UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

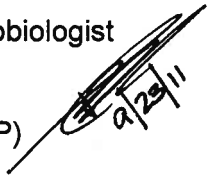
OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

September 23, 2011

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 777-113, Ottoman;
DP Barcode: 391679

From: Tajah Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)



To: Velma Noble PM31/Drusilla Copeland
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
One Philips Parkway
Montvale, NJ 07645

Formulation from the Label: Ingredient Statement not included on proposed label

I BACKGROUND

The product, Ottoman (EPA Reg. No. 777-113) is a registered disinfectant and soft surface sanitizer. According to the registrant's representative (Scientific & Regulatory Consultants), the current protocol was submitted "to support virucidal disinfection efficacy claims on soft surfaces" (enclosed letter, dated June 22, 2011). The referenced letter further states that "this protocol is written to cover various application methods, including trigger sprayer and aerosols....this action is submitted under Ottoman (EPA Reg. No. 777-113) which is a trigger product." Lastly, the referenced letter contends that "the submitted protocol contains only minor modification[s] for virus propagation and recovery as compared to the EPA approved soft surface bacterial disinfection protocol 'Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil' (MRID 48023001)." The protocol was authored by Reckitt Bencksier, dated March 2010.

The current data package contained the referenced letter from the registrant's representative (dated June 22,2011), one protocol (MRID No. 485174-01), and the proposed label.

II USE DIRECTIONS

The proposed product is intended to for use on soft surfaces as a virucide. According to the proposed label the soft surfaces include household fabrics and soft furnishings. Directions on the label provided the following instructions for the preparation and use of the product as a virucide for use on soft surfaces:

Trigger Spray Applications/Aerosol Spray Applications

For Fabric Spot Disinfection: Pre-clean surfaces prior to use. Spray 2-3 times from a distance of 6 to 8 inches until fabric is wet. Fabric must remain wet for [x] minutes/seconds. Let air dry. Wash treated clothing before using or wearing (baby toys, etc.).

To Kill Viruses: Fabric must remain wet for [x] minutes/seconds. Let air dry. For heavy fabrics, repeat application.

III AGENCY STANDARD FOR PROPOSED CLAIMS

There are no Agency standards for the proposed claims.

IV SYNOPSIS OF SUBMITTED PROTOCOL

Protocol Title: Inactivation of (Virus Name—Appendix) in the Presence of Organic Matter

Protocol Purpose: To determine the virucidal activity of a test substance used to disinfect representative fabric surfaces (i.e. polyester and cotton fabrics).

Description/Application of the Test Substance: The test substance is an aerosol spray/trigger/pump spray disinfectant. A description of the application of the test product and contact time will be described.

- For aerosol format products: A 2-3 second spray will be applied to the inoculated test surfaces from a distance of 6 to 8 inches for a ≤10-minute contact time.
- For trigger/pump spray format products: A 2-3 second spray/pumps will be applied to the inoculated test surfaces from a distance of 6 to 8 inches for a ≤10-minute contact time.

Experimental Design

Test System—Organism

- Virus name (ATCC VR-#)
- The "X" strain of Virus Name to be used in this study was obtained from the American Type Culture Collection (ATCC). (Note: Alternate sources of virus may be used provided they are obtained from a reputable supplier and adequate paperwork is obtained regarding the strain/source, transfer, propagation etc. of the virus, i.e. CDC). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected host cells. The cells are disrupted and cell debris removed by centrifugation. The high titer stock virus is aliquoted and stored at approximately -70°C until use. At the time of testing, if necessary, the virus is supplemented with fetal bovine serum to provide an organic soil load of at least 5%.
    - Alternately, influenza viruses may be prepared using 10-12 day old embryonic chick eggs as the host.

Host Cells

- The source of the host cells will be described in the final protocol and final report.
- Cultures are maintained at a target temperature of 37±1°C at 5-7% CO_2 and at the appropriate density.

