HANDBOOK OF DIAGNOSTIC VIROLOGY TESTING

Offered by

UW Medicine
Department of Laboratory Medicine
Community Services Programs

Third Edition 2004
UW MEDICINE
DEPARTMENT OF LABORATORY MEDICINE
VIROLOGY LABORATORY

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Laboratory Hours:
- Diagnostic Virology: 6am - 9pm, M-F, 9am - 5pm, Sat., Sun., & Holidays
- Retrovirus (HIV): 9am - 6pm, M-F
- Molecular (PCR) Virology: 7am - 6:30pm, M-F
- Chlamydia: 8am - 4:30pm, M-F

Clinical Virologist on-call: (206) 987-2131
Laboratory Medicine Resident on-call: (206) 598-6190

Websites:
- Virology Department: labmed.washington.edu
- Respiratory Virus Surveillance data: depts.washington.edu/rspvirus/

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Introduction

UW Medicine, Virology Laboratory performs a full range of diagnostic services including:

- Viral culture and antigen detection for most human pathogenic viruses, as well as detection of Clostridium difficile toxin.
- Antiviral Sensitivity testing for CMV and HSV
- HIV Genotypic Resistance Testing
- HIV 1 viral load, HIV 1 DNA PCR, HIV 1 p24 antigen, HIV Culture
- *Chlamydia trachomatis*, culture, antigen detection (dFA), NA identification by Aptima. *Chlamydia trachomatis* and *Chlamydia pneumoniae* ("TWAR") serologies.
- *Chlamydia pneumoniae* culture
- Molecular diagnostic testing includes polymerase chain reaction (PCR) assays for HBV, HCV, HIV, Adenovirus, HSV-1, HSV-2, CMV, EBV, HHV6, BK Virus, JC Virus, VZV, HHV8, as well as Enteroviruses and Parvovirus B19. Branch chain DNA (bDNA) assays are also available for HIV.
- Serological testing for:
  - Exanthem-producing viruses (Rubella, Measles, Parvovirus B19)
  - Hepatitis viruses A, B C and D
  - Herpes group Viruses (CMV, HSV-1, HSV-2, EBV, HHV8 and VZV)
  - Human Immunodeficiency Virus (HIV-1 and HIV-2)
  - Human T cell Leukemia Virus (HTLV-1 / HTLV-2)
  - Pericarditis and CNS associated viruses
  - Respiratory and Mumps viruses

The Virology Division provides direct clinical consultation services through the Virology physician on call (206) 987-2131. A 24-hour service is available for processing emergency specimens on evenings, weekends and holidays with the prior approval of the Virology physician on-call, or UWMC Laboratory Medicine resident on-call. The Virology physician on-call, and Virology technologist on-call can be reached by pager at (206) 987-2131. The UWMC Laboratory Medicine resident can be reached by pager at (206)598-6190. A nurse is on-call for organ transplant related issues, and is available via the Laboratory Medicine Resident on-call. Molecular Virology can be reached by pager at (206) 916-6572.

Clinical Virology Laboratory (206) 987-2088
Retrovirus Laboratory (206) 341-5210
Chlamydia Laboratory (206) 341-5300
Chlamydia Pager (206) 541-7932
Viral Hepatitis Laboratory (206) 731-3461
Molecular Virology Laboratory (206) 667-6999

During regular business hours, Community Services at (206) 598-6066 or (800)713-5198 can be consulted with most questions concerning virology specimens.

Accreditation:
Departmental laboratories are accredited by the College of American Pathologists, are CLIA registered, and participate in formal proficiency programs of CAP, CDC, AACC and Washington State Department of Health. All certifications can be found on our web site at http://depts.washington.edu/labweb/.
Shipping Address:
University of Washington Medical Center
Department of Laboratory Medicine
1959 NE Pacific Street, NW-220
Seattle, WA  98195

For packages sent by BUS or PLANE, and needing to be picked up, please add:

Call on Arrival (206) 598-6224

It is recommended that you notify the Community Services Office (206) 598-6066 or (800) 713-5198, 8am– 5pm. Monday through Friday, prior to sending packages by bus or plane. At times outside of these hours, please call the laboratory directly at (206) 598-6224 or (800) 713-5198.

Specimen Transport Service:

Courier service is provided at no charge* in the areas near Auburn, Bellevue, Bellingham, Bothell, Edmonds, Everett, Kent, Kirkland, Mt. Vernon, Olympia, Renton, Seattle, and Tacoma. The courier route includes bus and air freight depots. Stops are made at the Sea-Tac Airport as needed (please telephone (206) 598-6066 or (800) 713-5198 to schedule a pick-up). Stops are made at Greyhound Bus Terminal in the morning and afternoon, Monday through Friday. For more information, please contact the Community Services office, (206) 598-6066 or (800) 713-5198.

There is no STAT courier service.

All fees incurred for specimen transport other than via our courier service are the responsibility of the patient/sending location.

Supplies:

These supplies are routinely provided at no charge to laboratory users, please call Community Services office to place a request, (206) 598-6066 or (800) 713-5198.

- Blood culture collection tubes
- Chlamydia culture transport media
- Chlamydia NAAT collection urine kits
- Chlamydia NAAT Swab Kits
- Containers for timed urine or stool collections
- Laboratory requisition forms
- Mycoplasma culture transport media
- Plastic transport tubes
- Specimen bags
- Styrofoam mailing containers and blue ice packs
- Viral culture transport media

Client Billing:

Clients will be billed monthly by an itemized invoice that includes the date, patient’s name, specimen identification number, test performed, and the fee for each specimen completed during the month. Please note that our terms are payable upon receipt. If you have any questions pertaining to your account, please notify us immediately so that we may resolve them in a timely manner. Any adjustments will appear on the following month’s statements.

Out of state Client Billing
Community Services is not able to provide third party billing for out of state clients. All laboratory work that is ordered from out of state locations will be billed directly to the ordering location. Please note an ICD9 diagnosis code and a UPIN is required for all insurance billing.

* Please contact the Community Services office at (206) 598-6066 or (800) 713-5198 if you intend to use our courier service for non-departmental delivery.
Specimen Handling

Acceptable Specimens

- Culture swabs and tissues must be kept moist in Viral or Chlamydia Transport medium, M4 or M5. Specimens must not be leaking.
- Body fluids and wash specimens for viral culture should be transported in sterile containers, without dilution in transport media.
- Body fluids for Chlamydia culture; add no more than 0.5 mL fluid into Chlamydia Transport medium, M4 or M5.
- In general, most acute infections are diagnosed by isolating the agent from the patient (Viral or Chlamydia culture). In selected instances, e.g., Hepatitis A, Rubella, determination of IgM antibodies can be useful. An increase in antibody titer suggests recent infection, and thus workup requires acute and convalescent sera. Serologies requiring paired sera are as follows:

  Mumps titer  Respiratory syndrome viruses
  Measles titer  Pericarditis/CNS syndrome viruses
  VZV titer  Chlamydiae pneumoniae (4 weeks)

- Cerebrospinal fluids (CSF) for antibody determinations MUST be paired with a concurrent serum specimen. We recommend PCR sampling for CNS infections over antibody determinations.
- Single sera are appropriate for a serostatus screen or to determine evidence of past immunization for:

  CMV  EBV  Hepatitis A, B, C  HHV-8
  HIV-1  HSV-1 & 2 (type specific)  Measles  Mumps
  Parvovirus B19  Rubella  VZV  Chlamydia pneumoniae

  Chlamydia trachomatis

- Appropriate specimens for PCR vary depending on the assay.

Culture Swabs

Dacron or cotton swabs are recommended for viral cultures (Microdiagnostics, Inc. cotton and dacron blend swabs are best). Calcium alginate (“calgiswabs”) are inhibitory to Herpes simplex virus and should NOT be used for viral culture collection.

Dacron and dacron-cotton blend swabs from Puritan (Hardwood Products Corp., Maine) are recommended for Chlamydia culture. Cytobrushes are recommended for the collection of endocervical Chlamydia cultures on non-pregnant women. Do NOT use swabs with wooden sticks or calcium alginate swabs for Chlamydia cultures-they inhibit growth of the organism.

