	↑	↑	↑	↔	↔	↔	↓	↓
Multiple Paths Within Trend								
Absolute Level Change	12	58	5	26	20	6	17	20
Assessment	Deteriorating	Deteriorating	Deteriorating	Improving	Improving	Improving	Improving	Improving
Relative Level Change	17	15	Insufficient#	28	1	7.5	Insufficient #	9
Assessment	Deteriorating	Deteriorating	Insufficient#	Improving	Deteriorating	Improving	Insufficient#	Improving

KJ002 Severity of Pain in Baseline and Cramping with Glycerin Flush in Dosing Phase - Results of Single Subjects Analysis

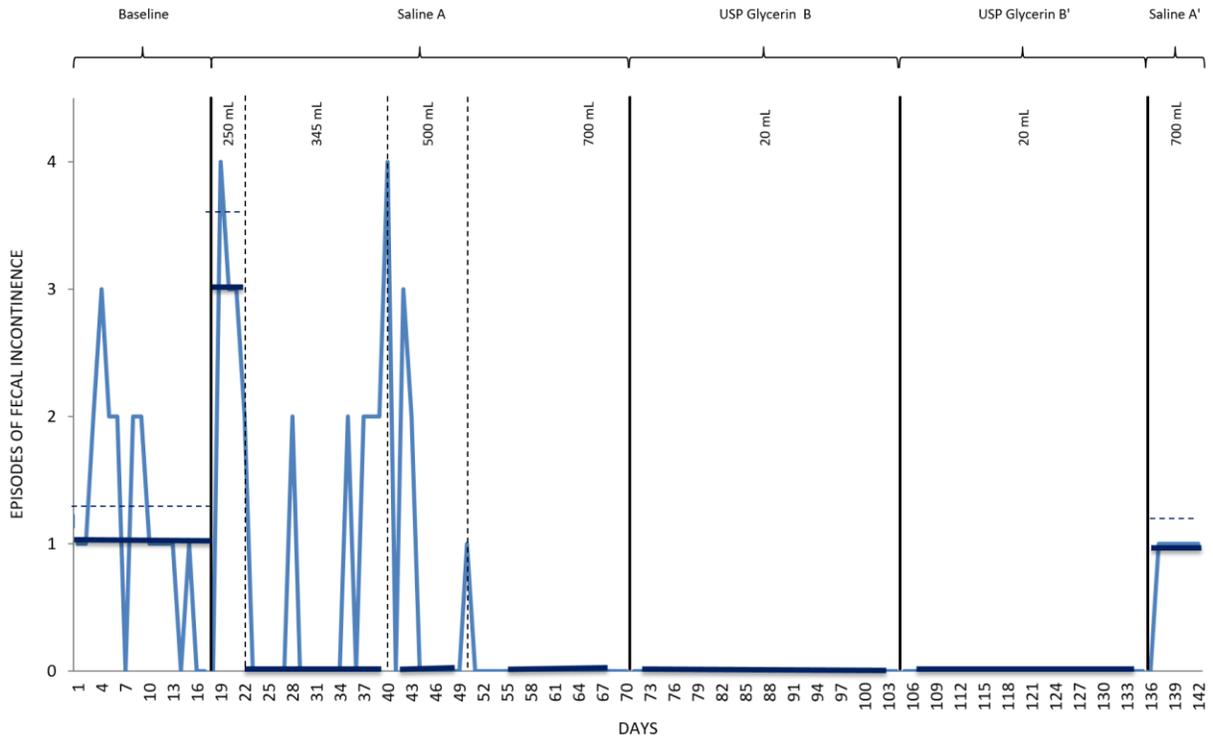
	Baseline	Dosing Glycerin 20mL 7 doses x 7d	Dosing Glycerin 25mL 11 doses x 30 days
	Severity	Severity	Severity
Median	0	0	0
Mean	0	0	0.133
Range	0-0	0-0	0-2
Stability Envelope	0	0	0
# data points inside envelope	17 of 17	7 of 7	28 of 30
% data points inside envelope	100%	100%	93%
Stability	Stable	Stable	Stable
Trend direction	↔	↔	↔
Multiple Paths Within Trend			
Absolute Level Change	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating

KJ002 Episodes of Cramping with Saline Flush in Dosing Phase - Results of Single Subjects Analysis

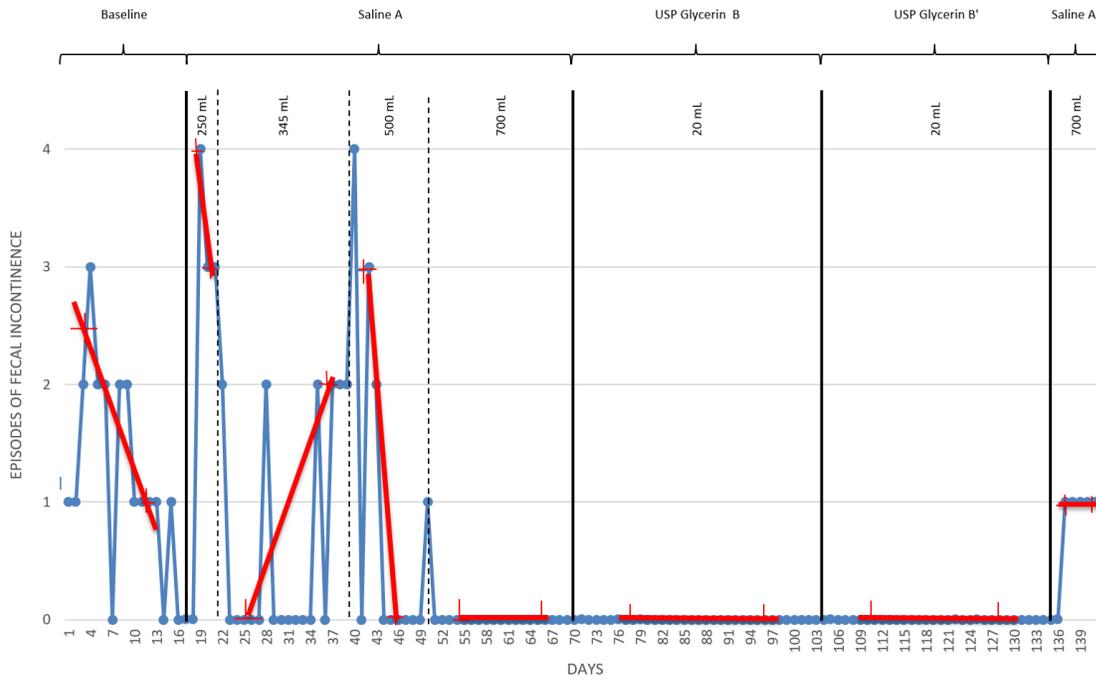
	Dosing Saline 450mL 2 doses x 2d Absolute	Dosing Saline 500mL 7 doses x 12d Absolute	Dosing Saline 600 5 doses x 12d Absolute	Dosing Saline 750mL 9 doses x 15d Absolute	Dosing Saline 900mL 2doses x 2d Absolute	Dosing Saline 1000mL 10 doses x 11d Absolute
Median	0	0	0	0	0	0
Mean	0	0	0	0	0	0
Range	0-0	0-0	0-0	0-0	0-0	0-0
Stability Envelope	0	0	0	0	0	0
# data points inside envelope	2 of 2	7 of 7	5 of 5	9 of 9	2 of 2	10 of 10
% data points inside envelope	100%	100%	100%	100%	100%	100%
Stability	Stable	Stable	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔	↔	↔
Multiple Paths Within Trend						
Absolute Level Change	0	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