Maintenance Media

- Minimum Essential Medium (MEM) + 0.1% Gentamicin (optional + 1-5% Fetal Bovine Serum (FBS)

Viral Cytopathogenic Effects (CPE)--Appendix

Media, Reagents, and Equipment

Materials: Including but not limited to

- Multiwell assay plates (e.g. 24 well). Number of assay plates necessary for a test is dependent upon the number of samples being tested, and the number of dilutions being tested.
- Sterile glass or serological pipettes
- 60 mm or 100 mm (diameter) sterile Petri plates
- Indelible marker
- Sterile test tubes with closures (for dilution blanks)
- Sterile test tubes containing 2.0±0.5g 3mm glass bead with closures
- Test tube racks
- Sterile volumetric flasks or cups
- Conical 50 ml tubes
- Sterile syringe columns with glass wool
- Forceps
- Sterile aspirating pipets
- Fabric Test Surfaces—Natural (i.e. 100% cotton) plain weave—78 x 76 threads per square inch. The fabric will be completely de-sized, bleached and without bluing or optical brighteners. The source of the fabric will be identified at the time of testing in the raw data and final report (e.g. Style 400M—obtained from Test Fabric Inc., Middlesex, NJ).
- Fabric Test Surfaces—Synthetic (i.e. 100% Polyester). The fabric will be completely de-sized, bleached and without bluing or optical brighteners. The source of the fabric will be identified at the time of testing in the raw data and final report (e.g. Style 400M—obtained from Test Fabric Inc., Middlesex, NJ).
- 3 mm sterile glass beads

Media and Reagents

- Cell culture maintenance medium (Eagles Minimum Essential Medium (MEM) supplemented with Gentamicin (optional) and FBS)
- Fetal Bovine Serum (FBS)
- Dulbecco's Phosphate-Buffered Saline (DPBS)(optional)
- 5% Sephadex LH-20 solution (optional)
- Trypsin reagent solution (optional)
- 70-99.9% Ethanol or equivalent
- Sodium carbonate (Na_2CO_3)
- 0.05% isoocyylphenoxypolyethoxyethanol (Triton X-100)

Equipment

- Biosafety hood
- Pipet aid
- CO_2 incubator
- Refrigerator/freezer
- -70°C Ultra Low Freezer
- Rees Scientific Environmental Monitoring System
- Inverted Microscope

- Vortex mixer
- Temperature recording device
- Aspirating pump
- Centrifuge
- Bunsen burner
- Timer

Test Fabric Preparation: In this assay, 1" x 1" fabric squares will be used in place of hard, non-porous surfaces to serve as the test surface to demonstrate disinfection on a fabric surface. Two (2) types of fabrics will be tested. Each fabric type will be tested in triplicate. These fabrics are described below:

- Natural (i.e. 100% cotton) plain weave—78 x 76 threads per square inch. The fabric will be completely de-sized, bleached and without bluing or optical brighteners. The source of the fabric will be identified at the time of testing in the raw data and final report (e.g. Style 400M—obtained from Test Fabric Inc., Middlesex, NJ).
- Synthetic (i.e. 100% Polyester). The fabric will be completely de-sized, bleached and without bluing or optical brighteners. The source of the fabric will be identified at the time of testing in the raw data and final report (e.g. Style 400M—obtained from Test Fabric Inc., Middlesex, NJ).

Prior to testing, each fabric will be processed in the following manner. Each fabric type will be processed separately. The documentation of this processing will be recorded.

- In a stainless steel pot, prepare a fabric scouring solution by adding 1.5 grams Sodium Carbonate (Na_2CO_3) and 1.5 grams of Triton X-100 to 3 liters of deionized water while heating.
- Add approximately 100 grams of test fabric per liter of scouring solution.
- Allow the solution to reach a rolling boil. Boil for ≥ 60 minutes but ≤70 minutes.
- Using gloved hands and/or tongs, remove the fabric from the scouring solution.
- Rinse thoroughly with deionized water until all traces (foaming) of the wetting agents are visibly noted to be gone. This can be achieved by running the deionized water to refill the pot. To aid in removing the scouring solution, the fabric can be rung out occasionally with gloved hands.
- Note: Only gloved hands should be used when handling the fabric from this point forward. This will avoid adding unwanted body oils to the fabric.
- Allow the fabric to air dry completely by hanging or draping for at least 24 hours.
- Using scissors and a ruler, cut 1" x 1" test squares from the dried fabric.
- Place each test square into separate glass Petri dishes.
- Steam sterilize for 20 minutes at 121°C.
- Cool and store the fabric test squares at ambient temperatures for ≤30 days.

