Figure 11. Preparing grids for electron microscopy by washing and negative staining. *A*: specimen grids positioned for application of virus suspension, washing, and negative staining. *a*: glass microscope slide; *b*: double-sided cellophane tape; and *c*: grids attached to the tape. One of them has a standing droplet of solution applied. *B*: a folded piece of blotting paper is being positioned to adsorb, and a Pasteur pipette is being positioned to deliver a solution to a grid positioned on the tape. *C*: at the initial stage of solution application, the blotting paper is positioned so that it touches the rim of the grid, and the first droplets are applied. *D*: as the washing proceeds, the blotting paper is slightly withdrawn from the grid while the application of the solution continues, insuring a steady flow of solution across the grid surface. The procedure is essentially the same whether the grids are to be washed with buffer, water, or bacitracin solution. Following the washing procedure (the final wash should be with water or bacitracin solution), several droplets of negative stain are applied, and the last droplet is removed as completely as possible by the blotting paper. The grid is then allowed to dry.

When the tissue extraction is complete, draw the extract into a Pasteur pipette and examine it. If it is more than slightly colored, it should be diluted with buffer until the color is just discernible. When a satisfactory concentration is obtained, place a droplet on a grid that has been mounted on the cellophane tape, and allow it to remain for 1 min. An extract that is too concentrated may present no problems, but an extract of greater dilution is generally easier to work with. Of course, the color of an extract is not a foolproof guide to its concentration, but in most cases will give acceptable results.

Remove the droplet from the specimen grid by touching the grid rim where it is attached to the tape with a folded corner of a piece of blotting paper folded in half. Immediately begin washing the grid with a washing solution (usually a buffer) dropped from a Pasteur pipette at a rate not exceeding the rate at which the blotting paper absorbs it. As the washing proceeds, begin withdrawing the blotting paper by pulling it away from the grid and across the tape for a distance of 6–8 mm, meanwhile maintaining contact between the blotting paper and the tape surface (see Figure 11). This tactic will promote thorough washing by producing a vigorous flow of buffer across the grid. Use at least 25–30 drops of washing solution (if you use "1-ml" bulbs on Pasteur pipettes, then one dropperful is generally about the right amount to use, and each piece of blotting paper can easily absorb 50–60 drops of liquid). As the blotter becomes saturated, switch ends, and change to fresh blotting paper as often as necessary to maintain a fast flow.