APPENDIX Q
 KJ003 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS
 DETAILING STABILITY AND TREND

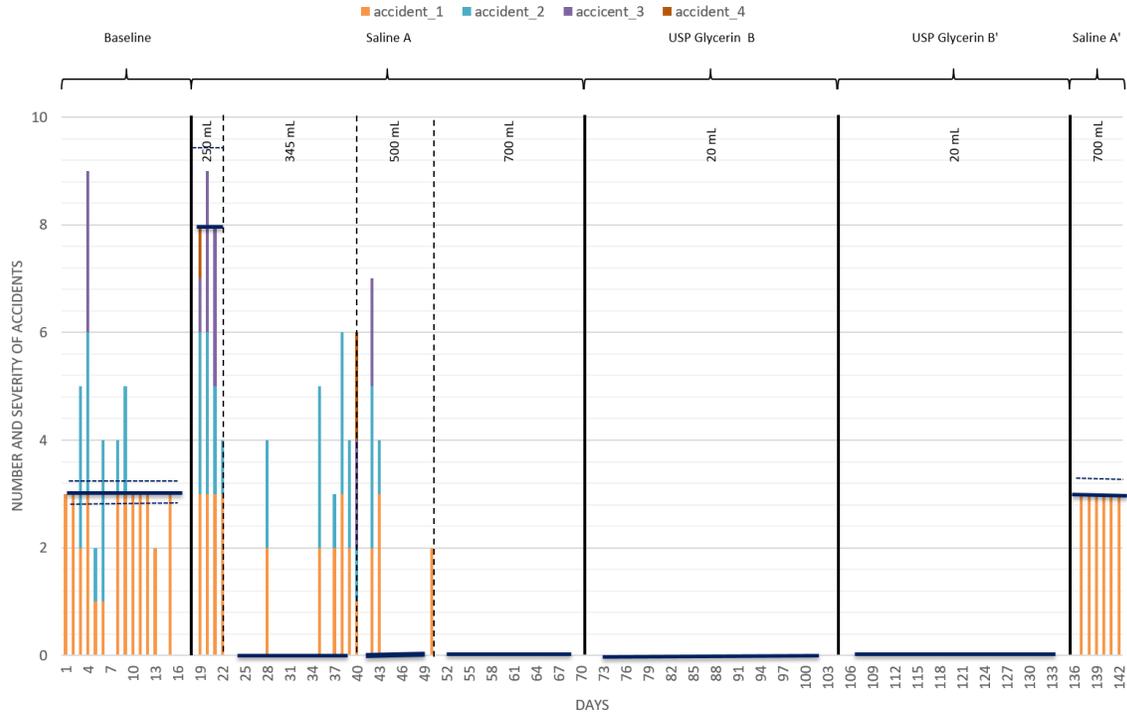
KJ003 STABILITY DATA FOR ABSOLUTE EPISODES OF INCONTINENCE



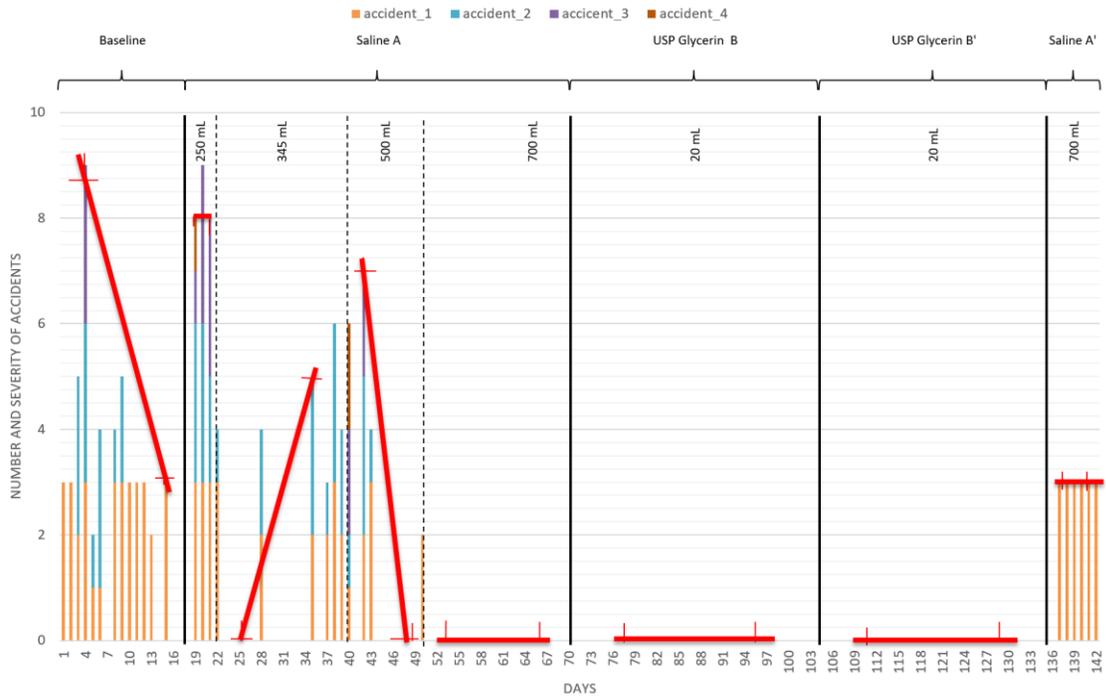
KJ003 TREND FOR ABSOLUTE EPISODES OF INCONTINENCE DATA



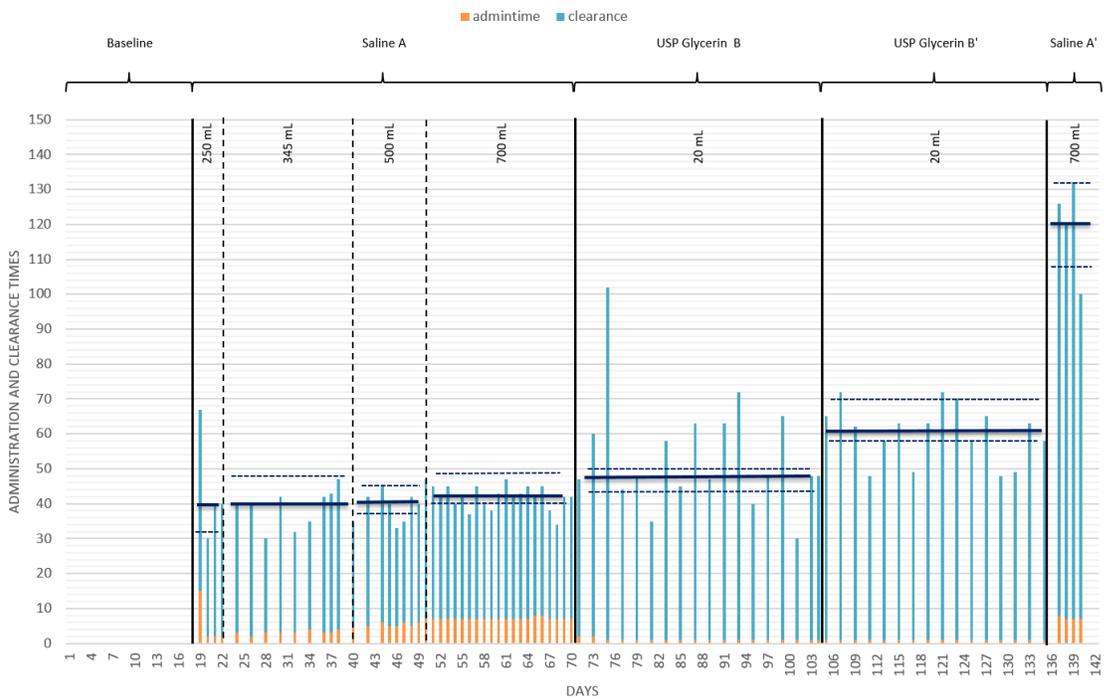
KJ003 STABILITY OF SEVERITY OF INCONTINENCE DATA



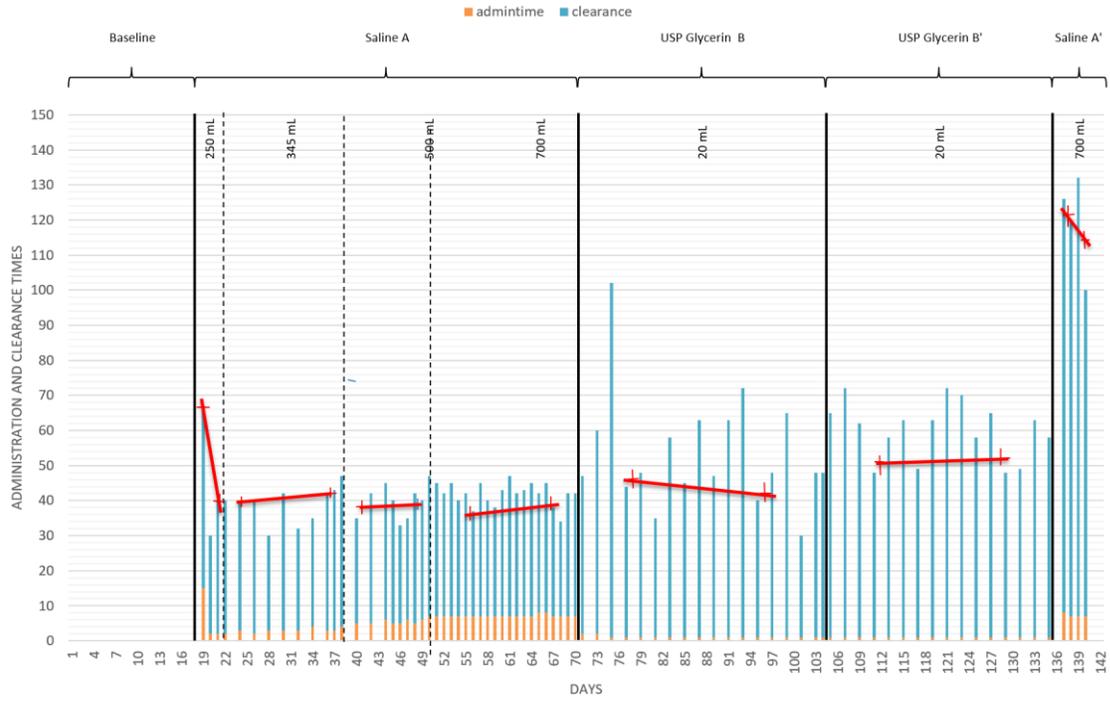
KJ003 TREND FOR SEVERITY OF INCONTINENCE DATA



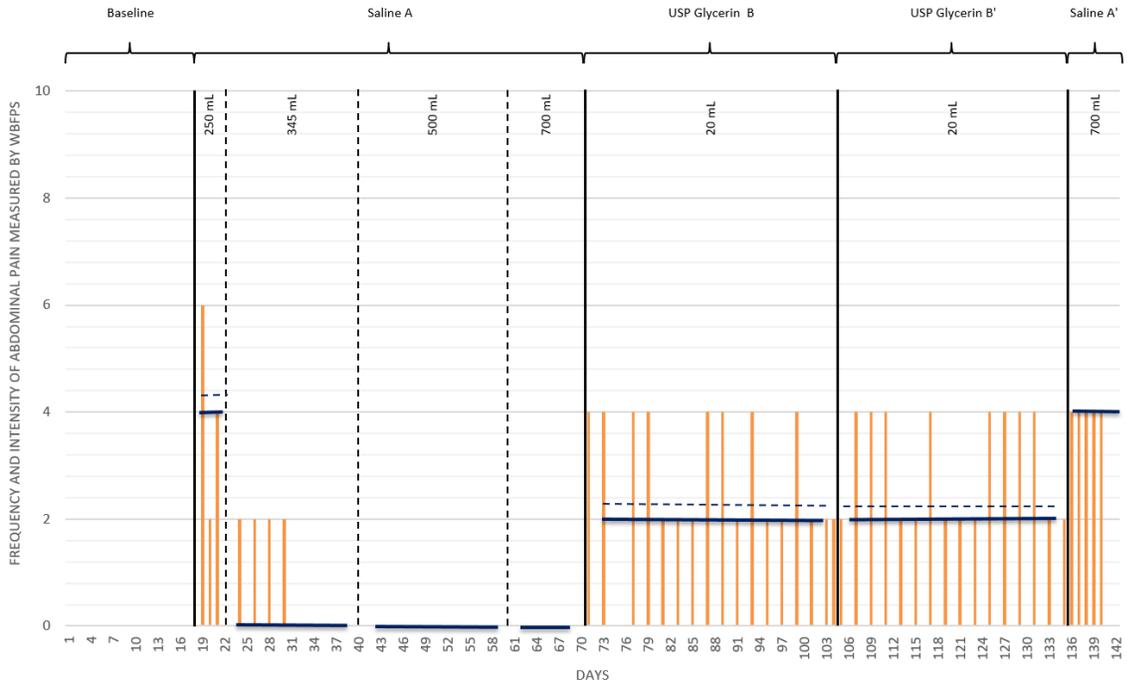
KJ003 STABILITY OF PROCEDURAL TIME DATA



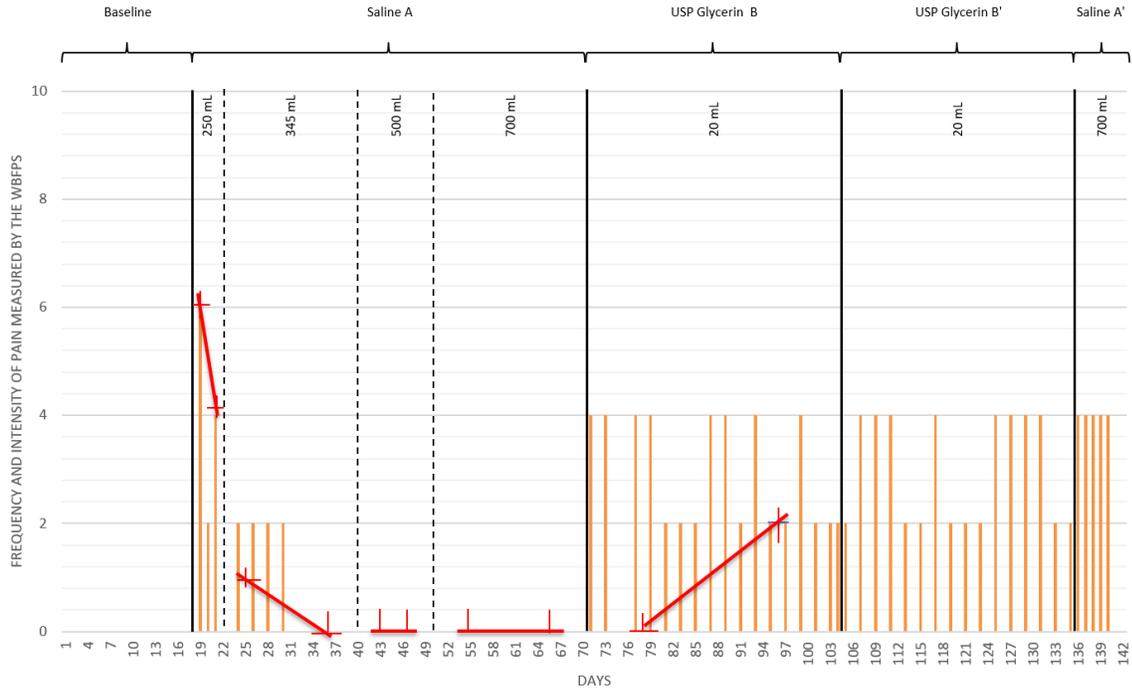
KJ003 TREND FOR PROCEDURAL TIME DATA



KJ003 STABILITY FOR CRAMPING WITH FLUSH DATA



KJ003 TREND FOR CRAMPING FROM FLUSH DATA



KJ003 Episodes of Fecal Incontinence in Baseline and on Saline Flush - Results from Single Subjects Analysis

