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| Processing, Inoculation and Incubation of Viral Cultures |
| **Purpose** | This procedure provides general instructions for the processing, inoculation and incubation of viral cultures |
| **Policy Statements** |  This procedure applies to all technical staff performing culture set up. |
| **Test Code** | VRSPVIRCHSVCRHSVRCMV |
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| **Sample** | 1. **Acceptable specimens**
* Refer to *Lab Test Directory* on StarNet
* Nasal specimens collected on large swabs are Nares and are acceptable for HSVC only. They are unacceptable for VRSP and VIRC. NP swabs (Minitips) must be used for nasopharyngeal swab (NP) collection.
* CSF is **NOT** acceptable for HSVC/RHSV due to low yield of HSV in culture from CSF. Herpes simplex PCR (HSVP) must be ordered for HSV. CSF is acceptable for VIRC.
1. **Specimen Collection and Transport**
* Refer to *Lab Test Directory* on StarNet
* Viruses are more stable at cold temperatures and their infectivity can decline rapidly at room temperature. Refrigerate specimens ASAP and do not leave specimens at room temperature for extended periods. Store specimens in VTM at -70° C if culture will be delayed beyond 24 hrs after collection.
* Do NOT refrigerate whole blood specimens (EDTA) for Viral Blood Cultures (BCV). Store at room temperature.
1. **Specimen assessment**
* Refer to the policy MCVI 2.1 *Specimen Rejection Criteria.*
* Reject: specimens with a transit time exceeding 2 hours after collection without refrigeration; specimen not submitted in appropriate container; calcium alginate or wooden swabs; dry swabs; improperly labeled specimen; insufficient volume; external contamination. If an unacceptable specimen is received, the physician will be requested before the specimen is discarded.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the [*Microbiology*and *Virology Policy Manual*](http://khan.childrensmn.org/Manuals/Lab/SOP/MCVI/MCVI.asp)**:**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.Culture attempts of avian influenza (H5,H7, H9) ,SARS coronavirus and MERS coronavirus should only be attempted in BSL 3 facilities and should be referred to MDH. |
| **CDSK Processing****Virology****Appendices**  | 1. Process specimens promptly .Refrigerate specimen up to 24 hours at 4°C. If specimens cannot be processed within 24 hours, then the specimen should be frozen at –70°C immediately upon receipt. If CSF specimens have viral cultures added to an existing refrigerated collection greater than 24 hours old, they may be run with a disclaimer.

A delay in inoculation results in a delay in reporting and results in the diminished likelihood of a positive result.1. Accession specimen in computer. See [MCVI 5.0 Micro/Viro Computer Training](http://khan.childrensmn.org/Manuals/Lab/SOP/MCVI/Comp/209737.pdf)
2. Viral transport media (VTM) is used to prevent specimen drying, to maintain viral viability and to retard the growth of microbial contaminants. Children’s uses 3ml VTM (Red cap) ,9ml VTM (blue cap 50 ml tube, frozen) and urine VTM (blue cap 15 ml centrifuge tube, frozen)
* **NP swabs (2 required)**: place into 3 ml VTM. Swabs should remain in the VTM by cutting the wire shafts. Vortex well.
* **Other Swabs (throat, skin, rectal swab (RS), etc)**: place into 3ml VTM. Vortex well.
* **Washings/aspirates**: place 1-2 ml into 3ml VTM depending on cellularity. Vortex well.
* **Stool**: Place 1ml/1 gram (pea sized) into thawed 9ml VTM. Vortex well.
* **Urine**: Place 3-5ml urine into thawed urine VTM. Vortex. Low volume urine: place 1.5-2.9 ml urine into thawed low volume urine VTM. Vortex.
* **Tissue**: Stomach specimen in 3ml VTM, transfer to same VTM tube.
* **CSF**: Do **NOT** put specimen into VTM. CSF is inoculated directly (0.1-0.2ml) per tube.
1. Follow processing instructions in the Media field of Micro label. Instructions are generated based on the Specimen Description code (SDES) entered.
* See VIRO 1.00.a1(Mpls) and VIRO 1.00.a2 (STP) Viral Specimen Processing Charts on StarNet and in the hoods for processing instructions.
1. Compare original specimen label with Sunquest labels. Match patient name, medical record number and accession number, assuring all match exactly. Label the original container with labels for all tests ordered on the specimen.
2. Place long bar code labels on VTM for all tests being performed on that specimen. Write your tech code on one bar code label when transferring specimen to VTM.
3. In the BSC, transfer the specimen to the correctly labeled VTM comparing patient’s name, medical record number, accession number and test information on the original specimen container with the labels on the VTM.
4. The following types of cell lines are used for viral isolation:

a. Diploid fibroblast cell line- MRC-5 SF(also called MRHF, HFF and SFIB) b. Established or heteroploid line- HEp-2 Mink lung/A549 (R-Mix) McCoy(mouse fibroblast) c. Primary cell line- PMK (also called RhMK)Continuous cell lines are serially propagated cell cultures that can be either diploid or heteroploid. Diploid cell lines are subcultures of a primary culture and have the same chromosomal make-up as the original tissue. Diploid cell lines are usually derived from normal tissue and maintain normal properties throughout subsequent cultivations and generally have limited sub-cultivations. Heteroploid cell lines are also subcultures of a primary culture, but transformation has occurred, so cells are considered abnormal. The cells can be propagated an indefinite number of times.1. The cell culture tubes have a single layer (monolayer) of metabolically active cells growing on the side of the tube opposite the indicator mark (^) on the tube. Tubes are incubated in slant racks with this indicator mark (^) up so the cells are bathed in media. Uninoculated tubes are located in Virology CO2 Incubator #1 (Upper) in labeled racks.
2. Select the appropriate tubes stated in the Media field of the Micro label and affix the accession number to each tube below the indicator mark (^) on the tube. The same accession number is placed on a cryotube, which is to hold the remaining specimen and be frozen at -70°C. Write date of inoculation on all tubes.
3. Respiratory specimens: Use PMK tubes with serum free media (SPMK). Fetal bovine serum contains substances that may be inhibitory to orthomyxoviruses and paramyxoviruses. Virology makes SPMK daily by removing the maintenance media from the PMK cells and replacing with 1 ml. of serum free MEM.
4. After processing specimens (refer to processing chart), use a sterile 1 ml pipette and inoculate 0.2 ml of specimen (0.1 minimum for CSF) in each tube or vial indicated. Inoculate in order: MRC-5 or SF, HEp-2, and PMK last to avoid contamination of simian viruses which may be present in the PMK.
5. To inoculate 6 tubes a total volume of 1.2 ml is required. If less than 1.2 ml is received, only inoculate 1 tube of each cell line with 0.2 ml.
6. Storage of specimen after inoculation:

Evenings and Nights- Store specimens in VTM in CDSK refrigerator in Virology rack.Day shift- Place 1.5-2ml of specimen in VTM in cryovial labeled with long bar code label. Write source on label. Place in virology rack with VTM tube in Virology refrigerator. Virology staff will freeze sample as appropriate after any subsequent testing. Urines for VIRC/RCMV are not frozen. Store urine samples in urine VTM in virology refrigerator for 5 days before discarding.1. The fluid medium in the cell culture contains phenol red as a pH indicator. Occasionally when urines are inoculated, the cell culture medium will become yellow indicating increased acidity. If the media appears bright yellow after inoculating the urine, use a sterile pipette to add 1 drop of 7.5% sodium bicarbonate to each tube prior to incubation.
2. After inoculation, loosen caps and place in appropriate racks slanted at 5° angle with the indicator mark (^) facing up. Caps remain tight on serum free PMK and HEp-2 tubes. Place rack in CO2 incubator at 32.5°-36.0°C.
3. Daily, one uninoculated tube of each cell line is incubated under the same conditions to be used as reference controls when screening for viral cytopathic effect (CPE).
4. After overnight incubation, transfer PMK tubes to roller drum to continue incubation for 9-10 days.
5. To avoid toxicity and degeneration of the monolayers, change the maintenance media after 24-hour readings on all cell cultures and at weekly intervals.
6. Observe monolayers for the development of CPE and /or hemadsorption. Tube cultures are examined for CPE at least every other day for the first two weeks of incubation.

Record all observations and manipulations on the cell line worksheets and in computer workups as appropriate. 1. Herpes simplex cultures are incubated for 7 days before reporting as negative.

Cultures for CMV, VZV and/or respiratory viruses are incubated for 21 days before reporting as negative.Viral cultures of CSF, stool, and rectal swabs, and oral sources are incubated for 14 days before reporting as negative.All other sources are incubated for 21 days.1. Hemadsorption of PMK tubes is performed on:
	1. Respiratory specimens at 10 days post inoculation or earlier as indicated (before passes, to evaluate or confirm CPE)
	2. Urine (if mumps is suspected) at 2, 6, and 10 days post inoculation

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| **References** | 1. Leber,A (Ed), Clinical Microbiology Procedures Handbook, 4th edition, American Society for Microbiology, Washington D.C., 2016. 2. Dunn J, Specimen Collection, Transport, and Processing: Virology In JH Jorgensen et al, (ed), Manual of Clinical Microbiology*,*11th edition, American Society for Microbiology, Washington DC,2015. 3. DHI Cells-SingleMixed product insert, Athens OH, June17, 2014. |
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial 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
2. Complete Microbiology/Virology Culture Desk New Employee Training Checklist
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 05/28/1986 | Initial Version |
| 1.1 | Helen Stefan | 09/01/1990 | Added HAd schedule for mumps |
| 1.2 | Helen Stefan | 01/1992 | Added where to place label on tube |
|  | 1.3 | Sonja Heck | 06/2005 | Changed HAd schedule due to addition of RMIX rapid culture |  |  |
| 1.4 | Helen Stefan | 07/02/2007 | Table; changed body fluids to inoculate directly unless bloody or insufficient quantity mix 1:2 with VTM, CSF Inoculate 0.2ml if sufficient quantity. Deleted centrifuge if grossly bloody. |
| 1.5 | Helen Stefan | 06/28/2010 | Corrected statement 1 from “store at 4°C no longer than 2 hrs” to Process specimens promptly. Refrigerate specimen up to 24 hours at 4°C. If specimens cannot be processed within 24 hours, then the specimen should be frozen at –70°C immediately upon receipt. If CSF specimens have viral cultures added to an existing collection greater than 24 hours old, they may be run with a disclaimer. |
| 1.6 | Helen Stefan  | 11/2/2010 | Added Examine tube cultures for CPE at least every other day. |
| 2 | Helen Stefan | 6/7/15 |  added sample and safety sections, added #3, #5, #9, updated references. Reformatted and renumbered for CMS |
| 3 | Helen Stefan | 7/10/15 | Added McCoy and Mink lung/A549 to cell line list. |
| 4 | Helen Stefan | 9/9/2015 | Adjusted CO2 Temp Range of 33-35 to Isensix defined ranges of 32.5-36.0 |
| 5 | Helen Stefan | 8/3/17 | Removed reference to red indicator dot for CellPro tubes and changed to indicator mark(^) for DHI tubes, added for the first 2 weeks of incubation in Virology #4, added MRHF, reiterated labeling requirements from MCVI 2.0,added documents in appendices,added updated references. |
| **Archived by:** |  | **Archived Date:** |  |