Transport Media

Viral Transport Media (VTM), C. trachomatis culture medium (CTM, M4 or M5), C. Pneumoniae culture medium (CPM or M5) and Chlamydia trachomatis Aptima Combo 2 medium. Viral and Chlamydia culture transport media contain different antibiotics and CANNOT be interchanged. VTM, CTM and CPM are stored at -20°C and must be thawed before use. M4 and M5 media are stored at refrigeration temperature. The shelf life of VTM is one year at -20°C and six months at 4°C. Chlamydia trachomatis Transport Media has a shelf life of six months at -20°C and two months at 4°C. Chlamydia pneumoniae Transport Media has a shelf life of 1 year at -20°C and two months at 4°C.
Specimen Collection

Viral isolation (culture) offers the broadest approach to identifying a viral agent. It is especially useful when the diagnosis is uncertain. However, many viral agents are fastidious and cannot be isolated in standard tissue culture. These agents (e.g. HHV6, HHV7, HHV8 and parvovirus) need to be identified by molecular technologies such as PCR.

Cultures should be obtained as early as possible after onset of illness. Appropriate specimens for culture vary according to syndrome and suspected agents. Generally, cultures are obtained by swabbing the sites with sterile swab and immediately immersing the swab into thawed transport media. Break off or cut the applicator stick short enough that the swab remains in the medium and cap fits tightly on the vial. Do not transport leaking vials.

**Aptima Combo 2, Urine**
Collect the first 20-30 mL of the stream. The lab does not want a clean catch. It is recommended that urine be collected at least 1 hour after last urination. Transport specimen in polypropylene containers, which are provided on request.

**Bone marrow**
Collect specimen in a heparinized syringe or transfer immediately into a heparinized blood tube. Store and transport to the laboratory at room temperature.

**Buffy coat culture**
Draw blood into 10 mL EDTA or heparinized tube. Buffy coat cultures should be stored and transported at room temperature.

**Cervix specimen**
Clean off vaginal secretions and debris from the cervix. For Viral cultures, swab the exocervix and endocervix. For Chlamydia cultures, swab the endocervix only. Cut or snap off the swab into the transport media vial. Cytobrushes are recommended for collection of endocervical Chlamydia cultures from non-pregnant females. For Aptima Combo 2 specimens, use the small blue swab for collecting all specimens; the large swab is provided only for cleaning the specimen site.

**Eyes, conjunctival Swab (Chlamydia):**
Pus accumulated at the inner canthus or the palpebral margin is NOT an adequate specimen. Both eyes should be sampled. For clinical purposes, both specimens may be pooled. Apply traction to the lower eyelid to pull the mucosa away from the globe. If the infant crying, a piece of tissue paper on the lid will provide better traction. Hold a flexible wire shafted swab about 2 cm. from the end used to sample the conjunctiva. Hold the swab vertically. Press the swab to the lower conjunctival sac. Vigorously rub the mucosal surface of the lower palpebral conjunctiva with the swab. If Chlamydia is present, slight bleeding may occur. Cut swabs off into the transport media.

**Fluid specimen**
For viral cultures, fluid specimens should be collected into sterile containers, without dilution in Viral Transport Media. For CSF's, submit a minimum volume of 1 mL for viral culture. For body fluids, submit a minimum of 5 mL for viral culture. For Chlamydia culture, fluid specimens should be pelleted in a high-speed centrifuge within 2 hours of collection and the pellet transferred into Chlamydia Transport medium, M4 or M5. If no centrifuge is available, transfer up to 0.5 mL of the specimen fluid to transport medium.

**Lesion swab**
Open vesicular-pustular lesions and vigorously rub the base to dislodge infected cells. Break off the swab into the transport media vial.

**Nasal wash (optimal specimen for RSV)**
Suction the patient if there is mucous in his/her nose. Place a mucous trap “in line” in the suction tubing and rapidly instill sterile saline into the nare. Immediately suction tubing and rapidly instill sterile saline into the nare aspirate with a suction catheter. As an alternative, squirt sterile saline into one nostril and suction back out immediately. Squirt the solution and mucous back into a sterile leak-proof container. Do not use Viral Transport Media (which contains penicillin) to collect nasal washes. Nasal wash specimens are not acceptable for Chlamydia culture.
Nasopharyngeal (NP) swab: Insert the swab through the nostril into the posterior nasopharynx. Rotate the swab when removing it. Cut swab off into the transport media. Nasopharyngeal swabs can be pooled in the same transport media vial with a throat swab. Sample both sides of the nasopharynx for Chlamydia culture. Swab and gently rotate it within the rectum; break it off into Chlamydia Transport Medium.

Rectal swab: For viral specimens, gently insert swab into rectum to dirty the swab; break off the swab into Viral Transport Medium. For Chlamydia specimens, clean the rectum of stool, then insert a second swab and gently rotate it within the rectum; break it off into Chlamydia Transport medium, M4 or M5.

Throat swab: Rub dry sterile swab over posterior pharynx or both tonsillar fossae. Break swab off into the transport media.

Urethral specimen (Chlamydia): Remove any external urethral discharge with a large swab or sterile gauze. For females, insert a wire shafted swab 1 to 2 cm. into the urethra and gently rotate several times; for males, insert a wire shafted swab 3 to 4 cm. into the urethra. Withdraw and cut or snap off the swab into the transport media vial. For Aptima Combo 2 specimens, use the small blue swab for collecting all specimens; the large swab is provided only for cleaning the specimen site.

Transport

Viral specimens should be placed in plastic bags (use a separate bag for each patient’s specimens). Requisitions should be attached to the outside of the plastic bag or in a separate pouch if available. Viruses are labile and speed of transport is essential. Transport to the laboratory immediately. PCR testing does not require immediate transport.

Chlamydia cultures must be stored and transported at refrigeration temperatures. If Chlamydia culture specimens can not be transported to the laboratory within two days of collection, freeze them at -70°C and transport on dry ice. Chlamydia trachomatis Aptima Combo 2 medium urine specimens are stable 30 days and swabs are stable 60 days at room temperature.

Reporting

Positive/Reactive results are called daily to the requesting laboratory or physician for the following tests:

- C. difficile toxin
- Chlamydia antigen (DFA)
- Chlamydia cultures
- Chlamydia trachomatis Aptima Combo2
- CMV antigenemia assay
- CMV rapid assay
- Hepatitis A IgM
- Hepatitis B surface antigen
- Herpes antigen (FA)
- Respiratory virus antigen (FA)
- Identification of viruses by culture isolation.
- Identification of viruses by PCR (except Hepatitis B, Hepatitis C and BK virus)
Diagnostic Tests and Profiles

Antigen Detection and Viral Cultures

**Clostridium difficile Toxin B Assay**

Patients suspected of having antibiotic-associated colitis should have 5-10 grams of fresh stool sent to the Virology Laboratory for a Clostridium difficile toxin assay. The stool should be placed in a sterile container. Rectal swabs are not acceptable. The stool can be stored at 4°C for 48 hours or frozen immediately at -20°C if transportation is delayed. The assay is set up daily. A preliminary result is ready 24 hours after inoculation and the final report is given at 48 hours.

If culture for *C. difficile* or Toxin A is desired, submit a small amount of stool in a clean container to the UWMC specimen processing services with a Microbiology Clinical lab request form.

**Buffy Coat Culture**

All buffy coat (peripheral blood leukocyte) cultures include both the Herpes group viral culture and the CMV antigenemia assay. The CMV antigenemia assay for buffy coat is stained for CMV antigen the day after it is drawn. The buffy coat specimen should be collected in either a heparinized or EDTA tube. Buffy coat cultures are maintained for 28 days before being finalized as negative.

**Herpes Simplex Virus Sub-typing or Identification of Non-HSV Isolate**

The Virology Laboratory can complete the identification of viruses isolated in other laboratories. HSV isolates are confirmed as HSV-1 or HSV-2 by FA. Non-HSV isolates are identified and confirmed by monoclonal antibody. Submit the infected cell culture as soon as possible after detecting cytopathic changes in the cell monolayer. Call the laboratory (206) 987-2088 to arrange this service.

Isolation of Epstein Barr Virus is not routinely available—see PCR or serologic detection methods. PCR for Human Herpes Virus type 6 (HHV6) and HHV8 are also available. Please see the Molecular Diagnostic (PCR) section for PCR tests.

**Viral Culture-Herpes Group**

Specimens submitted for this assay are processed primarily for the identification of Cytomegalovirus (CMV), Herpes simplex type 1 (HSV-1), Herpes simplex type 2 (HSV-2) and Varicella Zoster Virus (VZV). Appropriate specimens include lesions, genital sites, conjunctival swabs, throat swabs, rectal swabs, buffy coats (EDTA or heparinized), BAL fluid or tissues. First morning voids of urine are more concentrated and contain the highest titers of virus.

Specimens to rule out HSV-1 or HSV-2 are read daily for 5 days, and then every other day before being reported as negative after 14 days of observation. CMV and VZV are slow growing viruses and their cultures are maintained up to 28 days before being reported. All negative Herpes group cultures receive a preliminary report after 5 days incubation. Positive culture results are called to the ordering location or physician as soon as virus is detected. All positive cultures are confirmed by FA and HSV isolates are subtyped with monoclonal antisera.