	Baseline	Dosing Saline 230-250mL 3 doses 3d	Dosing Saline 345mL 10 doses x 18d	Dosing Saline 500mL 8 doses x 10d	Dosing Saline 700 mL 21 doses x 21d
	Absolute	Absolute	Absolute	Absolute	Absolute
Median	1	3	0	0	0
Mean	1.33	3.33	0.333	0.9	0.048
Range	0-3	3-4	0-2	0-4	0-1
Stability Envelope	0.2	0.6	0	0	0
# data points inside envelope	7 of 15	2 of 3	12 of 81	7 of 10	20 of 21
% data points inside envelope	47%	67%	66%	70%	95%
Stability	Unstable	Unstable	Unstable	Unstable	Stable
Trend direction	↓	↔	↑	↓	↔
Multiple Paths Within Trend					
Absolute Level Change	0	1	0	4	1
Assessment		Improving		Improving	Improving
Relative Level Change	1	0	0	2	0
Assessment	Improving			Improving	

KJ003 Severity of Fecal Incontinence on Saline Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 230-250mL 3 doses/3d Severity	Dosing Saline 345mL 10 doses/18d Severity	Dosing Saline 500mL 8 doses/10d Severity	Dosing Saline 700 mL 21 doses/21d Severity
Median	8	0	0	0
Mean	8.33	1.44	1.7	0.095
Range	8-9	0-6		0-2
Stability Envelope	1.6	0	0	0
# data points inside envelope	3 of 3	12 of 18	7 of 10	20 of 21%
% data points inside envelope	100%	66%	70%	95%
Stability	Stable	Unstable	Unstable	Stable
Trend direction	↔	↑	↓	↔
Multiple Paths Within Trend				
Absolute Level Change	0	0	6	2
Assessment			Improving	Improving
Relative Level Change	0	0	4	0
Assessment			Improving	

KJ003 Episodes and Severity of Fecal Incontinence on Glycerin Flush during Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 20mL 17 doses x 33d Absolute	Dosing Glycerin 20mL 17 doses x 33d Severity
Median	0	0
Mean	0	0
Range	0-0	0-0
Stability Envelope	0	0
# data points inside envelope	33 of 33	33 of 33
% data points inside envelope	100%	100%
Stability	Stable	Stable
Trend direction	↔	↔
Multiple Paths Within Trend		
Absolute Level Change	0	0
Assessment		
Relative Level Change	0	0
Assessment		

KJ003 Procedural Time for Saline and Glycerin Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 230-250mL 3 doses x 3d	Dosing Saline 345mL 10 doses x 18d	Dosing Saline 500mL 8 doses x 10d	Dosing Saline 700 mL 21 doses x 21d	Dosing Glycerin 20mL 17 doses x 33d
Median	40	40	40	42	48
Mean	45.66	39.1	39	42.095	53.82
Range	30-67	30-47	33-45	34-47	30-102
Stability Envelope	8	8	8	8.4	9.6
# data points inside envelope	1 of 3	7 of 10	5 of 8	17 of 21	8 of 12
% data points inside envelope	33%	70%	63%	81%	66%
Stability	Unstable	Unstable	Unstable	Stable	Unstable
Trend direction	↓	↑	↑	↔	↓
Multiple Paths Within Trend					
Absolute Level Change	37	7	5	5	1
Assessment	Deteriorating	Deteriorating	Deteriorating	Improving	Deteriorating
Relative Level Change	Insufficient #	2	3.5	0.5	0.5
Assessment	Insufficient #	Deteriorating	Improving	Deteriorating	Improving

KJ003 Episodes of Cramping with Saline Flush - Results of Single Subjects Analysis

	Dosing Saline 230-250mL 3 doses x 3d Absolute	Dosing Saline 345mL 10 doses x 18d Absolute	Dosing Saline 500mL 8 doses x 10d Absolute	Dosing Saline 700 mL 21 doses x 21d Absolute
Median	1	0	0	0
Mean	1	0.22	0	0
Range	1-1	0-1	0-0	0-0
Stability Envelope	0.2	0	0	0
# data points inside envelope	3 of 3	11 of 18	10 of 10	21 of 21
% data points inside envelope	100%	61%	100%	100%
Stability	Stable	Unstable	Stable	Stable
Trend direction	↓	↓	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	0	0	0	0
Assessment				
Relative Level Change	Insufficient #	0	0	0
Assessment	Insufficient #			

KJ003 Severity of Cramping with Saline Flush in Dosing Phase - Results of Single Subjects Analysis

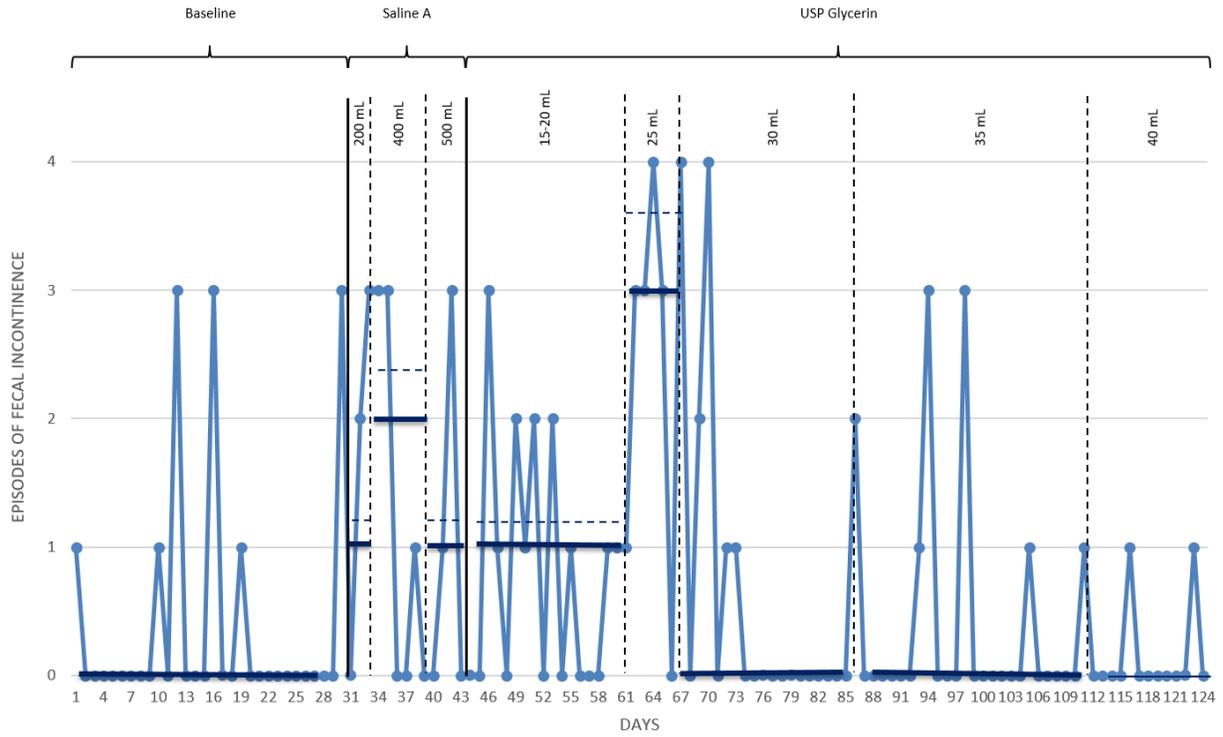
	Dosing Saline 230-250mL 3 doses/3d Severity	Dosing Saline 345mL 10 doses/18d Severity	Dosing Saline 500mL 8 doses/10d Severity	Dosing Saline 700 mL 21 doses/21d Severity
Median	2	0	0	0
Mean	4	0.444	0	0
Range	2-6	0-2	0-0	0-0
Stability Envelope	0.4	0	0	0
# data points inside envelope	1 of 3	11 of 18	10 of 10	21 of 21%
% data points inside envelope	33%	61%	100%	100%
Stability	Unstable	Unstable	Stable	Stable
Trend direction	↓	↓	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	2	0	0	0
Assessment	Improving			
Relative Level Change	Insufficient #	0	0	0
Assessment	Insufficient #			

KJ003 Episodes and Severity of Cramping with Glycerin Flush - Results of Single Subjects Analysis

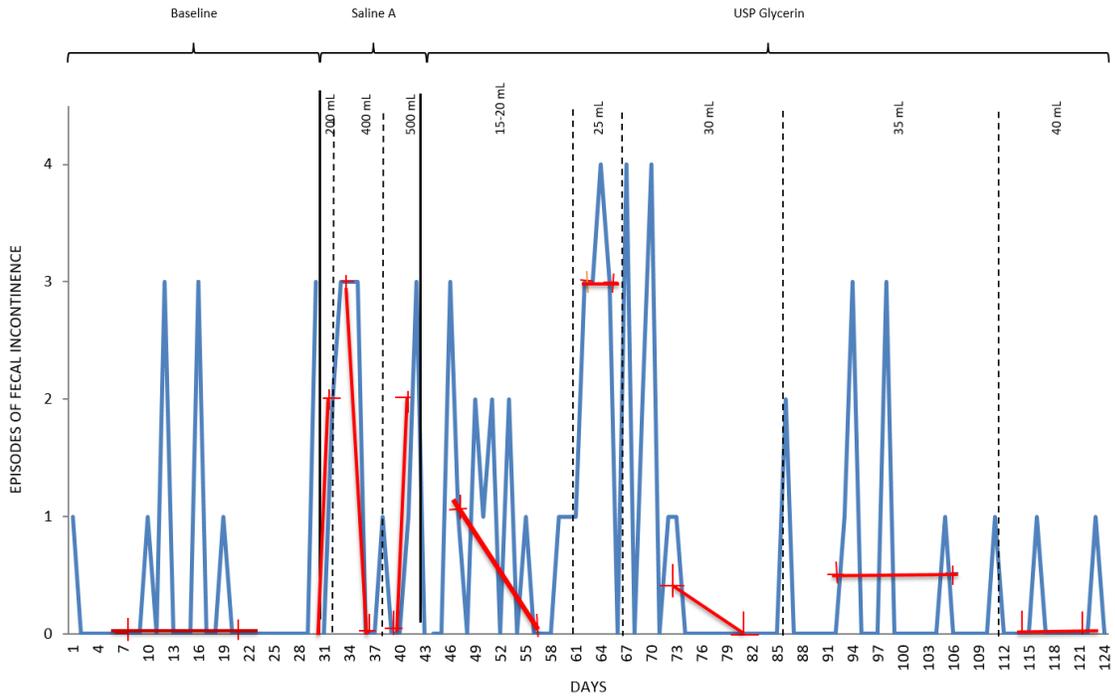
	Dosing Glycerin 20mL 17 doses x 33d Absolute	Dosing Glycerin 20mL 17 doses x 33d Severity
Median	1	2
Mean	0.515	1.576
Range	0-1	0-4
Stability Envelope	0.2	0.4
# data points inside envelope	17 of 33	17 of 33
% data points inside envelope	52%	52%
Stability	Unstable	Unstable
Trend direction	↑	↑
Multiple Paths Within Trend		
Absolute Level Change	0	0
Assessment	0	0
Relative Level Change		
Assessment		

