Lab Dept:	**Microbiology/Virology**
Test Name:	**HERPES SIMPLEX VIRUS CULTURE**

General Information

Lab Order Codes:	HSVC
Synonyms:	HSV Isolation; Herpes Culture; HSV Culture; HSV 1 and 2 Culture
CPT Codes:	87252 - Virus isolation; tissue culture inoculation, observation, and presumptive identification by cytopathic effect The following testing may be added if appropriate based on findings for organism identification (multiple additions are possible if more than one organism is identified or more than one method is needed for identification). 87253 – Virus isolation; tissue culture, additional studies or definitive identification
Test Includes:	Culture for Herpes Simplex Virus (HSV) **only**. **Rapid FA for HSV must be ordered with this test. Refer to** HSV Rapid FA. HSV is also detected in a routine viral culture.

Logistics

Lab Testing Sections:	Virology
Phone Numbers:	MIN Lab: 612-813-5806 STP Lab: 651-220-6555
Test Availability:	Daily, 24 hours
Turnaround Time:	7 days
Special Instructions:	● **Do Not** use calcium alginate swabs. ● Requisition must state **specific site** of specimen and **date/time** of collection. ● Specimens should be collected in the acute stage of the disease, preferably within 3 days and no longer than 7 days after symptoms develop.

Specimen

Specimen Type: Vesicle fluid, swab of base of lesion, tissue biopsy, nasopharyngeal swabs, nares swab, nasopharyngeal wash, conjunctival swab, throat swab, oral swab , genital swab, bronchial ,tracheal aspirates, blood, rectum swab, skin swab; Neonatal surface culture: Multiple sites can be cultured using a single swab (ending with a rectal swab).

Container: Swab transport system, sterile container, Lavender top (EDTA) Vacutainer™ tube, or viral transport media (M4 VTM) or BD/copan Universal Transport Media (available in Microbiology)

Volume: 1 swab; 1 - 2 mL washings/aspirates; 5 mL whole blood; 2 mini-tip NP swabs

Collection: **Blood:**

Venipuncture for patients greater than 26 weeks gestation OR greater than 2 weeks of age:

Prep with CloraPrep Sepp® Applicator with 2% CHG

1. Disinfect the stopper of the Lavender top tube (EDTA) with 70 % alcohol. Allow to dry.
2. Break the Sepp® ampule to release the 2% CHG.
3. Apply the CloraPrep® solution using a back-and-forth friction scrub for 30 seconds.
4. Allow the area to dry for 30 seconds.
5. If the site must be touched during venipuncture, disinfect the gloved fingers.
6. Collect 5 mL of blood and aseptically inoculate the Lavender top tube (EDTA).
7. Gently invert the tube 4-5 times to mix contents.
8. Forward unprocessed whole blood promptly at ambient temperature only.

Prep with CloraScrub™Swab with 3.15% CHG

1. Disinfect the stopper of the Lavender top tube (EDTA) with 70 % alcohol. Allow to dry.
2. Open the Chlorascrub™Swab package, do not unfold wipe.
3. Apply the Chlorascrub™ wipe using a back-and-forth friction scrub for 15 seconds.
4. Allow the area to dry for 30 seconds.
5. If the site must be touched during venipuncture, disinfect the gloved fingers.
6. Collect 5 mL of blood and aseptically inoculate the Lavender top tube (EDTA).
7. Gently invert the tube 4-5 times to mix contents.
8. Forward unprocessed whole blood promptly at ambient temperature only.

Venipuncture for patients less than 26 weeks gestation AND less than 2 weeks of age:

Prep with 2% tincture of iodine:

1. Disinfect the stopper of the Lavender top tube (EDTA) with 70 % alcohol. Allow to dry.
2. Scrub venipuncture site with 70% alcohol for 1 minute using the Frepp® applicator. Allow to dry.
3. Using the Sepp® applicator, apply 2% tincture of iodine to site starting at the center and moving outward in concentric circles. Allow to dry, approximately 30 seconds.
4. If the site must be touched during venipuncture, disinfect the gloved fingers.
5. Collect 5 mL of blood and aseptically inoculate the Lavender top tube (EDTA).
6. Gently invert the tube 4-5 times to mix contents.
7. Forward unprocessed whole blood promptly at ambient temperature only.
8. Following collection, remove the iodine using the Frepp® applicator or an alcohol pad.