Herpes group Viral cultures can be combined with either the Herpes group FA, the CMV antigenemia assay, the CMV rapid assay, or the VZV rapid assay. Because of the extreme lability of VZV, any culture for this virus is automatically combined with the Herpes group FA to detect VZV antigen.
Viral Culture-SARS or Avian Influenza

Clinical specimens from suspect Influenza A (H5N1) cases and SARS-CoV cases may be tested by PCR assays using standard BSL 2 work practices in a Class II biological safety cabinet. In addition, commercial antigen detection testing can be conducted under BSL 2 levels to test for influenza.

CDC does not recommend that virus isolation studies on respiratory specimens from patients who meet criteria for suspected avian influenza (A H5N1) be conducted unless stringent BSL 3+ conditions can be met. Therefore, respiratory virus cultures should not be performed in most clinical laboratories and cultures should not be ordered for patients suspected of having H5N1 infection. Contact the Washington State Epidemiologist at 361-2831 with questions.

The UW Virology laboratory has a BSL 3 facility and is capable of setting up cultures for either suspect A (H5N1) cases or SARS-CoV cases if the CDC clinical criteria are met for such cultures. For the safety of our laboratory technicians, please contact the laboratory before sending any specimens for culture, PCR or antigen detection assays to assure adequate safety conditions.

Specimen: 2 mL nasal wash or BAL preferred, NPT swabs acceptable, but less sensitive. For other specimens call the laboratory at (206) 987-2088. Transport at 2-8°C and send as soon as possible. Call lab when sending specimen.

Performed: Culture results in 10-14 days. PCR results as needed, M-F.

Viral Culture-Screen

Specimens that are submitted for a Viral culture screen are processed to detect Adenovirus, Coxsackie, Echoviruses, Herpes Group Viruses, Influenza, Measles, Mumps, Parainfluenza, Polio, Respiratory Syncytial Virus (RSV) and Rhinovirus. Appropriate specimens include throat swabs, nasopharyngeal swabs, nasal washes, rectal swabs, conjunctival swabs, lesion swabs, urine, CSF and tissues. Positive culture results are called the day the virus is detected. All positive cultures are confirmed by monoclonal antibody (FA). Order “Viral Culture: Screen”.

Specimens are read every other day for 14 days before a final report is issued. Some specimen sites are appropriate for CMV or VZV isolation which are slower growing viruses. In this case, a preliminary negative report is issued at 14 days and the final report follows in 28 days.

Viral cultures can be performed alone or combined with several antigen detection assays to yield a rapid preliminary result. The Viral culture screen can be combined with any of the procedures listed below:

Adenovirus Rapid Assay

For rapid detection of Adenovirus, the specimen is inoculated by centrifugation onto a monolayer and stained for Adenovirus antigen with monoclonal antibody at 24 and 72 hours post inoculation (in addition to standard viral culture). Swabs (in Viral Transport Media), body fluids or tissue can be submitted.

Specimen: Nasopharyngeal-throat swabs, conjunctival swabs and tissue in transport media. Bronchoalveolar lavage fluid or urine in sterile specimen cups. Refrigerate. A conventional tissue culture will also be done.

Performed: Daily, results in 1-3 days

Reference: Negative. All positive shell vial results are called.
Viral Culture-Screen, continued

**CMV Antigenemia Assay**

For rapid detection of Cytomegalovirus (CMV) in peripheral blood, the leukocytes are isolated, cytocentrifuged onto a glass slide, and stained with a fluorescent antibody to an early CMV antigen. Results from the CMV antigenemia assay are available within 24 hours of specimen receipt in the laboratory. Freshly drawn specimens received in the laboratory before 2pm Monday through Friday are processed the same day and read the following day. All peripheral blood specimens or buffy coat cultures (EDTA or heparinized) are set up for viral culture to increase the sensitivity of virus detection or for antiviral sensitivity testing of the isolate if desired. Transport blood at room temperature.

**Specimen:** 7-10 mL EDTA (Lavender top) (min. 5 mL). Green top - OK, but cannot be used for CMV PCR testing after Antigenemia test. Keep at room temperature. Note collection time.

**Performed:** M-F, specimen in lab by 2pm  
Sat & Sun, specimen in lab by Noon

**Reference:** Negative. All positive antigenemia assays are called.

Note: We prefer fresh specimens to be processed at the University of Washington Virology lab within 6-8 hrs of collection. We will accept specimen within 24 hrs of collection.

**CMV Rapid Assay**

For rapid detection of Cytomegalovirus (CMV), the specimen is inoculated by centrifugation onto a monolayer and stained for CMV antigen with monoclonal antibody at 24 and 48 hours post inoculation in addition to standard viral culture. Swabs (in Viral Transport Media), body fluids or tissue can be submitted. This test is recommended for all lung biopsies, bronchoalveolar lavage specimens and tissue specimens.

**Specimen:** Throat swabs in viral transport media, bronchoalveolar lavage fluid or urine in sterile specimen cups.

**Performed:** Daily, results in 24 or 48 hours.

**Reference:** Negative. All positive shell vial results are called.

**Herpes Group FA**

The Herpes group FA detects Herpes Simplex virus (HSV) or Varicella Zoster virus (VZV) antigens in infected cells. A slide is usually prepared at the Virology Laboratory from cells in the vial of transport media. HSV subtyping (HSV-1 vs. HSV-2) is done on culture isolates only. Lesions must be aggressively swabbed to obtain adequate numbers of cells for a valid assay. Order “Viral Culture: Herpes Group, plus Herpes Group FA”.

In addition to being more rapid than Viral culture, FA can detect viral antigens in infected cells even if the virus is no longer viable. Due to the extreme lability of Varicella Zoster Virus, FA is routinely performed on all VZV culture requests and is recommended on all skin/eye sites.

**Specimen:** Tissue, BAL fluid or swabs in viral transport media or sterile saline.

**Performed:** Daily

**Respiratory FA**

Fluorescent antibody (FA) staining of respiratory epithelial cells is the most rapid method to identify Respiratory Syncytial Virus (RSV), Parainfluenza, Adenovirus, and Influenza A and B. We recommend that a nasal wash be submitted-if not, then combine a throat and nasopharyngeal swab in the same vial of transport media. The Respiratory FA is offered only with a Viral culture; order “Viral Culture: Screen, plus Respiratory FA”, except during the “flu season” (Jan./Feb. - varies slightly every year). During this time we will perform a respiratory FA alone if requested to rule out Influenza. In Addition, a RSV direct FA is available during respiratory virus season (Nov. - April). This test is not recommended during the off season (May - Oct.). For a summary of seasonal virus detections, visit http://depts.washington.edu/rspvirus/
Viral Culture-Screen, continued

(Note: For immunocompromised patients, a full bronchoalveolar lavage workup is recommended. This includes CMV and RSV rapid assays and both a herpes group and respiratory with FA).

Specimen: Swabs or tissues in Viral Transport Media. Tracheal aspirates, bronchial washings in collection containers.
Performed: Daily-FA set up at 10am, results 5pm

Respiratory Syncitial Virus Rapid Assay
For rapid detection of Respiratory Syncytial Virus (RSV), the specimen is inoculated by centrifugation onto a monolayer and stained for RSV antigen with monoclonal antibody at 24 and 72 hours post inoculation in addition to standard viral culture. Nasal washes or NPT swabs are the specimen of choice; BAL fluid is appropriate as well.
Specimen: Nasal washes, naso-pharyngeal-throat swabs and bronchoalveolar lavages are the preferred specimens; Refrigerate. Back-up culture done also.
Performed: Daily, results in 1-3 days.
Reference: Negative. All positives shell vial results are called.

VZV Rapid Assay
For rapid detection of Varicella Zoster Virus (VZV), the specimen is inoculated by centrifugation onto a monolayer and stained for VZV antigen with monoclonal antibody at 48 and 96 hours post inoculation in addition to standard viral culture. Lesion specimens, skin sites, BAL fluid or tissue can be submitted.
Specimen: Dermal lesions unroofed and vigorously swabbed with cotton or dacron swabs or tissue are the preferred specimens; submit in viral transport media and refrigerate. Back-up culture done.
Performed: Daily, results in 2-4 days.
Reference: Negative. All positives shell vial results are called.