APPENDIX R
 KJ004 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS
 DETAILING STABILITY AND TREND

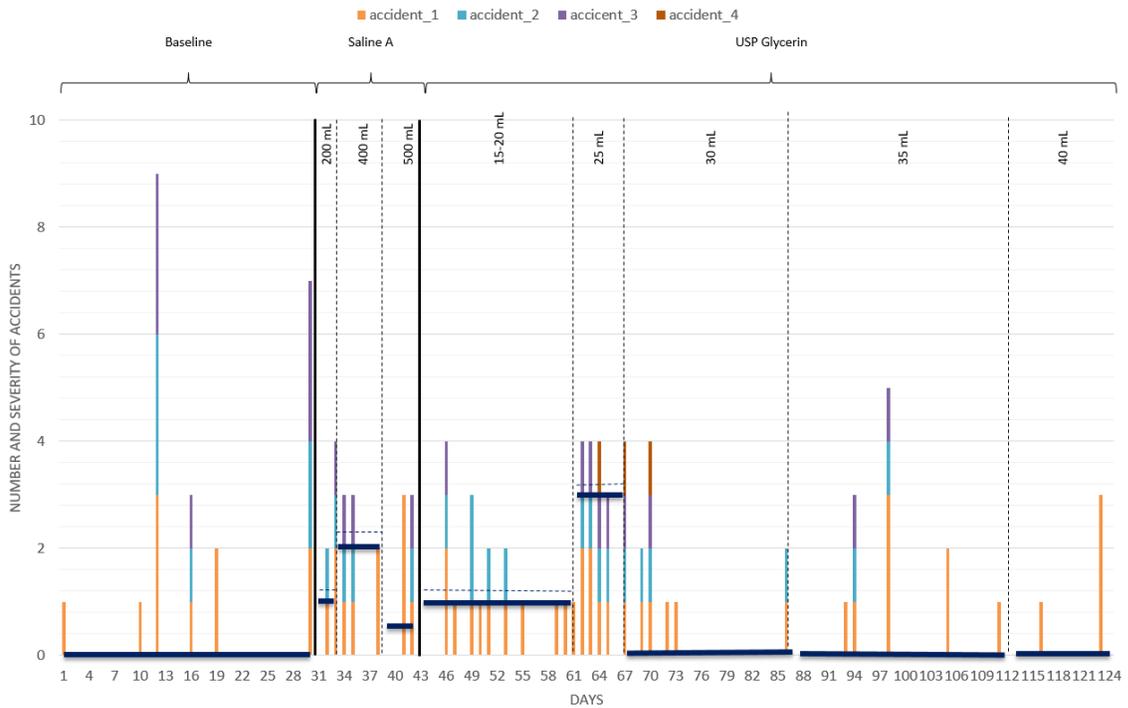
KJ004 STABILITY DATA FOR ABSOLUTE EPISODES OF INCONTINENCE



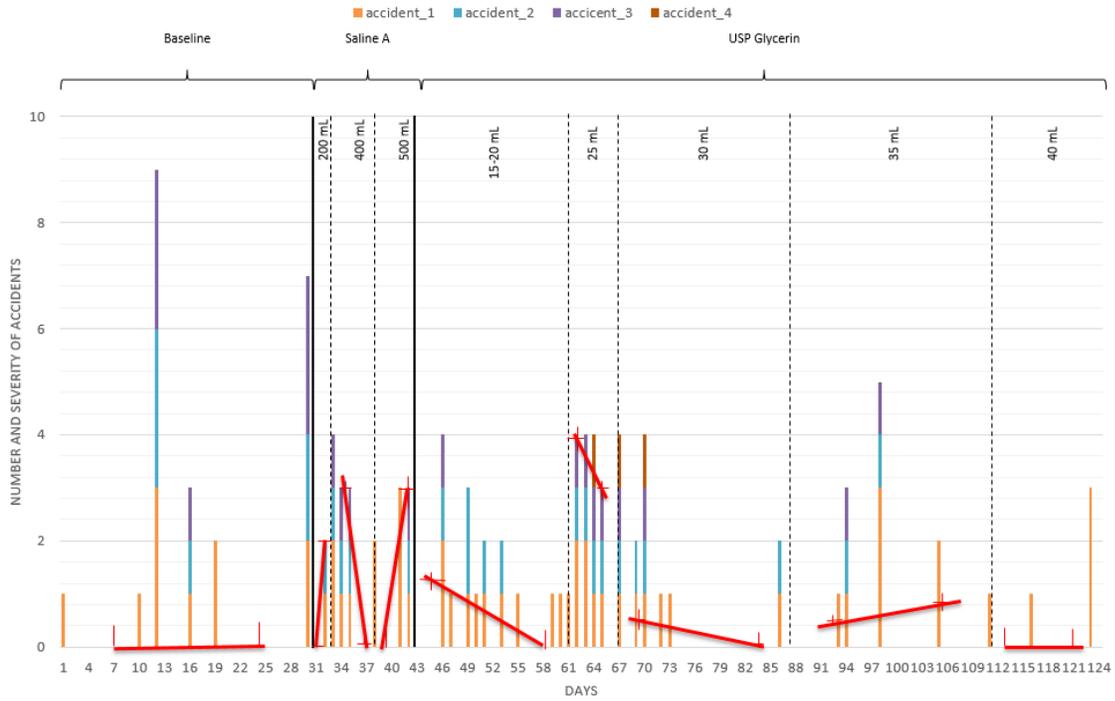
KJ004 TREND FOR ABSOLUTE FREQUENCY OF INCONTINENCE DATA



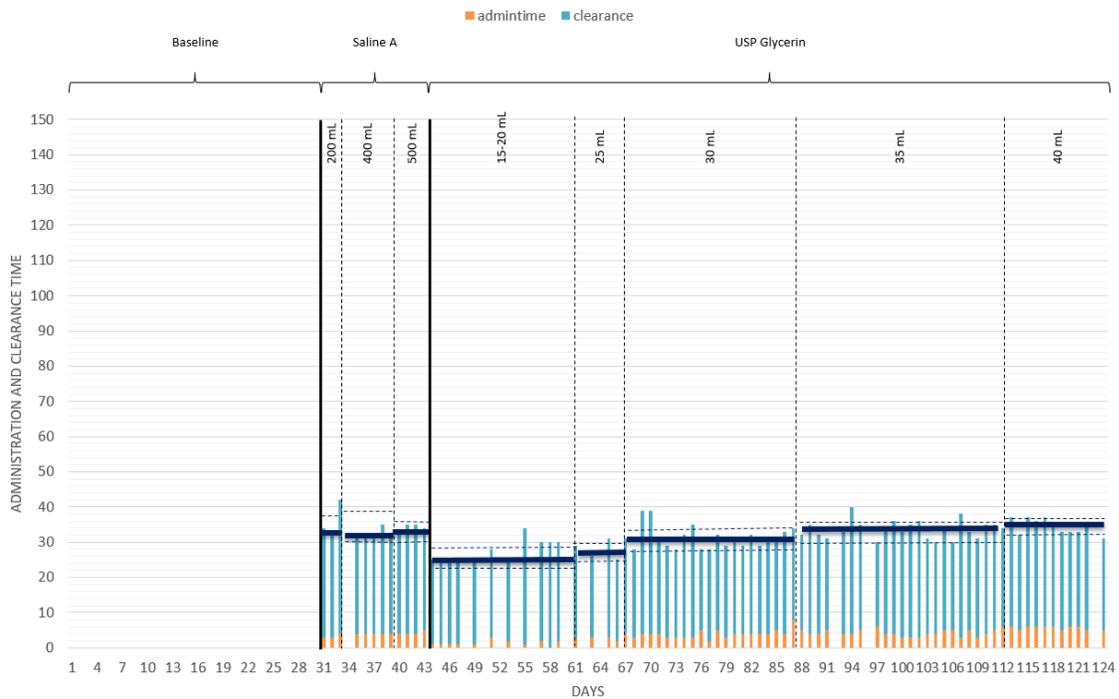
KJ004 STABILITY FOR SEVERITY OF INCONTINENCE DATA



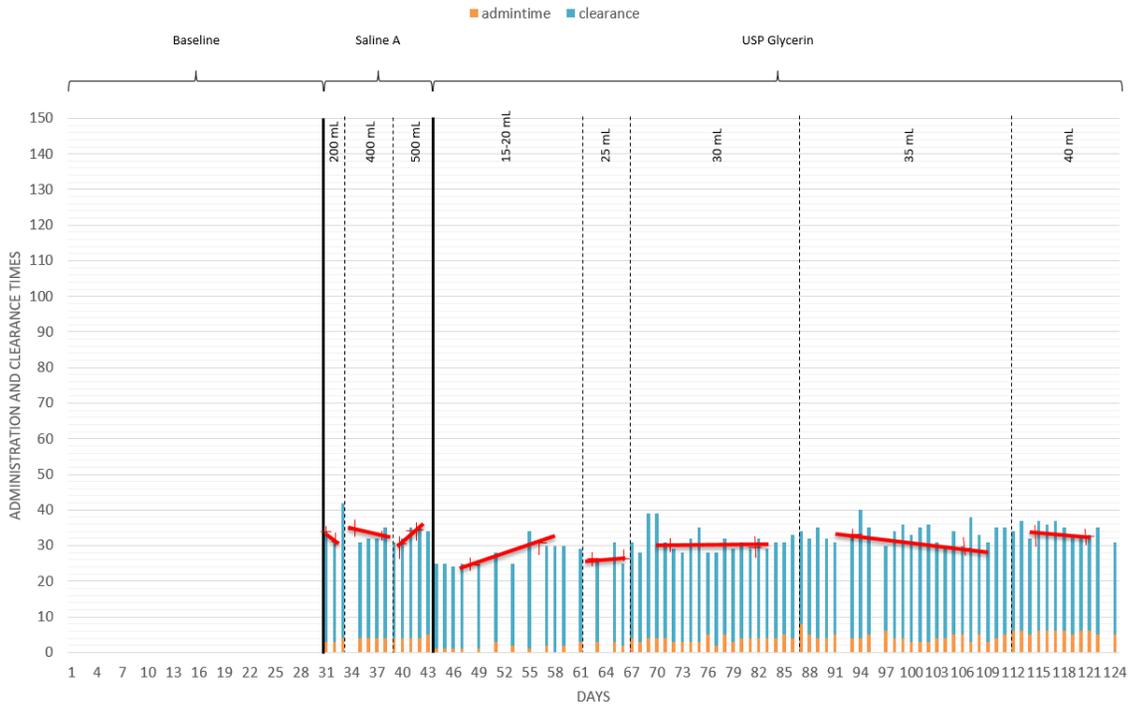
KJ004 TREND FOR SEVERITY OF INCONTINENCE DATA