Line Draw (All ages):

1. Prep catheter port by scrubbing the hub for 30 seconds using chlorhexidine gluconate (CHG) and allowing to dry.
2. Aseptically collect 5 mL of blood through the injection port. Blood may be collected without first drawing a discard.
3. **Aseptically inoculate the Lavender top tube (EDTA). Forward unprocessed whole blood promptly at ambient temperature only.**

Transport to the laboratory immediately. **Do not** centrifuge.

Bone Marrow:

Place 1 – 5 mL of bone marrow in Lavender top (EDTA) tube(s). Invert several times to mix bone marrow. **Do Not** centrifuge. Send in original Vacutainer™ tube. Forward unprocessed bone marrow promptly at ambient temperature only.

Throat Swab:

1. Depress the tongue with a tongue depressor so the swab does not touch the tongue.
2. Sample the posterior pharynx, tonsils, and inflamed areas with a sterile swab.

If specimen cannot be transported to the laboratory immediately, place swab in viral transport media (VTM) and refrigerate.

Tissue:

Submit specimen in a screw-capped, sterile container.

Transport to the Microbiology Laboratory immediately.

Skin/rectum:

1. Wash vesicles with sterile saline.
2. Open the vesicle and absorb vesicular fluid into a dry swab.
3. Vigorously scrape base of freshly exposed lesion with the same swab to obtain cells which contain virus.
4. For non-vesicular lesions, collect cells from base of lesion by using a swab premoistened with sterile saline.

If specimen cannot be transported to the laboratory immediately, place swab into viral transport media (VTM) and refrigerate.

Cervical:

1. Remove exudate prior to collection of specimen.
2. Gently insert separate large swab into endocervical canal past squamocolumnar junction. Rotate for 5 - 10 seconds.
3. To avoid contamination, withdraw swab while avoiding touching any vaginal surfaces.

If specimen cannot be transported to the laboratory immediately, place swab into viral transport media (VTM) and refrigerate.

Vaginal:

1. Wipe away excessive amount of secretion or discharge.
2. Obtain secretions from mucosal membrane of the vaginal vault with a sterile swab.

If specimen cannot be transported to the laboratory immediately, place swab into viral transport media (VTM) and refrigerate.

Nasopharyngeal:

1. Obtain 2 specimens using 2 NP swabs (i.e., MiniTip™ Culturette).
2. Gently insert swab through nose into posterior nasopharynx.
3. Gently rotate swab slowly for 5 seconds to absorb secretions.
4. Collect a second specimen in the same manner.

If specimen cannot be transported to the laboratory immediately, cut swabs into viral transport media (VTM) and refrigerate.

Bronchoscopy:

1. 1 – 2 mL of specimen obtained by physician through the biopsy channel of the bronchoscope.
2. Transfer specimen into a luki tube.
3. Transport to the Microbiology Laboratory immediately.

Nasopharyngeal washings:

1. Tilt patient's head back at a 70 degree angle.
2. Insert rubber bulb syringe containing 1-2 mL of sterile saline until it occludes the nostril.
3. Collect specimen (Minimum: 1 mL) with one complete squeeze and release the bulb.

4. Repeat in other nostril.
5. Dispense the specimen into a sterile screw cap container and transport to the laboratory immediately.

If the specimen cannot be transported to the laboratory immediately, place 1-2 mL of specimen in viral transport media (VTM) and refrigerate.

Nasopharyngeal aspirates:

1. Prepare suction set up on low to medium suction
2. Wash hands, Put on protective barriers. (e.g., gloves, gown, mask)
3. Place child supine and obtain assistant to hold child during procedure.
4. Attach luki tube to suction tubing and #6 french suction catheter.
5. Insert catheter into nostril and pharynx without applying suction.
6. Apply suction as catheter is withdrawn. If necessary, suction 0.5-1 mL of normal saline through catheter in order to clear the catheter and increase the amount of specimen in the luki tube.