Rotavirus/Enteric Adenovirus
These are EIA assays for the detection of antigens in stool. These viruses do not grow in standard tissue culture so this is the preferred method of detection.
Specimen: 5-10 grams of fresh stool
Performed: 1x/week, test day varies
Chlamydia Testing

**Chlamydia trachomatis Culture**

Chlamydia Transport Medium differs from Viral Transport Medium; they are not interchangeable. Wooden swabs and calcium alginate swabs inhibit the growth of Chlamydia and should never be used to culture for Chlamydia. Dacron swabs are recommended and may be requested from Community Services. A nasopharyngeal swab obtained from both sides of the posterior pharynx is the optimal method for culturing infants suspected of having infant pneumonia syndrome. For infants with conjunctivitis, swab the lower conjunctive of both eyes. Tissues can also be cultured by having either the swab or biopsy dropped into the Chlamydia Transport Medium. The optimal genital sites for chlamydia isolation are the urethra in men and the urethra and/or endocervix in women (separate swabs can be combined for culture). Rectal swabs should be collected from homosexual and bisexual males suspected of having a rectal chlamydial infection. Rectal swabs should never be combined with other specimen sites because of possible bacterial contaminants. Chlamydia specimens can be stored at 4°C for 48 hours without significant loss of titer or immediately frozen at -70°C if transportation is delayed. Double bag any specimen transported on dry ice to avoid exposure of the specimen to CO₂. Avoid freezing at -4°C or -20°C. Ship to UWMC, Room NW 220.

**Chlamydia trachomatis Antigen Detection**

Monoclonal antibodies are currently being used in a DFA assay to detect *C. trachomatis* in clinical specimens. For these tests; conjunctival, endocervical, nasopharyngeal, rectal or urethral swabs should be spotted onto clean glass slides. (Note: We prefer that you make the slide for Chlamydia at the time of specimen collection. Air dry the slide completely. Flood the slide with methanol and allow to air dry.) Either culture or nucleic acid amplification assays are preferable in women during menses to culture.

**Chlamydia Serologies**

Chlamydia serologies are also available. These include *C. trachomatis*, lymphogranuloma venereum, and *C. pneumoniae (TWAR)*. The indirect fluorescent antibody (IFA) assays determine IgG (past or persistent infections) and IgM (acute infection) levels. Only one serum sample is required (approximately 1 mL). Acute and convalescent (four weeks) sera are recommended for *C. pneumoniae* analysis. When analyzing results of Chlamydia serologies, it is helpful to note the date of onset and whether or not the specimen is considered to be acute or convalescent.

Specimen: IFA - *C. pneumoniae* - convalescent
- 7 mL red, SST - collected 4-6 weeks post onset.
IFA - *C. pneumoniae* - paired
- 7 mL red, SST - one for acute and convalescent sera.
IFA - *C. trachomatis*
- 7 mL red, SST or any body fluid. Body fluid specimen should be processed with concurrent serum specimen.
IFA - *C. trachomatis* - paired
- 7 mL red, SST. Convalescent sera should be collected 2 weeks post onset.

Performed: T, results W.
Chlamydia Nucleic Acid Amplification (NAAT) Gen-Probe Aptima

Gen-Probe Aptima Combo 2 assay collection kits are provided for your use. Please use the large swab to cleanse the specimen site of excess mucus and then use the small swab with blue shaft to obtain the specimen. Urethral specimens should be collected at least 1 hour after last urination. Urine should be transported in a polypropylene tube. Do not submit a clean catch.

Specimen: Urine, only the first voided 15-20 mLs (no clean catch). Urine not in transport medium needs to arrive in the lab within 24 hours of collection. Urine in transport medium is stable 30 days. Collect cervical or urethral swab using Gen-Probe Aptima Combo 2 assay collection kit.

Performed: T-F, Sunday

Chlamydia pneumoniae culture

M5 media is provided for Chlamydia pneumoniae culture upon request. Viral or Mycoplasma transport media should not be submitted. Chlamydia trachomatis media, CTM, or M4 media are acceptable but not optimal. Nasopharyngeal or BAL specimens are recommended. Swabs from spectrum corporation and those with wooden shafts or calcium alginate fibers inhibit chlamydia and should be avoided.

Specimen: Collect nasopharynx or throats swabs on dacron swabs. Bronchial alveolar lavage (BAL), bronchial washes or pleural fluid should be centrifuged first and the pellet suspended in M5 medium. Fluids only (not M5, M4 or CTM) will not be processed. Call the laboratory if further instructions are needed. Tissue in suspended in CPTM. Transport on dry ice.

Performed: M, Preliminary results in 3 days, final results 7-10 days
### Diagnostic Chart for *C. trachomatis* Infection

<table>
<thead>
<tr>
<th>Infection</th>
<th>Signs &amp; Symptoms</th>
<th>Confirmatory Test of Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Males</strong></td>
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</tbody>
</table>
| Non-gonococcal urethritis (NGU) | Discharge, dysuria | Urethral culture for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT |
| Epididymitis | Unilateral swelling, pain, tenderness, fever; often seen with NGU | Urethral culture for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT  
Serology (MIF); high titers of IgG (>256) or IgM are supportive evidence of the diagnosis |
| Proctitis | Rectal pain, discharge diarrhea, blood | Rectal culture for *C. trachomatis* |
| Reiter’s Syndrome | NGU, arthritis, conjunctivitis, skin lesions | Urethral culture for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT |
| LGV | Genital ulcer, inguinal adenopathy | Urethral and rectal cultures for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT  
Serology (MIF); presence of high IgG titers (>1:256) or IgM specific to LGV sero-variants. |
| **Adult Females** | | |
| Cervicitis | Macropurulent cervical discharge, ectopy, friability, edema | Endocervical culture for *C. trachomatis*  
Urine, cervical or vaginal specimens for *Chlamydia trachomatis* NAAT  
Serology (MIF); high titers of IgG (>256) or IgM are supportive evidence of the diagnosis |
| Salpingitis | Pelvic inflammatory disease | Endometrial or endocervical culture for *C. trachomatis*  
Urine, cervical or vaginal specimens for *Chlamydia trachomatis* NAAT  
Serology (MIF); high titers of IgG (>256) or IgM are supportive evidence of the diagnosis |
| Perihepatitis  
(Fitz-Hugh Curtis syndrome) | Pelvic tenderness, PID, macropurulent cervicitis, right upper quadrant tenderness | Urethral and cervical culture for *C. trachomatis*  
Urine, cervical or vaginal for *Chlamydia trachomatis* NAAT  
Serology (MIF); high titers of IgG (>256) or IgM are supportive evidence of the diagnosis |
| Urethritis | Dysuria & frequency w/o urgency or hematuria | Urethral and endocervical cultures for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT |
| LGV | Inguinal lymphadenopathy, rectal structure | Urethral and rectal cultures for *C. trachomatis*  
Urine for *Chlamydia trachomatis* NAAT  
Serology (MIF); presence of high IgG titers (>1:256) or IgM specific to LGV sero-variants. |
| **Neonates** | | |
| Conjunctivitis | Purulent conjunctival discharge 6-18 days post delivery | Conjunctival culture or Direct FA on conjunctival material for *C. trachomatis* |
| Infant Pneumonia | Afebrile, cough, diffuse rales, bilateral hyperinflation, interstitial infiltrates; at 1-4 months of age | Culture of nasopharynx for *C. trachomatis*  
Serology (MIF); high titer of IgG relative to maternal titer, or presence of IgM; |
Molecular Diagnostic (PCR) Testing

Additional Specimen Information for Molecular Testing:

A variety of specimens can be tested for the presence of viruses when molecular amplification methods are used to detect viral DNA or RNA. Serum, plasma, and non-cellular fluids can all be tested with a quantitative assay to determine the viral concentration (copies/mL or International Units/mL). For cellular samples, biopsies, swabs, bone marrows, etc., samples are tested with a qualitative assay to detect the presence or absence of virus (present or not detected). Paraffin embedded tissues can also be tested for the presence of virus after deparaffinization (which requires an additional billing charge). The best sample type for viral detection depends on the virus and associated clinical disease suspected. Specific tissue test codes exist for tissues routinely tested in the lab, HSV, CMV, EBV, HHV-6, BK, and VZV. If detection of a virus is needed for viruses other than these 6, request the “Virus Detection by PCR, Tissue” test and indicate on the requisition slip the virus to be tested. Refer to the following information for the most common sample types tested for each virus.