Description of Experimental Design

Number of Dilutions and Cultures for Virucidal Efficacy Study (Per Fabric Type)			
Test or Control Group	Dilutions Assayed**	Cultures Per Dilution	Total Cultures**
Host Control	NA	≥ 4	At least 4
Virus Suspension Titer Determination Control	-2, -3, -4, -5, -6, -7	4	24
Dried Virus Control (For Each Fabric Type)	-2, -3, -4, -5, -6, -7	4	24
Sample Batch #1 + Virus (Test in Triplicate for Each Fabric Type)	-1, -2, -3, -4, -5	4	20
Sample Batch #2 + Virus (Test in Triplicate for Each Fabric Type)	-1, -2, -3, -4, -5	4	20
Cytotoxicity Control (One Batch for Each Fabric Type)	-1, -2, -3, -4	4	16
Neutralization Effectiveness Control (One Batch for Each Fabric Type)	-1, -2, -3, -4	4	16
Neutralization Tier Confirmation	-1, -2, -3,	4	12

**This is an example dilution scheme. These dilutions can be expanded or collapsed based on the virus titer and test substance properties.

Host Cell Preparation: Multiwell plates (e.g. 24 well assay plates) containing the appropriate host cell line are received from a commercial source or prepared in-house and checked for confluence, contamination, etc., prior to test. Either the day before test, or on the day of the virucidal assay, growth media is aseptically removed from each well containing target cells. The cells can then be washed (i.e. 1-2 minutes) by adding 1-2 mL of wash solution (e.g. DPBS, EBSS or MEM without FBA) to each well, rocking the plate for 10-15 seconds, and removing the wash solution prior to adding the samples. This step is optional and can be performed if excessive cell debris is observed if for viruses sensitive to FBS (i.e. Rotavirus). If performed, the wash step will be performed immediately prior to re-feeding the plates and will be documented in the raw data and final report. The virus stock is removed from the freezer and thawed. A 0.2 mL aliquot of the virus stock is spread onto five areas of the fabric carrier.

- Inoculate 0.2 mL of the test culture onto each sterile carrier using an appropriate micropipette.
- The inoculum will be applied to five areas of the fabric carrier (the total inoculum of the five areas being 0.2 mL). Each corner and the direct center are the target areas.
- Test surfaces are allowed to dry at ambient temperature (24±5°C) in a laminar flow hood for up to 30 minutes until visibly dry. Alternately, viruses may be dried in a controlled temperature and humidity chamber. Actual drying conditions (temperature and humidity) will be recorded and documented in the raw data and final report.
- Aseptically transfer dried inoculated surfaces to a sterile Petri dish for treatment.

Treatment/Neutralization of the Test System (Inoculated Fabric Carrier) with the Test Substance: Test substance samples are used as per the Sponsor's instructions ensuring that the entire test surface area is covered. Note: Only one side (the inoculated surface) is treated with the test substance. For each batch tested, the test substance is applied directly to the dried virus and allowed to sit for the contact time at room temperature. At the contact time, 2.0 ml of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the inoculated/treated/neutralized test surface is transferred to a tube containing 2.0± 0.5 g of glass beads. The liquid remaining in the dish after neutralization will be recovered and added to the tube with the test surface. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger, (if desired) to reduce cytotoxicity. The collected sample will be considered the 10^{-1} dilution of the virus/test substance/neutralizer mixture or media/test substance/neutralizer mixture. Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media). If it is determined that the product has minimal toxic effects on the host cell, then Sephadex columns are optional.

Sephadex Gel Filtration to Remove Cytotoxic Effects

- Some test substances demonstrate cytotoxicity to a point that the assay acceptance criteria cannot be met or certain viruses die off in the presence of serum (i.e. Rotavirus). In such cases, Sephadex columns will be prepared and used in the assay. Sephadex is a filtration medium which binds to and removes components of the test substance that cause cytotoxicity and neutralizes the test substance. Assay controls (Dried Virus Control, Cytotoxicity/Neutralization Effectiveness) must also be passed through Sephadex columns if Sephadex columns were used during the test procedure.
- At least one day prior to start of the assay, prepare a 5% Sephadex slurry. Sterilize and store at least overnight at 2-8°C. To sterilize, autoclave the slurry for 20-25 minutes at 121°C/15 PSI, and allow it to cool to ambient temperature prior to use.
- Sephadex columns are prepared on the day of the virucidal assay procedure, but the assembly without Sephadex slurry may be prepared earlier and stored until needed. Prepare column by inserting a small wad of glass wool into a syringe barrel to cover the opening in the syringe tip. Place syringe barrel into a sterilized bottle or tube. Autoclave assembly and store at room temperature until needed. On the day of assay, pipet Sephadex slurry into the barrel and allow to drain to settle. Continue to add slurry until the settle column height reaches 8-10 mL in a 10 mL barrel, or 4-5 mL in a 5 mL barrel. Prior to neutralization, place full columns into a disposable centrifuge tube and cover the top of the tube/barrel assembly. Centrifuge at 600-650 x g in an IEC CRU-5000 centrifuge with a swinging bucket rotor for 3-4 minutes to remove the void volume in the column. The height of the column will pack down during this procedure.
- Discard the void volume.
- Neutralized test product/virus mixture, test product/medium mixture or virus/medium mixture will be passed through the Sephadex column utilizing a syringe plunger. The collected mixture (10-1 dilution), which was passed through the Sephadex column, will be serially diluted and plated as described.

