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|  Cell Culture Passes |
| **Purpose** | This procedure provides instructions for performing cell culture passes. |
| **Policy Statements** | • This procedure applies to all virology technical staff. |
| **Principle and Clinical Significance****Test Code** | Cell cultures showing marked degeneration, toxicity, or ambiguous CPE are passed by subculturing the culture medium and cells to new cell cultures. This dilutes out toxic effects of the inoculum and provides viable cells for viruses, which may be present. Identification of viral CPE can be made by passing the culture to the appropriate cell line and looking for characteristic CPE.PASS |
| **Materials** |  |  |  |
|  |  | **Supplies** | **Equipment** | **Media** |
|  | • Sterile 1 ml pipets | • BSC* Pipet Aid
* Vortex
 | •Uninoculated tubes of desired cell lines |
| **Sample** | Cell culture tube to be passed |
| **Special Safety Precautions** | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Microbiologists/virologist are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the [**Microbiology/Virology Policy Manual**](http://intranet.childrensmn.org/References/labsop/index.php?view=folder&folder=mcvi)**:**1. *Biohazard Containment*
2. *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
* All specimen handling and culture inoculations are to be done in the biosafety cabinets while wearing a lab coat and gloves.
* Passage of the culture is generally the first step taken to identify viral CPE so the virologist must be aware that unidentifiable patterns of cytopathic effect (CPE) could be caused by a potential agent of bioterrorism. Consult with the Lead MLS and Technical Director when unusual CPE is seen and contact the physician to see if the patient’s clinical presentation is consistent with smallpox or a hemorrhagic viral infection. If the patient’s history and/or disease is consistent, notify the Minnesota Department of Health and follow instructions to forward the culture and remaining specimens to the appropriate laboratory. If the patient’s history and/or disease is not consistent with a viral agent of bioterrorism, follow routine laboratory protocols

.**Orthopoxviruses:** Variola virus(smallpox),vaccinia virus (used for smallpox vaccine, and monkey pox will grow in cell lines used for herpesviruses. CPE is described as hypertrophic rounding of the cells in the monolayer. **Alphaviruses.(** Includes Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE), Venezuelan Equine Encephalitis(VEE) and Chikungunya): Cell lines that permit growth include MRC-5, A-549, Vero, LLCMK, and hamster kidney. CPE may only occur after passage. **Viral hemorrhagic fever viruses (VHF):** Filoviruses, arenaviruses, and SAHF viruses are cultivable in nonhuman primate and human cell lines, such as Vero and MRC-5. **Coxiella** : although not a virus will grow in cell culture and is highly infectious. *C. burnetii* can be inadvertently isolated in conventional cell cultures in a wide variety of cell lines, including all fibroblast cell lines. After an incubation period of 5 to 15 days, *C*. *burnetii-*infected cells are detectable as cytoplasmic inclusions. |
| **Procedure****Reporting** | 1. Scrape the cell monolayer off the side of the culture tube to be passed using a sterile 1 ml pipet until the media is slightly cloudy. Vortex well.
2. Label tubes as follows:

Passes to the same cell line: Label as Accession # P and number of pass (0000P1, 0000P2, etc.).Passes to different cell lines:  If pass is from a: MRC-5: Label as Acc # M and number of pass HEP-2: Label as Acc # H and number of pass RMK: Label as Acc # R and number of pass SF: Label as Acc # S and number of pass RMIX: Label as Acc # X and number of pass MRC-5 vial: Label as Acc# V and number of passExamples:MRC-5 to MRC-5 = 0000P1HEP-2P1 to HEP-2 = 0000P2MRC-5 to HEP-2 = 0000M1M1 to HEP-2 = 0000M2M1 on HEP to HEP-2= 0000M1H1Include source and date of inoculation on tubes.1. Inoculate 0.2 ml of the cell suspension into new cell cultures. Hold passes for HSV at least 3 days and passes for other viruses 7 days before reporting as negative.
2. Freeze remaining cell suspension at -70°C. Hold for one week.
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| Example of workup: |
| **Method Performance Specifications** | If CMV or varicella zoster virus is suspected passes should be made by trypsinizing the monolayer. See [VIRO 2.20 Trypsin Procedure](http://intranet.childrensmn.org/References/labsop/viro/proc/viro-2.20-trypsin-procedure.pdf) . |
| **References** | 1. Forbes, B.A., Bailey and Scott’s Diagnostic Microbiology, 12th edition, 2007, pg. 757.
2. Lennette, E.H., et al (Eds.), Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th edition, 1979, pg. 97.
3. Sentinal Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases, Unknown Viruses Section, American Society for Microbiology 3/2016,http://www.asm.org/index.php/guidelines/sentinel-guidelines.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Helen Stefan | 1986 | Initial Version |
| 1.1 | Helen Stefan | 1990 | Reformatted |
| 2 | Helen Stefan | 6/26/2013 | Numbered procedure, changed version designation to whole numbers, added safety considerations with unidentifiable patterns of CPE. |
|  | 3 | Helen Stefan | 9/30/14 | Added pass initial X for RMIX and V for MRC-5 vial passes |  |  |
| 4 | Helen Stefan | 4/5/2015 | Renumbered and reformatted for CMS from V 5.04 to VIRO 2.03, added PPE in Safety section, added acc # to labeling instructions |
| 5 | Helen Stefan | 5/27/16 | Added consult with Technical Director when unusual CPE is seen. |
| 6 | Helen Stefan | 8/4/17 | Added Coxiella, examples of Alphaviruses, Orthopox viruses and defined VHF in Safety Precautions section. Fixed hyperlinks. |
| **Archived by:** |  | **Archived Date:** |  |