Two specimens must be collected if both culture and PCR testing are requested. Sample collections for PCR must be done in a sterile manner to avoid contamination of any kind from the environment and samples should be placed in sterile tubes for transport. After collection, all FLUID SPECIMENS [PLASMA, SERUM, CSF, NON-CELLULAR FLUIDS] should be frozen and stored at -20°C. Plasma should be separated from cells within 2 hours of collection if possible. Shipment should be on dry ice if possible, or wet ice if necessary. WHOLE BLOOD should be collected in EDTA or CPD-A, stored at room temperature, and shipped immediately to arrive within 24 hours. Whole blood or plasma collected with HEPARIN ANTICOAGULANT ARE NOT ACCEPTABLE as heparin will inhibit PCR amplification reactions. BONE MARROW - it is also very important for bone marrow samples that they are not collected in sodium heparin anticoagulant, or that the syringe used to collect the specimen not be rinsed with heparin as this will inhibit the PCR reaction. Please collect the bone marrow into an unrinsed syringe and then rapidly place into an EDTA or CPD-A and mix rapidly to prevent clot formation. SWABS OF LESION areas should be obtained with a Culturette swab, and stored frozen. FRESH TISSUE specimens should be placed in a small amount of PBS or culture fluid and transported to the lab as quickly as possible on ice. FROZEN OR FIXED TISSUE should be shipped as is either frozen or at room temperature. Please consult the Molecular Virology, (206) 667-6999 for all questions concerning handling of other specimen types.

Adenovirus by PCR, Quant. - CSF, Plasma or Urine

Adenovirus infections are emerging as life-threatening complications in immunocompromised patients. In transplant recipients, adenoviruses can cause hemorrhagic cystitis, renal nephritis, enteritis, hepatitis, encephalitis, pneumonitis and multiple-organ failure. Disseminated infections frequently result in death. In patients with AIDS, adenoviruses can cause localized or disseminated disease. Adenoviruses can be isolated from CSF and brain from patients with meningoencephalitis. PCR assays allow sensitive and rapid diagnosis. Positive serum adenovirus PCR result can be used as an indicator of severe disseminated adenovirus infection. High adenoviral DNA levels in serum have been correlated with severe disseminated adenovirus infection in children with allogeneic stem-cell transplantation.

Specimen:  Plasma/Serum - 10 mL EDTA plasma (not heparin), or serum (plain red, SST or PPT)  
CSF - 1 mL  
Urine - 10 mL random or clean catch urine, no preservative.

Performed: M, Th, results next day.
**BK Virus PCR - Plasma, Serum, Tissue or Urine**

BK virus PCR detects the presence of BK virus DNA in a variety of clinical specimens. Most adults have childhood exposure to BK virus as indicated by the 60 to 80% of adults in the United States with positive tests for BK virus antibody. Some adults have low levels of BK virus in their urine but have no evidence of BK-related disease. Positive tests for BK virus are very rare in serum or plasma of normal adults. Conditions which lead to immunosuppression such as chemotherapy, bone marrow transplantation, high dose steroid therapy, leukemia, and lymphoma, can lead to “reactivation” of the virus and increased replication of BK virus in the kidney and/or bladder. Increasing levels of BK virus and very high levels of BK virus have been associated with late-onset hemorrhagic cystitis after bone marrow transplantation and nephritis after kidney transplantation. High levels of BK replication in either the kidney or bladder can be done by testing urine, serum, or plasma for BK virus by PCR. Extremely high levels of BK virus, greater than 1 x 10^6 copies/ml, are often seen in the urine of infected patients.

Specimen: Plasma/Serum - 10 mL EDTA plasma (not heparin), or serum (plain red, SST or PPT)
- CSF - 1 mL
- Urine - 10 mL random or clean catch - no preservatives.
- Biopsy - pea-sized amount of biopsy material, usually kidney.
- PBMC, BM - 5 mL lavender top.

Performed: Friday, results after 4:30pm same day.

**Cytomegalovirus (HHV-5) DNA by PCR - CSF, Plasma, Serum or Tissue**

The cytomegalovirus (CMV) quantitative PCR detects the presence of CMV DNA in a variety of clinical specimens. CMV is an important pathogen in transplant recipients, HIV-infected individuals, and in other types of immunosuppression conditions. The assay is best suited for evaluation of plasma viremia and CMV CNS infection. In general, a high systemic CMV load is associated with CMV disease. In addition, quantitative CMV testing is useful in monitoring response to therapy, successful therapy being associated with a decrease in viral load.

Specimen: Plasma/Serum - 10 mL EDTA plasma (not heparin), or serum (plain red, SST or PPT)
- CSF - 1 mL
- Biopsy - pea-sized amount of biopsy material, usually kidney, or swab.
- PBMC, BM - 5 mL lavender top.

Performed: Plasma/Serum/CSF - M-F, results after 6:30pm same day.
- Biopsy/PBMC/BM - M, Th, results after 6:30pm same day.

**Cytomegalovirus Drug Resist., Rapid UL97 - Plasma or Serum**

The test amplifies and sequences two overlapping regions of the UL97 gene spanning amino acids 446-638 of the UL97 protein. Clinical resistance to Ganciclovir has been shown to map to multiple amino acid mutations within this region. Mutations within the UL97 protein, which functions to phosphorylate Ganciclovir within infected cells, have been shown to result in decreased activity of the drug. Studies of the variety of mutations in the UL97 gene are ongoing to determine which mutations are most strongly associated with phosphorylase dysfunction and clinical drug resistance.

Eight different amino acid substitutions and 2 different deletions in the UL97 account for more than 90% of the reported cases associated with drug resistance. CMV strains isolated from these patients have shown strong in-vitro drug resistance when cultured with Ganciclovir. In addition, viral constructs containing these 10 specific mutations have been tested with in-vitro marker experiments and shown to result in decreased Ganciclovir phosphorylation. Thus these 10 mutations have been strongly associated in a variety of ways with Ganciclovir drug resistance. More than 30 additional mutations at other locations within the UL97 gene have been reported in one or more patients with clinical resistance but have not been as carefully studied with all available techniques. These mutations are therefore less well characterized at this time. Results from our UL97 sequencing test will contain the name of any mutation found and an interpretation which describes whether the mutation has been strongly associated with resistance at this time or not.
The other two common anti-viral drugs, Foscarnet and Cidofovir, do not require activation by the UL97 gene product, so this screening test cannot be used to measure CMV drug resistance to these two drugs. Mutations of the UL54 gene, the DNA polymerase, have been shown to be associated with resistance to Ganciclovir as well as Foscarnet and Cidofovir.

Specimen: Plasma/Serum - 10 mL EDTA plasma (not heparin), or serum (plain red, SST or PPT)
CSF - 1 mL
Performed: M, results next day.

Enterovirus by PCR - CSF, Plasma or Serum

Enterovirus is the most common cause of aseptic meningitis. PCR detection of enterovirus RNA in CSF is the most rapid way to diagnose enteroviral meningitis. The assay detects Polio, Coxsackie A and B, Echoviruses, and Enteroviruses 68, 69, 70, and 71. During enterovirus season from July – October, the test is run each afternoon, M-F. During the off-season, the test is run 2 or 3 times a week, depending on volume. Sample types other than CSF which may contain Rhinoviruses may give positive results with this assay.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 2 mL
Performed: July-October - M-F, results after 4:30pm same day
November-June - 2-3 times per week, results after 4:30pm same day

Epstein-Barr virus (HHV-4) DNA by PCR – CSF, Plasma, Serum or Tissue

Epstein-Barr virus (EBV) quantitative PCR detects the presence of EBV DNA in clinical specimens, most commonly plasma or serum. EBV is the cause of infectious mononucleosis usually seen in children and young adults, which is typically a self-limiting disease. The EBV DNA test is useful for the diagnosis of infectious mononucleosis very early in the disease or when serological testing for EBV is non-diagnostic. Normal adults will have no detectable EBV DNA in their plasma/serum, although normal adults previously infected with EBV will have low levels of EBV DNA in their lymphocytes. For this reason, our test is performed on plasma or serum and not on whole blood or cells. A second and more common use of the EBV DNA test is the detection of “reactivation” of EBV virus in patients who have undergone immunosuppression secondary to chemotherapy or after organ or bone marrow transplantation. Patients with reactivation may have low levels of EBV in their plasma and circulating lymphocytes. Occasionally, immunosuppressed patients may develop a rapidly proliferating B cell lymphoproliferative disease, PTLD. PTLD is an EBV driven-B cell malignancy that can be rapidly fatal if not treated early. PTLD can also be seen in AIDS and HIV-1 infected individuals, and also may be seen as a form of newborn primary immunodeficiency. Development of these rapidly replicating EBV-associated lymphomas can be detected by observing rapidly increasing and very high levels of EBV in the serum or plasma of affected individuals.