Host Cell Plating and Incubation: A 0.1 mL aliquot of each dilution is then aseptically pipette into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2 mL of the appropriate media. The assay plates are placed under the appropriate incubation conditions (i.e. 37±1.0°C with 5-7% CO_2), and the cell are observed for toxicity or characteristic viral CPE after the designated time period (Appendix).

If cytotoxicity is suspected or known, after ≥ 22 hours incubation, the plates will be observed for cytotoxicity and any dilutions exhibiting toxicity or potential toxicity can be aspirated and re-fed with the appropriate maintenance media. If media change out is performed on test plates, it must also be performed on the same dilutions for all controls, except neutralization validation plating. This will be documented in the raw data.

Documentation of the various steps in the procedure as well as results will be recorded in the appropriate data sheet.

Controls

Viral Suspension Titer Determination: The viral suspension will be diluted and plated to determine the initial viral titer of the test virus. A 0.1 mL aliquot of each dilution is aseptically pipette into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2 mL of the appropriate media. The assay plates are placed under the appropriate incubation conditions (i.e. 37±1.0°C with 5-7% CO_2), and the cell are observed for toxicity or characteristic viral CPE after the designated time period (Appendix).

Dried Virus Control: A 0.2 mL aliquot of the virus inoculum will be inoculated onto the five areas of the fabric test surface and allowed to dry for up to 30 minutes. The actual drying conditions (temperature and humidity) will be recorded in the raw data and final report. The dried virus control will be performed for each fabric test surface type using 2.0 mL of media or neutralizer in place of the test substance. At the contact time, 2.0 ml of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the inoculated/treated/neutralized test surface is transferred to a tube containing 2.0±0.5 g of glass beads. The liquid remaining in the dish after neutralization will be recovered and added to the tube with the test surface. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger, (if desired) to reduce cytotoxicity. The collected sample will be considered the 10^{-1} dilution of the virus/test substance/neutralizer mixture or media/test substance/neutralizer mixture. Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media). A 0.1 mL aliquot of each dilution is then aseptically pipette into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2 mL of the appropriate media. The assay plates are placed under the appropriate incubation conditions (i.e. 37±1.0°C with 5-7% CO_2), and the cell are observed for toxicity or characteristic viral CPE after the designated time period (Appendix).

This control will determine the relative loss in virus infectivity resulting from drying, neutralization and Sephadex neutralization (if applicable) alone.

The results from this control will be compared with the test results to confirm recovery of at least four $\log_{10}$ of infectious virus, for each fabric type, following drying and neutralization. This titer will be compared with the titers of the test results for that particular fabric type to reach the acceptable test criteria.

Cytotoxicity and Neutralization Effectiveness Controls: The cytotoxicity control will be performed for each fabric type to determine the level of cytotoxicity present ($TCID_{50}$).

The Neutralization Effectiveness Control will determine if residual active ingredient is present after neutralization.

Perform the previous procedure, under Dried Virus Control, utilizing 0.2 mL of cell culture media as the final inoculum, in place of the virus suspension during the inoculation procedure and dry for up to 30 minutes. Treat the surface as performed in the test. At the contact time, 2.0 ml of an appropriate neutralizer will be added to the test surface and the plate will immediately be swirled to mix. Using sterile forceps, the media inoculated/treated/neutralized test surface is transferred to a tube containing 2.0±-0.5 g of glass beads. The remaining liquid volume is recovered and added back to the tube containing the test surface and glass beads. Immediately, vortex the tube for 10-15 seconds. Pass the liquid through Sephadex columns using a syringe plunger, (if desired) to reduce cytotoxicity. The collected sample will be considered the 10^{-1} dilution of the virus/test substance/neutralizer mixture or media/test substance/neutralizer mixture. Serial tenfold dilutions are then carried out in cell culture media or appropriate neutralizer (e.g. 0.5 mL of a virus dilution is added to 4.5 mL of cell culture media). A 0.1 mL aliquot of each dilution is then aseptically pipette into each of four wells of host cells, which were previously aspirated of media, washed (optional) and re-fed using approximately 1-2 mL of the appropriate media. Four wells of each dilution will be used for the Cytotoxicity Control and four wells of each dilution will be used for the Neutralization Effectiveness Control. The assay plates are placed under the appropriate incubation conditions (i.e. 37±1.0°C with 5-7% CO_2), and the cell are observed for toxicity or characteristic viral CPE after the designated time period (Appendix).

