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Section: <b>Virology Manual</b>	Subject Title: <b>Appendix XIV Quality Control of Cell Cultures Used for Routine Virus Isolates</b>	
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## Appendix XIV

### QUALITY CONTROL OF CELL CULTURES USED FOR ROUTINE VIRUS ISOLATES

#### Tube Culture

Upon receipt of cell culture tubes, the record of the date received, vendor, lot number, and passage number is kept in the QC binder for cell lines. The monolayer is checked microscopically for sterility and appearance of an acceptable confluent monolayer.

#### A. Uninoculated Negative Controls:

Reserve 4 tubes of each lot cells for use as controls and label as follows:

N1, date;  
N2, date;  
C, date;  
V, date

- a. Negative Controls with refeed: (2 tubes, N1; N2)

Select one tube on Thursday and one tube on Monday to set up along with inoculated specimens. Incubate, observe and refeed these tubes in parallel with patient inoculated cultures to monitor monolayer quality. They can also be used to provide a baseline for comparison of inoculated cultures when reading for CPE and immunostaining. CMK and HFF tubes are kept for 3 weeks, HEp-2 and RD for 2 weeks

- b. Unopened Negative Controls without refeed: (2 tubes, C; V)

These tubes are left unopened and observed to identify toxicity and contamination originating with the vendor. All tubes, CMK, HEp-2, HFF and RD tubes are kept for only one week. One tube (C) is kept in the clean room and one (V) is placed on the Virology drum.

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Record sterility and cell appearance for these tubes into the LIS Manual  
(REGISTRATION OF TUBE CULTURE MEDIA)

**B. Positive Controls:**

Each week scrape from an HFF tubes containing the following QC strains to propagate the QC strains in the new lot of HFF tubes. Examine microscopically for CPE and record the results into the LIS as growth control. At the same time also inoculate MRC-5 shell vials to perform quality control for MRC-5 shell vials (see II. **Shell Vial Cell Lines (MRC-5 cell suspension below)**)

HSV-1 (ATCC VR-5539)  
HSV-2 (ATCC VR-540)  
CMV (ATCC VR-807)

The shell vials are then stained with HSV-1, HSV-2 and CMV IEA monoclonal antibodies at 24 hours. Record the results into the LIS as shell vial growth control as well as stain controls.

Each month (and whenever necessary), remove from the liquid nitrogen storage, cryovials of above strains to propagate the QC strains in HFF tubes.

Additional positive controls may be set up with the following strains and for the following reasons:

Coxsackie B1 (ATCC VR-28)  
RSV (ATCC VR-284)  
Influenza A (ATCC VR-544)  
Varicella-zoster (ATCC VR-1367)

- i) Low isolation rates
- ii) Comparison of cell lines
- iii) Vendor changes
- iv) Proficiency test failures
- v) Training purposes
- vi) Consistent problems with negative controls
- vii) Preparation of QC material (i.e. Positive control slides)

Consult a senior technologist to determine the cell lines and viruses to be set up.

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## II. Shell Vial Cell Lines (MRC-5 cell suspension)

Upon receipt of a shipment of cells, initial and date the record sheet accompanying the shipment. The record should contain vendor, lot number, passage number and QC data. File in the QC binder for cell lines.

Before seeding the shell vials, aspirate about 30 mL of MRC-5 cell suspension into a 125 cm<sup>2</sup> tissue culture flask, label on the side of flask with “MRC-5, date and ‘Pre’”.

After seeding shell vials, aspirate about 30 mL of MRC-5 cell suspension into another 125 cm<sup>2</sup> tissue culture flask, label on the side of flask with “MRC-5, date and ‘Post’”.

Reserve 6 shell vials for use as negative and positive controls as follows:

### A. Negative Controls for this week, also become Positive Old Lot for following week (3 Vials)

These are incubated at 36°C and observed daily for one week or more to identify toxicity and contamination originating with the vendor. Results are recorded on the QC chart.

Three shell vials are reserved to QC the next shipment in parallel after the cells are added.

### B. Positive Controls (3 Vials)

Each week, usually 2-3 days after seeding the shell vials, HFF tubes containing HSV-1 (ATCC VR-5539), HSV-2 (ATCC VR-540) and CMV (ATCC VR-807) are scraped from and used to inoculate 6 MRC-5 shell vials, 3 from the current lot and 3 from the previous lot (the same 3 vials were used as Negative Controls for a week). The shell vials are then stained with HSV-1, HSV-2 and CMV IEA monoclonal antibodies after 1 day incubation. Record the results into the LIS daily QC under the following codes:

- a. VHSV1D HSV1 daily SLIDE SV QC
- b. VHSV1D HSV1 daily SLIDE SV QC
- c. VCMV-D CMV-IE daily SLIDE SV QC
- d. VQCSV Shell Vial MRC-5 Quality Control