Exfoliation Technique for Detection of Non-Cytopathic BVD in Cell Cultures

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Culture cells to confluency in a 25cm2 flask. Pour off medium; add back 10 ml of serum-free media. Using a cotton swab, scrape off about half of the cell sheet and triturate with a pipette. Pour off medium; add back 10 ml of serum-free media. Using a cotton swab, scrape off about half of the cell sheet and triturate with a pipette. Pellet cell suspension at low RPM for 5 minutes. Pour off supernatant by quickly inverting, leaving about 0.2ml of medium to drain back down on the pellet. After letting the tube stand for 30 seconds or so, tap the tube 15-20 times on the bench top to disperse the cells. Using a Pasteur pipette *without* a bulb, allow the cell suspension to enter the pipette tip via capillary action. Apply the suspension by moving the tip of the pipette around on a glass slide, preferably one masked with teflon.

Dry the suspension in a dry 37°C incubator for 15-30 minutes and fix in pure acetone for 10 minutes at room temperature. Dry in incubator again for 15 minutes to remove any residual water.

Then perform the standard direct or indirect FA procedure using BVD-specific reagents. As few as 5% of cells may be expressing antigen. This technique is more sensitive than growing cells directly onto the slide.