EBV PCR can also be performed on tissue specimens; however, all individuals with prior exposure to EBV will have small numbers of lymphocytes containing the latent EBV genome in essentially all tissues and peripheral blood lymphocytes. Thus, the simple detection of EBV in a tissue sample is not sufficient to make the diagnosis of PTLD. Please consult the laboratory (206) 667-6999 with requests for testing of tissue specimens.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Biopsy - pea-sized amount of biopsy material or swab.
PBMC, BM - 5 mL lavender top.
Performed: Plasma/Serum - M-F, results after 6:30pm same day
Tissues - M,Th, results after 6:30pm same day
Hepatitis B by PCR – Plasma or Serum

The most sensitive marker of HBV replication is the PCR assay. Most acute and chronic HBV infections have evidence of 1 or more viral proteins, HBV surface, core or e antigen, as well as have HBV DNA in the blood. The HBV DNA test is more useful than the antigen detection tests when the virus concentration is low (<5,000 copies/mL) or when HBV mutants are present which do not produce normal protein antigens. The HBV DNA test is most often used to confirm HBV Ag positive tests, to help clarify confusing HBV serological tests, to screen transplant candidates and donors very sensitively, and to follow levels of HBV DNA during anti-viral therapy. Response to therapy has been associated with significant decreases in the HBV DNA quantity in the blood. HBV DNA has also been used to test chronic hepatitis patients and determine the presence or absence of low level HBV replication. The HBV DNA test may also be useful in testing early in the acute infection prior to the development of the antibody response.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
Performed: Tuesday, results by 4:30pm same day

Hepatitis C RNA – Plasma or Serum

The HCV RNA test is used to confirm positive HCV serological tests and to demonstrate the presence of HCV RNA in the blood. Most individuals who have an HCV infection become life-long carriers of the virus. A positive test for HCV in the serum indicates active replication of the virus in the liver and possible liver damage. Once liver damage is confirmed and therapy initiated the HCV RNA test is useful for monitoring of response to therapy. Measurement of HCV RNA levels during antiviral therapy provides a direct measurement of the amount of ongoing viral replication. Drugs that inhibit HCV replication dramatically reduce the absolute levels of viral nucleic acid in serum. Likewise, patients who fail to respond to therapy show no decrease in viral nucleic acid level. Therefore, quantitative nucleic acid assays can be used to monitor direct antiviral response after therapy. At the end of therapy, the test is then used to periodically determine the continued lack of or the resumption of HCV replication.

The HCV assay is now performed by the RT-PCR method and sensitivity for the assay is <60 International Units/mL. The RT-PCR assay is also linear from 60 to 1 x 10^8 International Units/mL. Extensive in-house evaluations demonstrated excellent correlation of the quantitative results with those of the Bayer bDNA test.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
Performed: M-F, results by 4:30pm next day.

Hepatitis C RNA Genotype – Plasma or Serum

The virus genotype present in an HCV-infected patient is a major predictor of the likelihood of clinical response to therapy and therefore the genotype test result is used to tailor the patient treatment protocol. The test should be done prior to the initiation of anti-viral therapy when the virus titer is highest to ensure that enough virus is present in the sample for the test to be accurate. Six major genotypes of HCV and numerous subtypes have been described. Numerous studies have documented differences in response to therapy based on the HCV genotype present. HCV genotype 1 infections are less responsive to therapy than are Genotypes 2 and 3 and may require 12 months of combination therapy (interferon-a plus ribavirin) to obtain a significant response. However, with genotypes 2 or 3, optimal therapeutic responses usually require only 6 months of combination therapy.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 2 mL
Performed: M, T, results Thursday and Friday.
**Herpes Simplex Virus (HSV-1, HSV-2) DNA PCR – CSF, Plasma, Serum or Tissue**

Primary infection in children and adults is usually a localized skin or mucous membrane lesion. HSV DNA can usually be detected on swabs taken from localized lesions or from saliva or genital fluids. The virus then almost always becomes latent in the ganglia innervating the affected area. Recurrent skin lesions can then later be seen in the same localized area caused by reactivation of the virus. Occasionally, the virus can spread more systemically and cause more widespread skin lesions and organ and/or neurological disease. Disseminated HSV infections are an important cause of morbidity and mortality in neonates.

The PCR test is the most sensitive way to detect the presence of HSV DNA in CSF and is thus the most sensitive way to confirm suspected HSV encephalitis in adults, children, and neonates. The HSV PCR test is positive in up to 95% of CSF specimens from HSV encephalitis patients. Because HSV is not normally found in CSF, even low viral levels are associated with HSV encephalitis. HSV DNA can be detected immediately after the onset of neurologic symptoms, after the initiation of acyclovir therapy, and up to 3 weeks after the onset of symptoms.

Significant and life threatening infections with HSV can be seen in immunosuppressed patients who have “reactivation” of previously acquired HSV. HSV can cause a variety of severe infections in transplant cases with organ, bone marrow, and neurologic involvement, and often causes a severe interstitial pneumonia that is usually fatal. Detection of HSV DNA in these more systemic illnesses can be done with plasma or serum specimens from the patient.

The DNA PCR test detects either HSV-1 or HSV-2 (HHV-1, HHV-2). If distinction between HSV-1 and HSV-2 in a positive specimen is clinically indicated, type-specific PCR can be performed from the extracted sample. Please call the lab to add on this test if desired (206) 667-6999.

**Specimen:** Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Biopsy - pea-sized amount of biopsy material or swab.
PBMC, BM - 5 mL lavender top tube

**Performed:** Plasma, Serum, CSF - M-F, results by 6:30pm same day.
Biopsy, BM, PBMC - M, Th, results 6:30pm next day.

**HHV-6 DNA by PCR – CSF, Plasma, Serum or Tissue**

Most adults have been previously exposed to HHV-6 during childhood. Rarely, HHV-6 causes high fevers and sepsis in infants 3-24 months old and it also appears to be a common cause of febrile seizures. HHV-6 can be present in normal adult, previously exposed individuals in saliva, PBMC’s and other tissues while the virus is almost always absent in plasma, serum and CSF from the same individuals. Occasionally, overwhelming HHV-6 infections can be seen in neonates and children and can be confirmed by HHV-6 DNA detection in either CSF or plasma. Immunocompromised individuals who have been previously exposed to HHV-6 may “reactivate” the virus and have a more systemic viral illness due to HHV-6 infection. Detection of these infections is also best done with plasma or CSF specimens.

**Specimen:** Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Biopsy - pea-sized amount of biopsy material or swab.
PBMC, BM - 5 mL lavender tube

**Performed:** Plasma, Serum, CSF - M-F, results by 6:30pm same day.
Biopsy, BM, PBMC - M, Th, results 6:30pm next day.
HHV-8 DNA by PCR (KS or Kaposi’s Sarcoma Virus) – Plasma or Tissue

Human Herpes Virus 8, also known as Kaposi’s sarcoma associated herpes virus (HHV-8, KSHV), has been found in the cells of Kaposi’s sarcoma, body cavity lymphomas (primary effusion lymphoma), and Castleman’s disease. The viral genome has also been identified in B-Cell lymphomas occurring in HIV-positive subjects. HHV-8 genomes can be identified by in-situ hybridization to be present in endothelial and spindle cells of Kaposi’s sarcoma tissues. However, a large number of HIV-1+ individuals have been shown to have exposure to HHV-8 virus, so the presence of virus is not diagnostic for the KS-related lymphomas. Rising levels of HHV-8 in plasma or serum is correlated with increasing infected-tissue burden and is the best indication of advancement of the sarcoma or lymphoma. A low level of HHV-8 DNA in peripheral blood has been described for both healthy and HIV negative individuals and is an indication of either low viral replication or the presence of latent virus. The best specimens for testing are biopsies (usually skin biopsies or lymph node biopsies) or plasma.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Performed: T, results by 4:30pm next day.

Human Parvovirus B19 PCR Assay – Bone Marrow, Plasma or Serum

Parvovirus B19 causes a wide spectrum of disease including erythema infectiosum, aplastic crisis in patients with hemolytic anemias, hydrops fetalis, acute arthritis, persistent anemias and neutropenia in immunocompromised patients. PCR amplification of Parvovirus B19 DNA can be used for early diagnosis of B19 infection, as B19 DNA may precede the appearance of IgM antibody.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Performed: Tues, Fri, results after 4:30pm same day.

JC Virus PCR – Brain Biopsy Tissue or CSF

JC virus is a frequent cause of self-limiting infection in children and by the time of adulthood, a significant number of individuals will have been exposed to the virus. Rarely, the virus infects the myelin-producing cells and causes progressive multifocal leukoencephalopathy (PML). Greater than 90% of affected individuals will have JC virus DNA in their CSF. The affected brain tissue will also contain high levels of the virus. JC virus has also been reported in urine of patients who are immunosuppressed. About 1/3 of kidney transplant patients have “reactivated” JC virus DNA in their urine, and a significant subset of patients have both JC and BK virus present. Unlike BK virus, JC has not been reported to cause clinical disease in these patients.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Performed: T,F, results by 4:30pm same day.

