BELLCO GLASS, INC. USA
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ROLLER VESSEL CELL CULTURE

Vessel Preparation

Cleanliness of the bottles is critical. Common tissue culture glassware cleaning procedures may be inadequate. Included in these instructions are suggested rollerbottle cleaning protocols.

Sterilization

Autoclave the bottle and cap as a unit with cap loosened to allow venting. Exhaust autoclave slowly, allowing bottles to cool before removing from the autoclave. Rubber liners can become detached if caps are tightened while bottle is still hot or autoclave is exhausted to quickly. Cell Production roller technique may require some experimentation with respect to optimum rate of rotation of the bottle culture, the number of cells in the inoculum, etc. generally mammalian cells grown in monolayer culture will adapt well to the rolling bottle technique.

THE FOLLOWING RECOMMENDATIONS ARE OFFERED AS GENERAL INFORMATION ONLY SINCE CULTURE CHARACTERISTICS VARY WITH DIFFERENT CELLS AND VIRUSES.

Seeding

1-3 x 10 6 per 20mL/inch of usable linear length of the bottles. Thoroughly disperse the cells and shake the bottle thoroughly before placing on the roller.

Media

Many investigators indicate that the initial medium added at the time of seeding of cells is sufficient to obtain a confluent monolayer, however, it is quite normal that media may have to be changed after two or three days. Media should be pre-warmed to 37°C since addition of cold media will cause the cells to aggregate at either end of the bottle rather than attach to the surface.

Bottle Speed

Recommended speed is 0.1 to 0.3 rpm during the first 24 hours of attachment and a maximum of 2 rpm afterward.

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The optimal speed for a particular cell type may vary and this must be determined experimentally. The formation of well-dispersed homogeneous cultures is dependent upon the rate at which the cells settle and attach to the glass. The more rapidly they attach the more even the distribution of cells on the glass surface. The rate of attachment is dependent on the temperature of the medium, rotational velocity of the bottle and of course the cells involved.

Characteristics
The growth of cells in roller bottle culture differs from that in stationary cultures in two respects. The first concerns the distribution of the cells on the glass surface. Stationary culture can be readily made even and homogeneous.

Even in the 'best' roller bottle cultures, small areas of very dense growth are scattered throughout the surface of the bottle. The second difference relates to maximum attainable cell density (number of cells per unit of glass surface). Very heavy stationary cultures begin to deteriorate after a few days. This deterioration can be delayed but not averted by replacing the medium frequently. Roller bottle cultures deteriorate less rapidly. The cells in roller bottle cultures can tolerate a density nearly two times higher than that of stationary cultures.

Yield
The yield depends upon the cell line with 4 x 10 7 cells per linear inch of usable bottle length being approximate. This totals 4.7 x 10 8 cells per 840cm bottle.

Cleaning Protocol

1. 7X and Chlorox. Add 50mL of full strength 7X and Chlorox to roller and fill with hot tap water. Roll overnight on roller on roller apparatus or stand bottle upright at room temperature. Empty bottle and rinse (fill completely) 4 times with tap water, 4 times with deionized water and once with sterile distilled, deionized water OR wash in dishwasher using long rinse cycle.

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2. Chromic Acid
Prepare chromic acid by adding one 25mL bottle of Chromerge (Fisher Chemicals) to a standard 9 lb. Bottle of sulfuric acid. Add 25mL of this solution to a dry roller bottle, cover the neck with para-film and roll overnight on roller apparatus. Pour off acid and rinse by filling bottle completely 4 times with tap water, 4 times with deionized water and once with sterile distilled,deionized water. Drain and let dry.

3. Nitric Acid
Add 25mL of a 30% solution of nitric acid to each roller bottle, cover and roll overnight on a roller apparatus. Rinse as above, drain and let dry.

4. Dry Heat Sterilize
To dry-heat sterilize roller bottles, cover neck with aluminum foil and place in oven at 350°F for at least 4 hours.

NOTE: Since nitric acid and chromic acid may leave residues over a long period of use, it is best not to use these methods every time the roller bottles are cleaned.

PREPARATION OF ROLLER BOTTLES FOR TISSUE CULTURE
The following treatments have been effective in imp roving cell attachment and growth under certain conditions. For example the attachment of Vero cells is enhanced by sodium hydroxide treatment; duck embryo fibroblasts attach more readily to sodium carbonate-treated bottles. Build-up may occur, so it is best not to use these procedures without an occasional acid wash.

1. Sodium Hydroxide Treatment
Fill clean roller bottle with a .01M solution of NaOH, cover neck with aluminum foil and autoclave for45 minutes on liquid cycle. Empty bottle, fill once with distilled, deionized water, rinse and drain. Oven sterilize dry roller bottle at least 4 hours at 350°F.

2. Magnesium Acetate Treatment
Fill clean bottle with a 1mM solution of (CH3COO)2MG4H2
O (0.2 g/L). Let stand at room temperature for 4 hours. Empty bottle but do not rinse and let drain. Oven sterilize bottle for at least 4 hours at 35°F.

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3. Sodium Carbonate Treatment

Fill clean bottle with a 0.1% solution of Na2CO3 (1 g/L). Cover neck with aluminum foil and autoclave standing up in a shallow pan of water for 45 minutes on liquid setting. Let bottle cool, empty and rinse one with distilled water. Drain and then oven sterilize for at least 4 hours at 350°F.

4. Collagen treatment

Dissolve 50mg. Of collagen in 83mL glacial acetic acid which has been diluted 1/1000 in sterile distilled, deionized water. Stir overnight in cold room to dissolve. Spin at 1500 rpm for 15 minutes, remove clear supernatant and discard pellet. Freeze supernatant at -20°C in 10mL aliquots.

Acid wash roller bottles overnight and rinse (by filling bottle completely) 4 times with tap water, 4 times with deionized water and once with sterile distilled, deionized water. Drain bottles and oven-sterilize. Add 10mL of collagen to roller bottle and close with sterile cap. Roll overnight on roller apparatus.Decant collagen, add 50mL Tris buffer and keep bottle on roller apparatus until needed.