KJ004 STABILITY FOR PROCEDURAL TIME DATA



KJ004 TREND FOR PROCEDURAL TIME DATA



KJ004 Episodes and Severity of Fecal Incontinence in Baseline Phase - Results from Single Subjects Analysis

	Baseline Absolute	Baseline Severity
Median	0	0
Mean	0.387	0.71
Range	0-3	0-9
Stability Envelope	0	0
# data points inside envelope	25 of 31	25 of 31
% data points inside envelope	80%	80%
Stability	Stable	Stable
Trend direction	↔	↔
Multiple Paths Within Trend		
Absolute Level Change	3	6
Assessment	Deteriorating	Deteriorating
Relative Level Change	0	0
Assessment	Zero Celerating	Zero Celerating

KJ004 Episodes and Severity of Fecal Incontinence on Saline in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 200mL 2doses x 2d Absolute	Dosing Saline 200mL 2doses x 2d Severity	Dosing Saline 400mL 4 doses x 6d Absolute	Dosing Saline 400mL 4 doses x 6d Severity	Dosing Saline 500mL 4 doses x 4d Absolute	Dosing Saline 500mL 4 doses x 4d Severity
Median	1	1	2	2.5	0.5	0.5
Mean	1	1	1.666	6.5	2	2
Range	0-2	0-2	0-3	0-4	0-3	0-3
Stability Envelope	0.2	0.2	0.4	0.5	0.1	0.1
# data points inside envelope	0 of 2	0 of 2	0 of 4	2 of 6	0 of 4	0 of 4
% data points inside envelope	0%	0%	0%	33%	0%	0%
Stability	Unstable	Unstable	Unstable	Unstable	Unstable	Unstable
Trend direction	↑	↑	↓	↓	↑	↑
Multiple Paths Within Trend						
Absolute Level Change	2	2	2	2	3	3
Assessment	Deteriorating	Deteriorating	Improving	Improving	Deteriorating	Deteriorating
Relative Level Change	Insufficient #	Insufficient #	3	3	2	3
Assessment	Insufficient #	Insufficient #	Improving	Improving	Deteriorating	Deteriorating

KJ004 Episodes of Fecal Incontinence on Glycerin Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 20mL 9 doses x 16d Absolute	Dosing Glycerin 25mL 4 doses x 6 d Absolute	Dosing Glycerin 30mL 20 doses x 20 d Absolute	Dosing Glycerin 35mL 23 doses x 25d Absolute	Dosing Glycerin 40mL 12 doses x 12d Absolute
Median	1	3	0	0	0
Mean	0.875	2.333	0.7	0.36	0.167
Range	0-3	0-4	0-4	0-3	0-1
Stability Envelope	0.2	0.6	0	0	0
# data points inside envelope	5 of 16	3 of 6	14 of 20	20 of 25	11 of 12
% data points inside envelope	31%	50%	70%	80%	92%
Stability	Unstable	Unstable	Unstable	Stable	Stable
Trend direction	↓	↓	↓	↔	↔
Multiple Paths Within Trend					
Absolute Level Change	1	1	2	1	0
Assessment	Deteriorating	Improving	Improving	Deteriorating	Zero Celerating
Relative Level Change	0.5	0	0.5	0	0
Assessment	Improving	Zero Celerating	Improving	Zero Celerating	Zero Celerating

KJ004 Severity of Incontinence on Glycerin Flush during Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 25mL 4 doses x 6 d Severity	Dosing Glycerin 30mL 20 doses x 20d Severity	Dosing Glycerin 35mL 23 doses x 25d Severity	Dosing Glycerin 40mL 12 doses x 12d Severity
Median	3.5	0	0	0
Mean	2.667	0.7	0.48	0.333
Range	0-4	0-4	0-5	0-3
Stability Envelope	0.7	0	0	0
# data points inside envelope	3 of 6	14 of 20	20 of 25	11 of 12
% data points inside envelope	50%	70%	80%	92%
Stability	Unstable	Unstable	Stable	Stable
Trend direction	↓	↓	↑	↔
Multiple Paths Within Trend				
Absolute Level Change	1	2	1	0
Assessment	Improving	Improving	Deteriorating	Zero Celerating
Relative Level Change	1	0.5	0	0
Assessment	Improving	Improving	Zero Celerating	Zero Celerating

KJ004 Episodes and Severity of Pain In Baseline - Results from Single Subjects Analysis

	Baseline Absolute	Baseline Severity
Median	0	0
Mean	0	0
Range	0-0	0-0
Stability Envelope	0	0
# data points inside envelope	31 of 31	31 of 31
% data points inside envelope	100%	100%
Stability	Stable	Stable
Trend direction	↔	↔
Multiple Paths Within Trend		
Absolute Level Change	0	0
Assessment	Zero Celerating	Zero Celerating
Relative Level Change	0	
Assessment	Zero Celerating	Zero Celerating

KJ004 Episodes of Cramping with Saline Flush in Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 200mL 2dose x 2d Absolute	Dosing Saline 400mL 4 doses x 6d Absolute	Dosing Saline 500mL 4 doses x 4d Absolute
Median	0	0	0
Mean	0	0	0
Range	0-0	0-0	0-0
Stability Envelope	0	0	0
# data points inside envelope	2 of 2	4 of 4	4 of 4
% data points inside envelope	100%	100%	100%
Stability			
Trend direction	↔	↔	↔
Multiple Paths Within Trend			
Absolute Level Change	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating

KJ004 Episodes of Pain with Glycerin Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 20mL 9 doses x 16d Absolute	Dosing Glycerin 25mL 4 doses x 6 d Absolute	Dosing Glycerin 30mL 20 doses x 20 d Absolute	Dosing Glycerin 35mL 23 doses x 25d Absolute	Dosing Glycerin 40mL 12 doses x 12d Absolute
Median	0	0	0	0	0
Mean	0.111	0	0	0.043	0
Range	0-1	0-0	0-0	0-1	0-0
Stability Envelope	0	0	0	0	0
# data points inside envelope	8 of 9	6 of 6	20 of 20	22 of 23	12 of 12
% data points inside envelope	89%	100%	100%	96%	100%
Stability	Stable	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔	↔
Multiple Paths Within Trend					
Absolute Level Change	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

KJ004 Severity of Pain with Glycerin Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 20mL 9 doses /16d Severity	Dosing Glycerin 25mL 4 doses/6 d Severity	Dosing Glycerin 30mL 20 doses/20 d Severity	Dosing Glycerin 35mL 23 doses/25d Severity	Dosing Glycerin 40mL 12 doses/12d Severity
Median	0	0	0	0	0
Mean	0.667	0	0	0.348	0
Range	0-6	0-0	0-0	0-8	0-0
Stability Envelope	0	0	0	0	0
# data points inside envelope	8 of 9	6 of 6	20 of 20	22 of 23	12 of 12
% data points inside envelope	89%	100%	100%	96%	100%
Stability	Stable	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔	↔
Multiple Paths Within Trend					
Absolute Level Change	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

KJ004 Procedural Time for Saline Flush During Dosing Phase - Results of Single Subjects Analysis

	Dosing Saline 200mL 2doses x 2d	Dosing Saline 400mL 4 doses x 6d	Dosing Saline 500mL 4 doses x 4d
Median	33	32	33.5
Mean	33	34.4	33.25
Range	32-34	31-42	31-35
Stability Envelope	6.6	6.4	6.7
# data points inside envelope	2 of 2	5 of 5	4 of 4
% data points inside envelope	100%	100%	100%
Stability	Stable	Stable	Stable
Trend direction	↓	↓	↑
Multiple Paths Within Trend			
Absolute Level Change	1	7	4
Assessment	Improving	Improving	Deteriorating
Relative Level Change	Insufficient #	3	3.5
Assessment	Insufficient #	Improving	Deteriorating

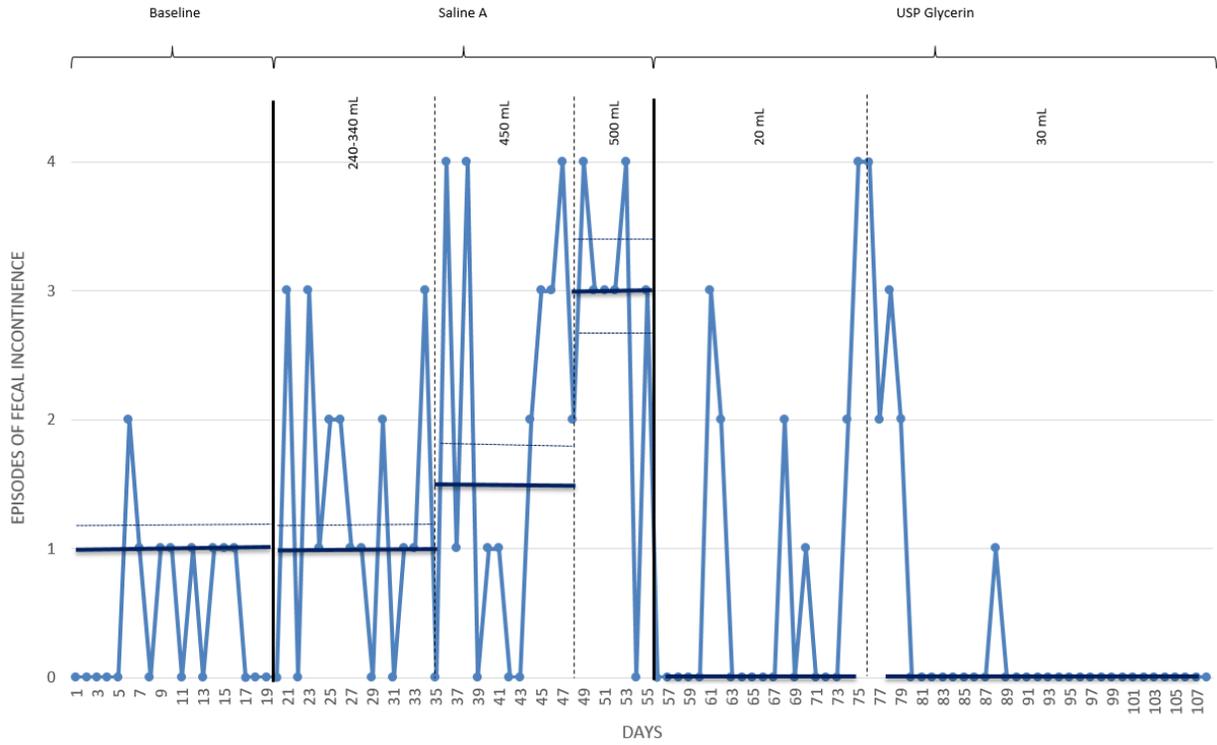
KJ004 Procedural Time Chart for Glycerin Flush in Dosing Phase - Results of Single Subjects Analysis