Metapneumovirus Virus by PCR

HuMPV is a newly recognized pathogen that is related to RSV. Both RSV and huMPV are members of the Pneumovirinae subfamily and both cause mild to severe respiratory infections. Nearly all children have experienced huMPV by age 5. The virus has been associated with wheezing in children as well as bronchiolitis and pneumonia.

Our studies of pediatric nasal wash samples here at CHRMC indicate that as many as 7% of samples during “respiratory season” contain huMPV. In Seattle, the incidence of huMPV appears to follow RSV, with a peak in April-May. The best diagnostic method currently available is RT-PCR.

Specimen: 3 mL nasal wash specimen (min.2 mL). Transport at 2-8°C.
Performed: M, Th, results next day.
**SARS Coronavirus by RT-PCR - Fluids**

This RNA virus is a previously unrecognized strain of coronavirus that causes severe atypical pneumonia. First recognized in November, 2002, it has infected more than 8000 people worldwide and resulted in more than 800 deaths. The primary means of transmission is by close person-to-person contact or from direct contact with infectious material from a person who has SARS.

The detection of the genomic RNA from SARS-CoV is based upon reverse transcription of specific genomic RNA sequences followed by real-time PCR amplification. The SARS-CoV RT-PCR test is intended to be used in combination with clinical symptoms and other diagnostic test results to help diagnosis patients suspected of have a SARS-CoV infection. All positive results are confirmed by performing a second RT-PCR assay that targets a different SARS-CoV gene.

Specimen: 0.5 mL of sputum, BAL, or nasal wash, a combined throat and nasal swab, or a rectal swab. Transport at 2-8°C.

Performed: as needed, results will usually be available within 1 or 2 days.

**Vaccinia Virus by PCR - Skin or Mucous Membrane Lesion**

Vaccinia virus is used to immunize high-risk persons against smallpox or to protect laboratory technologists who work with vaccinia virus in the research setting. Occasionally, vaccinia virus can be transferred from the active vaccination site to a site elsewhere on the skin through transfer of infection, usually by the fingers. A more generalized infection is sometimes seen in one of three forms – *eczema vaccinatum* as a complication in children or adults who suffer from chronic dermatitis; *true generalized vaccinia*, which is a limited, non-fatal infection that occurs in persons with normal skin and a delayed antibody response to vaccination; or *progressive vaccinia* (*vaccinia gangrenosa*), which is rare and represents a slow necrotizing progression from the initial vaccination site or elsewhere due to a continued vaccinia viremia. In addition, vaccinia virus may be transferred to a close contact, usually a household member or sexual partner. As such, vaccinia virus should be considered in the differential diagnosis of dermatitis and genital ulcer disease.

To identify vaccinia in skin lesions and distinguish this orthopoxvirus from variola virus (the etiologic agent of smallpox) and varicella-zoster virus (the herpes virus that causes chickenpox and shingles; see *Varicella-zoster virus (VZV/HHV-3) DNA by PCR*), the UW Clinical Retrovirology Laboratory uses electron microscopy (EM) in combination with real-time reverse polymerase chain reaction (PCR) amplification assays.

Specimen: Touch prep on microscope slide; swab or biopsy – pea-sized amount of material, in a dry, screw-cap tube.

Performed: On an as needed basis, initial EM results within one working day of specimen receipt; PCR results within two working days. Notification of Laboratory Medicine Resident recommended to prioritize and coordinate sample testing.

**Varicella-Zoster Virus (HHV-3) DNA by PCR – CSF or Plasma**

VZV infections cause chicken pox in children or zoster/shingles in adults. Primary infections with VZV are becoming less common due to vaccination of children with VZV vaccine. Occasionally, systemic infections can be seen in neonates and children which can be very severe and involve the skin, internal organs, and the brain. Neonatal infections are particularly severe in infants born to VZV seronegative mothers. VZV can also cause the skin disease shingles in adults many years after the primary infection and is particularly severe when it occurs in immunocompromised patients including HIV-1 positive individuals and in bone marrow transplant patients. Systemic infections can also often cause significant brain disease including aseptic meningitis and encephalitis that can be detected by the presence of VZV in the CSF. Plasma samples can be used to document systemic infections while skin biopsy and swab material can detect skin and organ disease due to the virus.
Specimen:  Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL
Biopsy - pea-sized amount of biopsy material or swab.
PBMC, BM - 5 mL lavender tube
Paraffin - fresh specimen is preferred.

Performed: Plasma, Serum, CSF - M, W, F, results by 6:30pm same day.
Biopsy, BM, PBMC - M, Th, results 6:30pm next day.

**Virus Detection by PCR, Tissue**

Fresh tissue biopsies, needle biopsies, bone marrow, whole blood or PBMC, or any other sample type with cellular material can be tested to detect either DNA or RNA viruses. Fresh biosies and other fluids containing cells should be kept cool and send to the laboratory as soon as possible. Bone marrow and blood specimens should be drawn in EDTA, CPD-A, or PPT tubes and must not be drawn into heparinized tubes or through heparin-wetted syringes as heparin inhibits the DNA amplification reaction. Paraffin imbedded samples can also be tested, but because of tissue fixation variation, false negative results are sometimes seen. If testing for HSV, EBV, CMV, HHV-6, BK, or VZV, please order the specific qualitative virus test. For HBV, HCV, JC, Parvo, B19, West Nile, HHV-8, please order the “Virus Detection by PCR, Tissue” test.

Specimen: Biopsy - pea-sized amount of biopsy material or swab.
PBMC, BM - 5 mL lavender tube
Paraffin - fresh specimen preferred, but paraffin testing available.

Performed: Extraction on Monday and Thursday, results available depending on virus to be tested.

**West Nile Virus by PCR – CSF or Serum**

The West Nile Virus PCR assay can be used in conjunction with the IgM serology assay as an aid in the diagnosis of viral meningitis or systemic infections due to West Nile Virus. The assay is specific for West Nile virus (Lineage 1 or the New York 1999 strain) and does NOT detect the closely related West Nile Lineage 2 (Uganda) strain. The Lineage 1 (NY99) strain has been identified in the USA and Canada while the Ugandan strain has not yet been seen in North America and is not as strongly associated with the occurrence of meningitis. The PCR assay is also negative when tested with other mosquito-born Flaviviruses including St. Louis, Eastern and Western Equine, and Murray Valley encephalitis viruses. Sensitivity of the PCR assay is much lower (around 60% in early studies) than that of the IgM serology test but is significantly more specific because the serological test is cross-reactive with a number of other Flaviviruses. The low sensitivity of the PCR assay is due to the short period of viremia (a few days) and to low levels ($1 \times 10^3$ to $1 \times 10^5$ copies/mL) of the virus seen in the blood or CSF.

Specimen: Plasma/Serum - 10 mL EDTA (not heparin) or serum (plain red, SST or PPT)
CSF - 1 mL

Performed: May - October - M-F, results same day
Nov. - April - 2-3x/week, results same day
Transplant Related specimens - Th, results same day
HIV Diagnostic and Molecular Testing

Rationale for approaching the virological diagnosis of HIV infection:

The figure shows an approximate sequence and time course of virological and immunological events during primary Human Immunodeficiency Virus type-1 (HIV-1) infection. After infection, virus may replicate in mucosal and lymphoid tissue that drains the inoculation site, for variable periods lasting from 1 to 2 weeks but occasionally up to 6 months. Following this period of viral replication, there is hematological dissemination (day 0 in the figure). Subsequently, there is a rapid rise in detectable viral RNA (squares) in plasma on approximately day 10 and HIV-p24 antigen (triangles) on day 15. Anti-HIV antibody (circles) appears between days 20-30 post viremia. Seroconversion is associated with containment of viremia and establishment of an HIV RNA setpoint that correlates with the subsequent risk of disease progression and probability of secondary transmission. (after Busch MB & Satten GA, Am J Med 1997; 102 (5B): 117-124)
HIV1&2 Antibody Screen

Antibodies to HIV-1 and HIV-2 are detected by enzyme immunoassay (EIA). Reactive results are confirmed by HIV-1 Western blot. The HIV Western blot identifies antibodies against eight HIV-1 encoded proteins: p18, p24, p31, gp41, p51, p55, p65/66, gp120/p160. Criteria accepted by CDC/ASTPHLD are used for determining a positive HIV Western blot. These criteria require antibodies against any two of the following HIV-1 proteins: p24, gp41, gp120/160. Specimens showing reactivity to HIV-1 protein(s), but not fulfilling the criteria for a positive result, are reported as Indeterminate. All indeterminate Western blots are further tested in supplemental HIV-1 and HIV-2 specific assays. Specimens showing reactivity to non HIV-1 proteins are not assigned an indeterminate status but are instead reported as “Antibody to non-HIV-1 encoded proteins”. A negative Western blot has no detectable bands, i.e. no antibodies reacting to either HIV-1 or non-HIV-1 proteins. Supplemental assays are performed on EIA-reactive specimens which do not confirm by HIV-1 Western blot. Specimens may be forwarded, upon request, for HIV-2 specific Western blot if supplemental assays indicate HIV-2 antibodies may be present. HIV-1 and -2 EIA screens are run daily, Monday through Friday. HIV-1 Western blot confirmation assays are run on Tuesday, Thursday and Friday.