One lot, one repetition (for each fabric type) of the test substance will be used for the cytotoxicity and neutralizer effectiveness controls. This control will be processed exactly as the test procedure but instead of viral inoculum, appropriate media will be added to the surface and dried as described previously. Post test, recovery and Sephadex neutralization, if applicable, the sample will first be diluted and plated for the cytotoxicity control.

Each plated dilution of the neutralized test substance (and Sephadex filtered, if applicable) will be challenged with a 0.1 mL aliquot of low titer virus (which delivers 1-1000 viral particles) to determine the dilution(s) at which virucidal activity, if any is retained. A 0.1 mL aliquot of the diluted virus will be added to the four wells of host cells which were previously inoculated with 0.1 mL of the neutralized test samples. Those dilutions which are toxic to the cells and/or do not exhibit virus replication are not included in the $\log_{10}$ reduction calculations of the germicidal activity.

Neutralization Verification Control: The diluted virus used for the neutralization effectiveness control will be plated into host cells as previously described to confirm a low level of viral inoculum (1-1000 viral particles).

Host Cells Viability Control: At least four wells will be inoculated with an appropriate medium during the incubation phase of the study. This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the test media and fetal bovine serum.

Calculations

Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman and Karrer.

Log of 1st dilution inoculated – [((sum of % mortality at each dilution/100) -0.5) x (logarithm of dilution)]

Calculation of log reduction (for each fabric type):

Dried Virus Control $TCID_{50}$—Average Test Substance $TCID_{50}$ = Log Reduction*
*Note: EPA requires complete inactivation of the test virus

Assay Evaluation Requirements

The success criteria for soft surfaces will be identical to the EPA virucidal requirements for hard surfaces. EPA Pesticide Assessment Guidelines, Subdivision G: Product Performance § 91-30 (d)(5), Recommended Methods; Disinfectants –Efficacy Against Viruses (Hard Surfaces) state that a procedure for virucidal assay must provide the following information:

- If desired, a 5% soil load of fetal bovine serum will be added to the viral inoculum to simulate an organic soil load.
- The virus must be recovered from a minimum of four determinations per each dilution in the assay system (e.g. cell culture system).
- The activity of the germicide against the test virus from a minimum of four determinations per each dilution in the assay system is determined.
- Cytotoxicity controls: the effect of the germicide on the assay system for a minimum of four determinations per each dilution.
- The viral preparation method, recovery, and neutralization will be documented in the raw data (The data submitted to the Agency must include this information).
- The $TCID_{50}$ values calculated for each assay.
- The test results shall be reported as the reduction of the virus titer by the activity of the germicide ($TCID_{50}$ of the control less the TCID of the test substance activity), expressed as $\log_{10}$, and calculated by a statistical method (e.g. Spearman Karrer).
- When cytotoxicity is observed in the assay system, at least 3 $\log_{10}$ reductions in the viral titer must be demonstrated beyond the cytotoxic level. The calculated viral titer must be reported with the test results.

Study Acceptance Criteria

A valid test requires:

- The product must demonstrate complete inactivation of the virus at all dilutions.
- When cytotoxicity is evident, a ≥ 3 $\log_{10}$ reduction in the viral titer must be demonstrated beyond the cytotoxic level.
- The virus titer recovered from the dried control must be $\geq 10^4$.
- The neutralization effectiveness control must demonstrate adequate neutralization of the test substance using low number (1-1000) of viral particles.
- Host cell controls must be free from contamination and remain viable throughout the length of the assay.

V PROTOCOL CONCLUSIONS

1. The submitted protocol supports the use of virucidal claims on cotton and synthetic fabrics. The virucidal efficacy data provided must support cotton and/or synthetic fabric types consistent with the same fabric type used support disinfection claims. The registrant must demonstrate efficacy as a disinfectant on soft surfaces prior to submitting efficacy data to support virucidal claims on soft surfaces.