	Dosing Glycerin 15-20mL 9 doses x 16d	Dosing Glycerin 25mL 4 doses x 6 d	Dosing Glycerin 30mL 20 doses x 20 d	Dosing Glycerin 35mL 23 doses x 25d	Dosing Glycerin 40mL 12 doses x 12d
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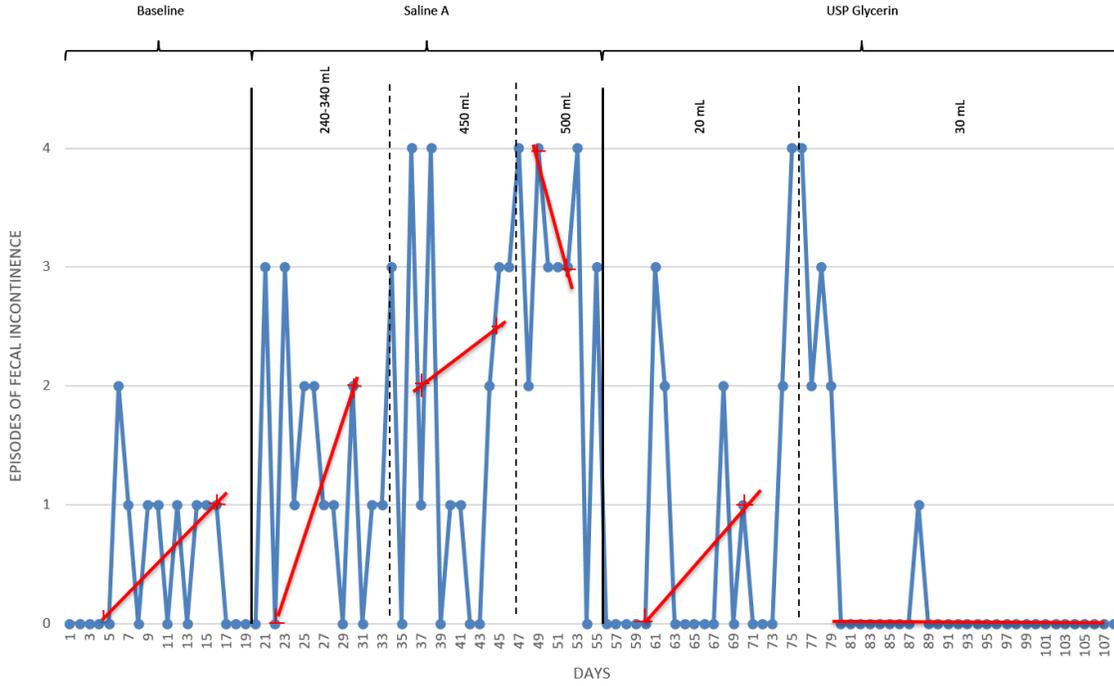
Procedural Time					
Level	Absolute	Absolute	Absolute	Absolute	Absolute
Median	25	27.5	31	34	34.5
Mean	27.33	21.75	31.25	33.65	34.417
Range	24-34	25-31	28-39	30-40	31-37
Stability Envelope	5	5.5	6.2	6.8	6.9
# data points inside envelope	6 of 9	3 of 4	16 of 20	21 of 23	12 of 12
% data points inside envelope	66%	75%	80%	91%	100%
Stability	Unstable	Unstable	Stable	Stable	Stable
Trend direction	↑	↔	↔	↓	↓
Multiple Paths Within Trend					
Absolute Level Change	5	4	2	1	4
Assessment	Deteriorating	Improving	Deteriorating	Deteriorating	Deteriorating
Relative Level Change	5	Insufficient #	0	0	1
Assessment	Deteriorating	Insufficient #			Improving