If the specimen cannot be transported to the laboratory immediately, place 1-2 mL of specimen in viral transport media (VTM) and refrigerate.

Conjunctiva:

Do Not use a dry swab to collect an eye culture.

1. Moisten swab with sterile saline.
2. Retract lower lid and firmly swab conjunctival surface with enough pressure to collect epithelial cells. Avoid eyelid border and lashes.

If specimen cannot be transported to the laboratory immediately, place swab into viral transport media (VTM) and refrigerate.

Anterior Nares:

1. Insert swab, pre-moistened with sterile saline, approximately 2 cm into the nares.
2. Rotate the swab against the nasal mucosa.

If specimen cannot be transported to the laboratory immediately, place swab into viral transport media (VTM) and refrigerate.

Special Processing: Place specimen into viral transport media upon arrival in the laboratory. Swabs should remain in the VTM.

Transport/Storage: **Onsite collections:** Transport to the laboratory immediately.

Offsite collections: Place swab specimens in VTM and refrigerate. Store and ship blood specimens at room temperature. Specimens must be promptly transported to the laboratory with the next available courier, not to exceed 24 hours from the time of collection. However, delayed transport causes a delay of test results.

Sample Rejection: Non-blood specimens with a transit time exceeding 2 hours after collection without refrigeration; specimen not submitted in appropriate transport container; improperly labeled specimen; insufficient volume; external contamination. If an unacceptable specimen is received, the physician or nursing unit will be notified and another specimen will be requested before the specimen is discarded.

Interpretive

Reference Range: No Herpes Simplex Virus isolated after 7 days.

Critical Values: Positive results in systemic infections will be called to the physician or nursing unit.

Limitations:

● HSV can only rarely be cultured from the CSF of patients with HSV 1 encephalitis. The virus is occasionally isolated from spinal fluid of patients with HSV 2 meningitis and of neonates with congenital herpes. **HSV PCR is the method of choice for detecting HSV in CSF**.

● A negative result does not eliminate the possibility of HSV infection.

Methodology: Cell culture with confirmation of HSV cytopathic effect with fluorescein-labeled monoclonal antibodies specific for HSV type 1 or 2.

Additional Information: Genital transmission of HSV infection to sexual partners and neonates involves subclinical shedding of HSV by women with genital herpes. Such shedding is detectable by culture. Culture can provide evidence of acyclovir resistance, and detect potential for transmission of HSV to neonates.

Detection of live virus may be useful in cases of suspected congenital herpes infection when the ability to distinguish between the presence of active, replicating virus and inactive virus or viral nucleic acid is important.

References:

Cook, JH, and M Pezzlo (2010). Specimen receipt and accessioning. Section 1. Aerobic bacteriology, 1.2.1-4. In HD Isenberg, L Garcia (ed) Clinical Microbiology Procedures Handbook. American Society for Microbiology, Washington DC, 3rd Edition

Miller, J Michael (1999) A Guide To Specimen Management in Clinical Microbiology, American Society for Microbiology, Washington DC

Miller, J Michael, and HT Holmes (1999) Specimen Collection, Transport, and Storage In PR Murray et al, (ed), Manual of Clinical Microbiology, 7th edition, American Society for Microbiology, Washington DC, pp 33-104

American Academy of Pediatrics (2012) Red Book: 2012 Report of the Committee on Infectious Diseases. Pickering LK, 29th ed, Elk Grove, IL: Academy of Pediatrics

Updates:

3/24/2010: CPT Updates
9/3/2013: Addition of Nasopharyngeal washes and updated specimen transport information.
7/21/2014: Clarification of specimen storage requirements.
8/26/2014: Added specimen types: skin and rectum swab.
11/20/2014: Offsite information added.
1/21/2015: Added Neonatal surface culture.
6/8/2015: CPT update, 87252 previously x2.