2. The list of testable viruses and conditions is included in Appendix 1.

VI LABEL RECOMMENDATIONS

The proposed label claims are unacceptable:

- "fast acting"; the Agency has not determined the contact time consistent with this descriptor;
- "at the source"; this statement is false and misleading. The soft surface may be a source, but it is not exclusively the source; and
- "healthier"; as this implies heighten efficacy.

Appendix 1

Virus (Strain)	Host	Maintenance Medium	Virus Type [1]	Incubation Time	Typical Cytopathogenic Effect (CPE)
Adenovirus	A259, HEK293, HeLa, HEp-2, MRC-5, WI-38	EMEM + 1-2% FBS	H	7-21 Days	Formation of tightly associated grape-like clusters or lattice-like of enlarged, refractile, rounded cells.
Coronavirus	L132/WI 38	EMEM + 2% FBS	L	7-14 Days	Granulation and vacuolation of cells, degeneration of cells
Coxsackie A	RMK, MRC-5, WI-38	EMEM + 2-5% FBS	H	5-12 Days	Highly refractile, rounded cells throughout monolayer or in loose clusters, degeneration and detachment of cells.
Coxsackie B	RMK, HeLa, Hep-2, Vero	EMEM + 2-5% FBS	H	5-12 Days	Highly refractile, rounded cells throughout monolayer or in loose clusters, degeneratio. and detachment of cells.
Echovirus	RMK, MRC-5, WI-38, Vero	EMEM + 2-5% FBS	H	5-12 Days	Highly refractile, rounded cells throughout monolayer or in loose clusters, degeneration and detachment of cells.
Feline calicivirus	CRFK	EMEM + 2-5% FBS	H	1-7 Days	Refractile rounding and sloughing, degeneration and detachment of cells.
Herpes Simplex Virus (1 or 2)	A549, HeLa, Hep-2, MRC-5,Vero, WI-38	EMEM + 2-5% FBS	L	7-14 Days	Grape-like clusters of rounded, refractile cells with or without syncytia.
Influenza A/B	10-12 day ECE	N/A	L	2-7 days	N/A; Virus propagation is detected by the presence of hemagglutinin
Influenza A (H1N1, Example: A/WS/33)	RMK, MDCK	EMEM + 1% FBS	L	5-10 days	Cellular detachment, degeneration of the cell sheet, giant cell / syncytia formation
Influenza A (H1N1, Example: A/Swine/Iowa/15/30)	RMK, MDCK	EMEM + 1% FBS	L	5-10 days	Cellular detachment, degeneration of the cell sheet, giant cell / syncytia formation
Measles	RMK, Vero	EMEM + 2% FBS	L	7-14 days	Formation of large, refractile multinucleated syncytia cells
Mumps	RMK, Vero	EMEM + 2% FBS	L	7-14 days	Variable with increased rounding, granularity, progressive degeneration and syncytia.
Poliovirus Type 1	Vero, HeLa, WI-38	EMEM + 2-5% FBS	H	5-12 Days	Highly refractile, rounded cells with rapid destruction of monolayer.
Respiratory Syncytial Virus	HeLa,HEp-2,MRC-5, WI-38	EMEM + 1-2% FBS	L	7-14 Days	Formation of large, multinucleated refractile syncytia/giant cells, detachment of cells
Rhinovirus	MRC5/WI-38	EMEM + 1-2% FBS	H	7-14 Days	Highly refractile, rounded cells throughout monolayer or in loose clusters.
Rotavirus	MA-104	EMEM + 1-5 ug/ml Trypsin	H	5-12 Days	Degeneration of cell sheet.
Vaccinia	Vero	EMEM + 2% FBS	L	7-14 Days	Cell rounding; and formation of cytoplasmic bridges.

Key:
EMEM = Minimum Essential Medium (Earle's Salts) FBS = Fetal Bovine Serum ECE = Embryonated Chick Eggs Typical Growth/ Incubation conditions: Coronavirus and Rhinovirus = 32-34°C and 5-7% CO_2. All other viruses = 36.0-38.0 °C and 5-7% CO_2.

[1]H = hydrophilic or Non-enveloped; L = Lipophilic or Enveloped

Note: All stock viruses will be received from a reputable source (i.e. ATCC, CDC, etc.) with the proper paperwork to ensure traceability as to source, passage number, titer, etc. and stored in the Deep Freezer at approximately -70°C.

Note: The viruses specified in Appendix 1 are examples and other viruses can be added as needed.