APPENDIX S
 KJ005 WITHIN SUBJECTS ANALYSIS CALCULATIONS INCLUDING GRAPHS
 DETAILING STABILITY AND TREND

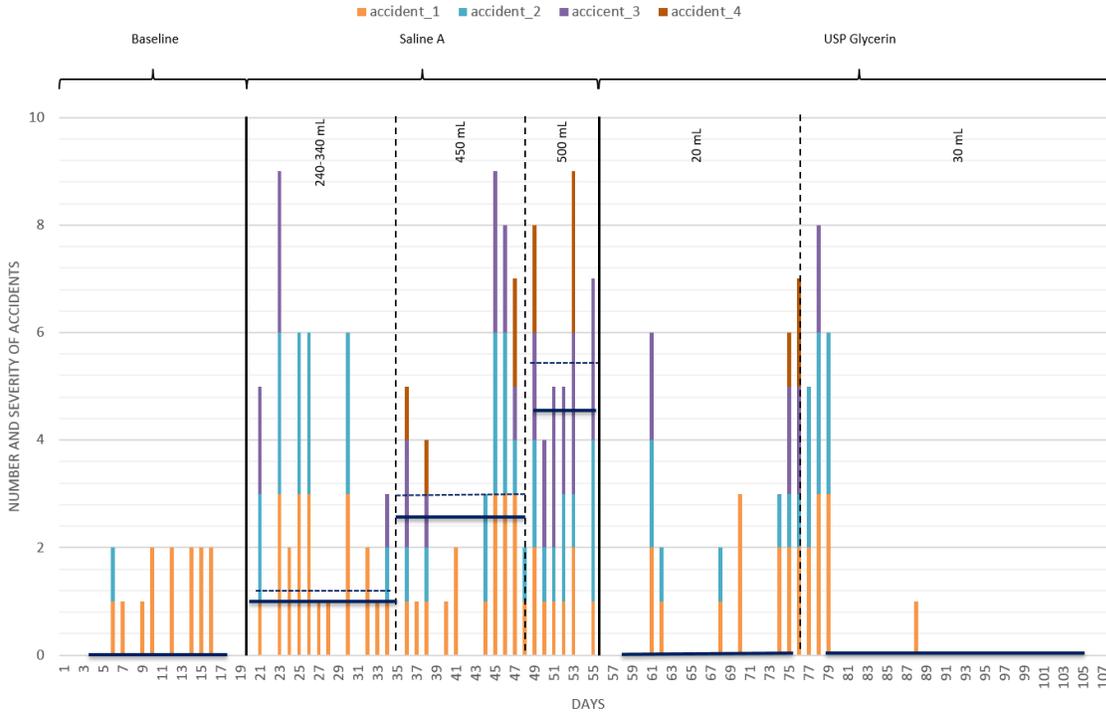
KJ005 STABILITY OF ABSOLUTE FREQUENCY OF INCONTINENCE DATA



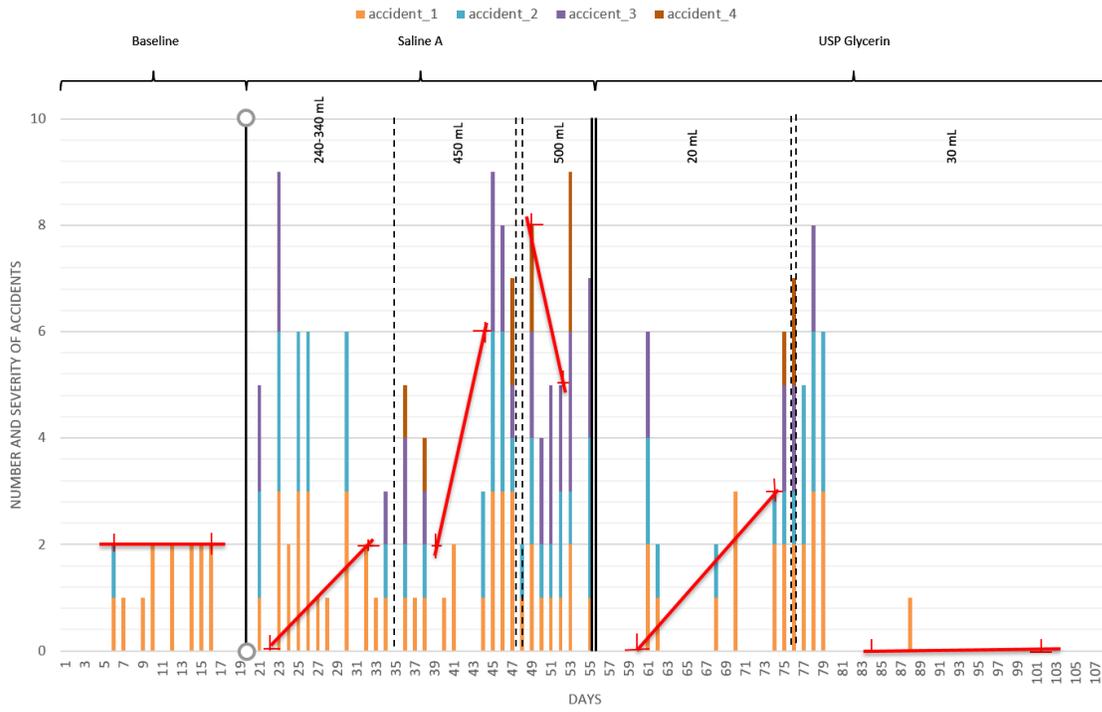
KJ005 TREND FOR ABSOLUTE FREQUENCY OF INCONTINENCE DATA



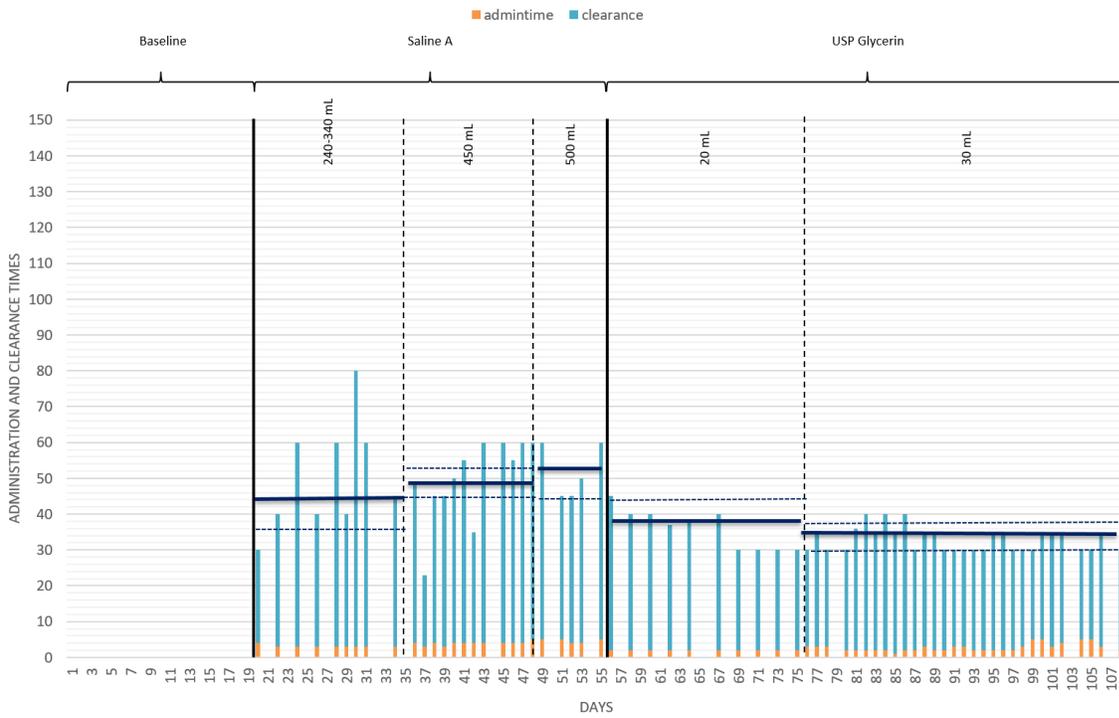
KJ005 STABILITY FOR SEVERITY OF INCONTINENCE DATA



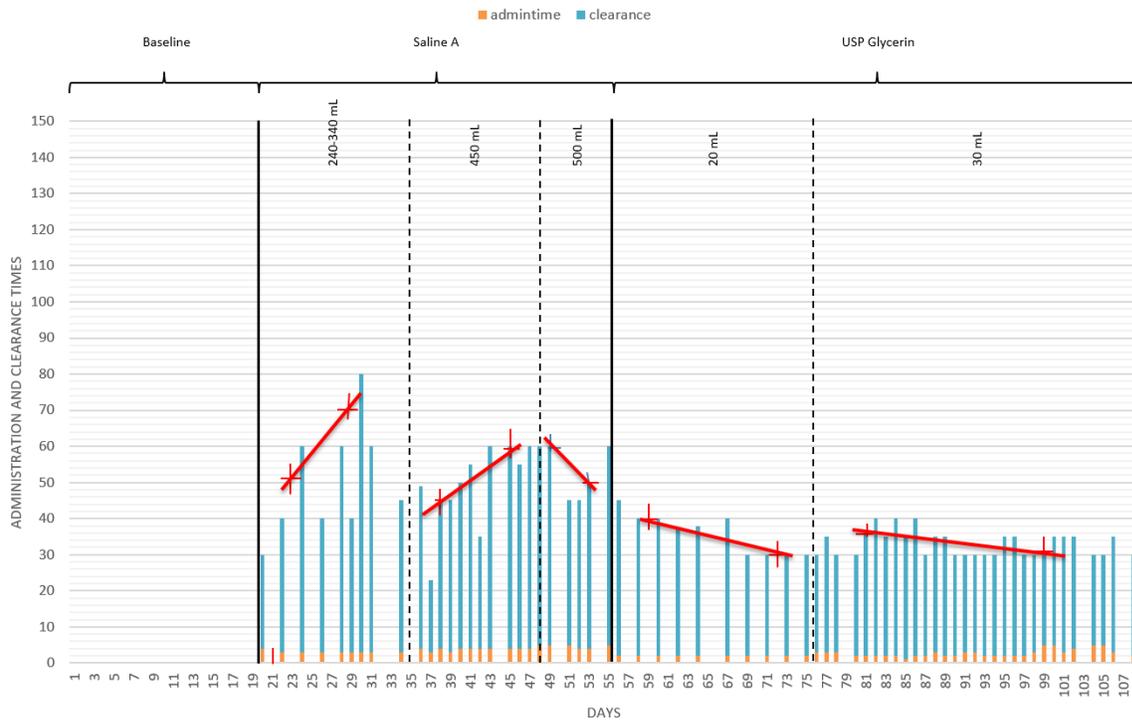
KJ005 TREND FOR SEVERITY OF INCONTINENCE DATA



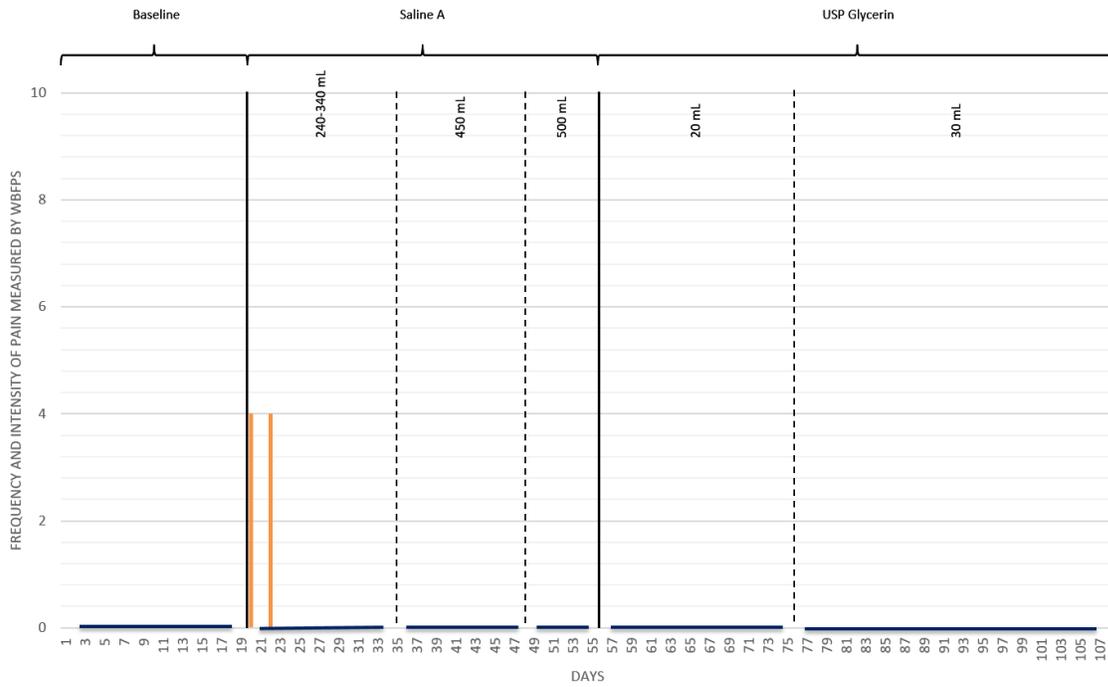
KJ005 STABILITY FOR PROCEDURAL TIME DATA



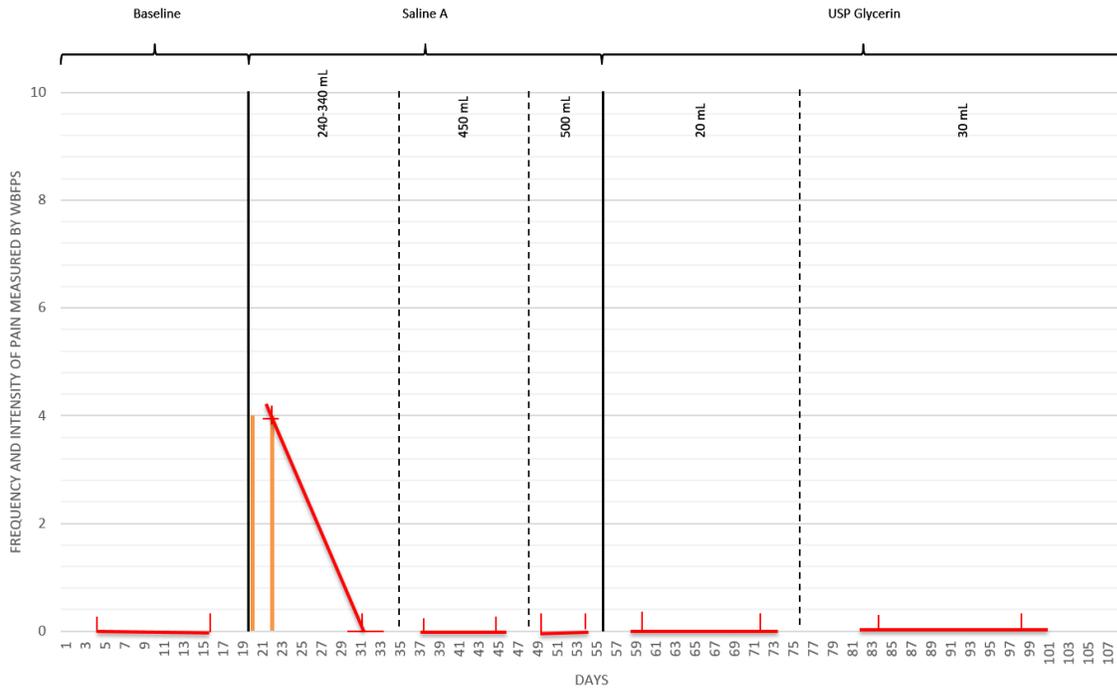
KJ005 TREND FOR PROCEDURAL TIME DATA



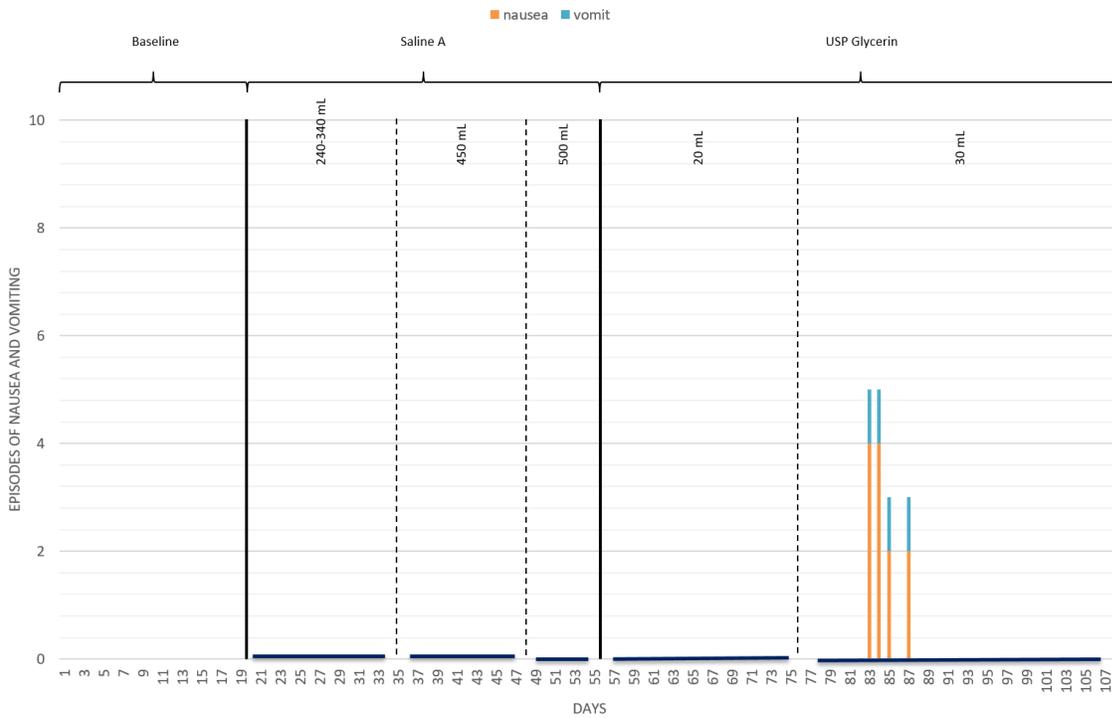
KJ005 STABILITY OF CRAMPING WITH FLUSH DATA



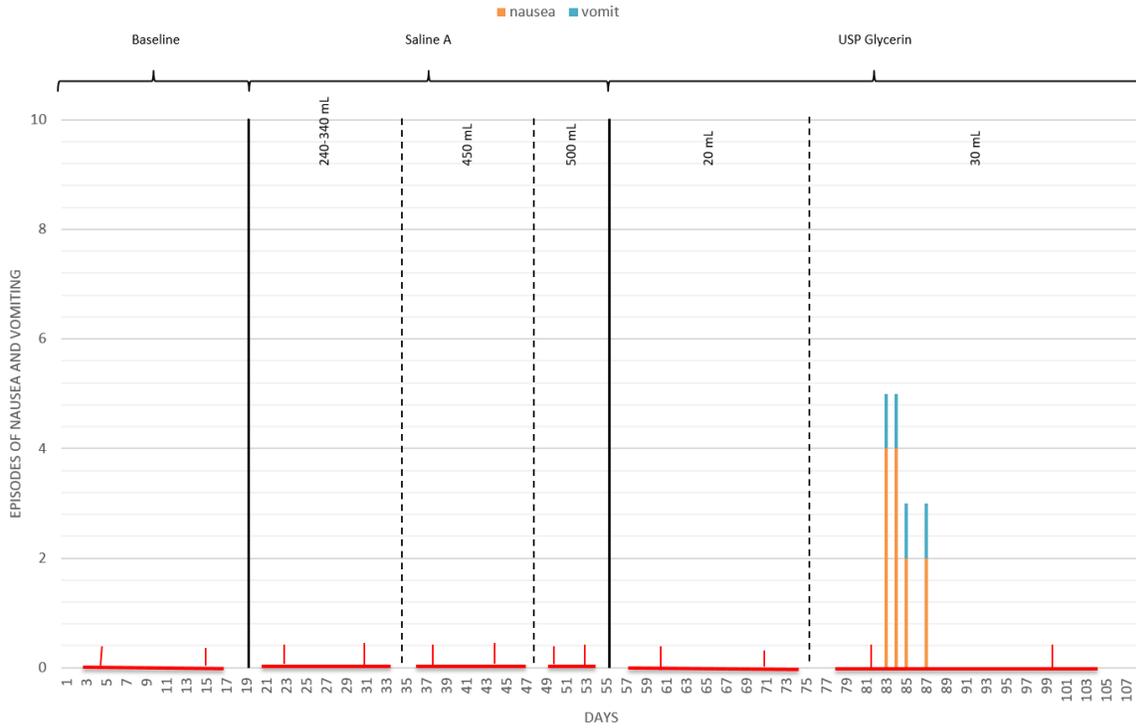
KJ005 TREND DATA FOR CRAMPING FROM FLUSH



KJ005 STABILITY OF VEGAL SYMPTOMS DATA



KJ005 TREND DATA FOR EPISODES AND SEVERITY OF VEGAL SYMPTOMS



KJ005 Episodes and Severity of Incontinence on Saline in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 240-340mL 10 doses/16d Absolute	Dosing Saline 240-340mL 10 doses/16d Severity	Dosing Saline 450mL 11 doses/12d Absolute	Dosing Saline 450mL 11 doses/12d Severity	Dosing Saline 500mL 6 doses/8d Absolute	Dosing Saline 500mL 6 doses/8d Severity
Median	1	1	1.5	2.5	3	4.5
Mean	1	1.875	1.92	3.333	7.667	4.25
Range	0-3	0-6	0-4	0-9	0-4	0-9
Stability Envelope	0	0	0.3	0.5	0.6	0.9
# data points inside envelope	6 of 16	6 of 16	0 of 12	1 of 12	3 of 8	2 of 8
% data points inside envelope	38%	38%	0%	8%	36%	25%
Stability	Unstable	Unstable	Unstable	Unstable	Unstable	Unstable
Trend direction	↑	↑	↑	↑	↓	↓
Multiple Paths Within Trend						
Absolute Level Change	3	3	0	2	2	6
Assessment	Deteriorating	Deteriorating		Deteriorating	Deteriorating	Deteriorating
Relative Level Change	0	0	1.5	3.5	0	1
Assessment	Zero Celerating	Zero Celerating	Deteriorating	Deteriorating	Zero Celerating	Deteriorating

KJ005 Episodes and Severity of Incontinence on Glycerin in Dosing Phase - Results of Single Subjects Analysis

	Dosing Glycerin 20mL 9 doses/9d Absolute	Dosing Glycerin 20mL 9 doses/9d Severity	Dosing Glycerin 30mL 31 doses/33d Absolute	Dosing Glycerin 30mL 31 doses/33d Severity
Median	0	0	0	0
Mean	0.43	0.68	0.36	0.818
Range	0-3	0-6	0-4	0-8
Stability Envelope	0	0	0	0
# data points inside envelope	15 of 19	15 of 19	28 of 33	28 of 33
% data points inside envelope	79%	79%	85%	85%
Stability	Unstable	Unstable	Stable	Stable
Trend direction	↑	↑	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	4	6	4	7
Assessment	Deteriorating	Deteriorating	Improving	Improving
Relative Level Change	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

KJ005 Procedural Time for Saline and Glycerin in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 240-340mL 10 doses/16d	Dosing Saline 450mL 11 doses/12d	Dosing Saline 500mL 6 doses/8d	Dosing Glycerin 20mL 9 doses/9d	Dosing Glycerin 30mL 31 doses/33d
Median	45	50	55	38	35
Mean	51.66	48.82	53.33	36.66	31.968
Range	30-80	23-60	45-60	30-45	30-40
Stability Envelope	9	10	11	7.6	7
# data points inside envelope	5 of 9	6 of 10	4 of 6	5 of 9	27 of 31
% data points inside envelope	56%	60%	67%	56%	87%
Stability	Unstable	Unstable	Unstable	Unstable	Stable
Trend direction	↑	↑	↓	↓	↓
Multiple Paths Within Trend					
Absolute Level Change	15	11	0	15	0
Assessment	Deteriorating	Deteriorating	Zero Celerating	Improving	Zero Celerating
Relative Level Change	12.5	15	10	10	5
Assessment	Deteriorating	Deteriorating	Improving	Improving	Improving

KJ005 Episodes and Severity of Pain at Baseline - Results from Single Subjects Analysis

	Baseline Absolute	Baseline Severity
Median	0	0
Mean	0	0
Range	0-0	0-0
Stability Envelope	0	0
# data points inside envelope	18 of 18	18 of 18
% data points inside envelope	100%	100%
Stability	Stable	Stable
Trend direction	↔	↔
Multiple Paths Within Trend		
Absolute Level Change	0	0
Assessment	Zero Celerating	Zero Celerating
Relative Level Change	0	0
Assessment	Zero Celerating	Zero Celerating

KJ005 Episodes and Severity of Pain with Saline Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Saline 240-340mL 10 doses x 16d Absolute	Dosing Saline 240-340mL 10 doses x 16d Severity	Dosing Saline 450mL 11 doses x 12d Absolute	Dosing Saline 450mL 11 doses x 12d Severity	Dosing Saline 500mL 6 doses x 8d Absolute	Dosing Saline 500mL 6 doses x 8d Severity
Median	0	0	0	0	0	0
Mean	0.123	0.5	0	0	0	0
Range	0-1	0-4	0-0	0-0	0-0	0-0
Stability Envelope	0	0	0	0	0	0
# data points inside envelope	14 of 16	14 of 16	12 of 12	12 of 12	8 of 8	8 of 8
% data points inside envelope	88%	88%	100%	100%	100%	100%
Stability	Stable	Stable	Stable	Stable	Stable	Stable
Trend direction	↓	↓	↔	↔	↔	↔
Multiple Paths Within Trend						
Absolute Level Change	0	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

KJ005 Episodes and Severity of Pain with Glycerin Flush in Dosing Phase - Results from Single Subjects Analysis

	Dosing Glycerin 20mL 9 doses x 9d Absolute	Dosing Glycerin 20mL 9 doses x 9d Severity	Dosing Glycerin 30mL 31 doses x 33d Absolute	Dosing Glycerin 30mL 31 doses x 33d Severity
Median	0	0	0	0
Mean	0	0	0	0
Range	0-0	0-0	0-0	0-0
Stability Envelope	0	0	0	0
# data points inside envelope	9 of 9	9 of 9	9 of 9	9 of 9
% data points inside envelope	100%	100%	100%	100%
Stability	Stable	Stable	Stable	Stable
Trend direction	↔	↔	↔	↔
Multiple Paths Within Trend				
Absolute Level Change	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating
Relative Level Change	0	0	0	0
Assessment	Zero Celerating	Zero Celerating	Zero Celerating	Zero Celerating

KJ005 Episodes and Severity of Vegal Symptoms with Glycerin Flush - Results of Single Subjects Analysis

Condition Sequence	Dosing Glycerin 20mL 9 doses x 9d Absolute	Dosing Glycerin 20mL 9 doses x 9d Severity	Dosing Glycerin 30mL 31 doses x 33d Absolute	Dosing Glycerin 30mL 31 doses x 33d Severity
Median	0	0	1	2
Mean	0	0	0.194	0.581
Range	0-0	0-0	0-1	0-4
Stability Envelope	0	0	0.2	0.4
# data points inside envelope	9 of 9	9 of 9	25 of 31	25 of 31
% data points inside envelope	100%	100%	81%	81%
Stability	Stable	Stable	Stable	Stable
Trend direction				
Multiple Paths Within Trend				
Absolute Level Change	0	0	0	0
Assessment	↔	↔	↔	↔
Relative Level Change	0	0	0	0
Assessment	↔	↔	↔	↔

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BIOGRAPHICAL SKETCH

Kim's major was nursing, graduating with a Master of Science degree in the spring of 1994 and a Doctor of Philosophy in the fall of 2017. Her clinical specialization and research focus are dysfunctional elimination in children. Kim is a practicing pediatric nurse practitioner and the Clinical Director of the Dysfunctional Elimination Program at Nemours Pediatric Specialty Care in Jacksonville, Florida. This program is the first reported independent nurse-practitioner run referral center in a free-standing pediatric subspecialty ambulatory setting in the country and the first pediatric program to combine subspecialty services including urology, gastroenterology, and psychology in a single point of care for this population.