Specimen: 1.5 mL serum (red or SST); plasma acceptable (EDTA or heparin)
Performed: EIA Screen: M-F, results next day for Non-reactives;
Western blot for EIA-Reactives: T, Th, F

HIV1 RNA Quantitation

Quantitation of HIV RNA copy number is available to monitor antiviral therapy and to predict disease progression in HIV infected persons. HIV RNA quantitation may be useful to indicate when an HIV infected person should start anti-retroviral therapy and when such therapy should be adjusted. (Alone, this assay is not recommended nor approved for diagnosing HIV infection. However, in conjunction with a positive DNA PCR or a reactive EIA, the RNA quantitation may be diagnostic.) High levels of RNA are found during acute infection and in patients who are more likely to have disease progression. Inhibition of cell-free HIV, as reflected by RNA copy number, is associated with better CD4 response and clinical response in some patient populations.

To quantify HIV RNA, the UW Clinical Retrovirology Laboratory uses a real-time reverse transcription (RT)-polymerase chain reaction (PCR) amplification platform with enhanced sensitivity and broader dynamic range compared to available commercial assays. The Real-time RT-PCR assay for HIV RNA quantification has been validated against the commercial bDNA and ultrasensitive (US) RT-PCR assays.

• The dynamic range for HIV RNA detection by Real-Time RT-PCR is 30 to 1,000,000 copies/mL of plasma.

HIV clade B is the predominant virus causing HIV/AIDS in North America and Europe. To provide HIV RNA quantification for non-clade B viruses, the Roche Monitor® US-RT-PCR version 1.5 assay should be ordered.

• The dynamic range for HIV RNA quantitation by US-RTPCR is 50 to 100,000 copies/mL of plasma.

Real Time RT-PCR

Specimen: 7 mL EDTA (purple) preferred. ACD (yellow tube) acceptable, but yields lower copy number due to dilution effect of liquid anticoagulant. Note: serum (red top) and heparin (green top) are unacceptable.
Performed: Set up, in batches, M-F, results within 5 days.
US RT PCR

Specimen: 7 mL EDTA (purple) preferred. ACD (yellow tube) acceptable, but yields lower copy number due to dilution effect of liquid anticoagulant. Note: serum (red top) and heparin (green top) are unacceptable.
Performed: Set up Tuesday, results late Wednesday

HIV1 proviral DNA Detection

The detection of cell associated Human Immunodeficiency Proviral DNA by polymerase chain reaction (PCR) amplification is one of the most sensitive non-serologic methods for confirming HIV infection. In addition to HIV culture, this assay is recommended for confirming HIV infection in the neonate. HIV DNA PCR may also be used as a supplemental test to determine the significance of an indeterminate HIV Western Blot serology result.

Specimen: 10 mL ACD (yellow tube), preferred. Lavender - ok (Green top - not acceptable)
Performed: First Tuesday of each month, results ready the following afternoon.

HIV1 Culture

Culture is an extremely sensitive virologic method for documenting HIV infection, especially in neonates whose serologies are complicated by the presence of maternal antibody. Since cultures must be processed within 30 hours of collection, specimens should not be obtained Friday PM through Sunday AM.

Specimen: 20 mL ACD (yellow top tubes-preferred) (Green or Lavender-ok]
2 mL spinal fluid or fresh semen in sterile container.
Performed: M-F

HIV1 Genotypic Resistance Assay

This assay is performed once a week and an interpretive report is sent (see example on following page). The assay involves sequencing of the HIV pol gene, after which mutations in the gene can be compared to sequences known to confer resistance to different classes of antiretroviral drugs. The assay is most useful in patients who lose viral suppression on antiretroviral therapy and should be performed before switches in therapy are entertained.
Specimen requirement is a 10 mL EDTA tube at room temperature or frozen plasma at -70°C, shipped on dry ice, is also acceptable.

Specimen: 7 mL EDTA (Lavender) top tube
Performed: 1x/week, results in 14-21 days
HIV Genotypic Resistance Assay Report

Patient Name: 
Specimen Date: 
Patient/Location ID #: 
Accession #: 
Ordering physician: 
Tissue analyzed (plasma/PBMC): Plasma viral RNA

Sequence analysis using the ABI-310 was performed on DNA reverse-transcribed from the plasma viral RNA. All mutations that lead to amino acid changes are reported for the patient’s specimen when compared to Los Alamos HIV-1 subtype B consensus sequence:

**Protease Gene:** Amino acids analyzed 1 TO 99

Mutations associated with resistance to protease inhibitors:

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<tr>
<th>Generic Name</th>
<th>Trade Name</th>
<th>No Evidence of Resistance</th>
<th>Possible Resistance</th>
<th>Low Level Resistance</th>
<th>High Level Resistance</th>
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<tbody>
<tr>
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Other mutations causing amino acid changes, but not frequently associated with drug resistance:

**Reverse Transcriptase Gene:** Amino acids analyzed 1 TO 230

Mutations associated with resistance to reverse transcriptase inhibitors:

**Gp41 Gene:** Amino acids analyzed 20 to 70

Mutations associated with resistance to Enfuvirtide

Other mutations causing amino acid changes, but not frequently associated with drug resistance:

**Interpretation** of resistance profile based on the mutations detected in this patient’s HIV-1:

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Trade Name</th>
<th>No Evidence of Resistance</th>
<th>Possible Resistance</th>
<th>Low Level Resistance</th>
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**Non-Nucleoside Reverse Transcriptase Inhibitors**

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**Entry Inhibitors**

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<td>Enfuvirtide</td>
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</table>

Reporting Center: University of Washington, Laboratory Medicine, Virology Division Room G800 4800 Sand Point Way, NE, CH-82 Seattle, WA 98105 Phone: (206) 526-2088
HIV Genotypic Resistance Assay Report (continued)

Notes on the interpretation

HIV-1 strain analyzed: subtype B of pol.

Legend for the interpretation

No Evidence of Resistance  The genotype of the patient’s HIV-1 has no known mutations which suggest phenotypic resistance to the anti-retroviral drug noted.

Possible Resistance  The genotype of the patient’s HIV-1 has mutations which, when associated with other mutations, may cause phenotypic resistance to the anti-retroviral drug noted.

Low Level Resistance  The genotype of the patient’s HIV-1 has mutations which have been associated with low level phenotypic resistance to the anti-retroviral drug noted.

High Level Resistance  The genotype of the patient’s HIV-1 has mutations which have been associated with high level phenotypic resistance to the anti-retroviral drug noted.

Note: This interpretation was based on the 29 January 2004 Drug Resistance Key which accompanies this report. The Drug Resistance Key is periodically updated. You may always obtain the most recent version of the Drug Resistance Key (below) to see if new genotype information might alter the interpretation of the patient’s HIV.

Guidelines for Interpretation of Results:

1. Please refer to the Drug Resistance Key for more detailed information concerning associations between mutations and HIV-1 resistance. If you do not have a current Drug Resistance Key from this facility, please call Community Services at (phone # 206-598-6066) to obtain one. Information pertaining to HIV-1 drug resistance can also be found in the Los Alamos Database at: http://hiv-web.lanl.gov, on which the Drug Resistance Key is based.

2. In preliminary studies genotypic testing predicted HIV-1 resistance to therapies, but was less predictive of susceptible HIV-1.

3. Specific mutations in the HIV-1 polymerase gene (pol) have been closely associated with a lack of or loss of treatment effect to certain antiretrovirals, e.g. the M184V and T215Y/F mutations are commonly associated with HIV-1 resistance to lamivudine (3TC) and zidovudine (ZDV), respectively. However, HIV-1 resistance to other antiviral drugs is less well understood, either because no single mutation has been identified that confers resistance, or because human cellular factors may contribute to resistance, e.g. by pumping the drug out of the cell.

4. Assessing HIV-1 resistance is complicated by the replication kinetics of resistant mutants. Resistant mutants are often less fit than wild type virus and thus, when the selective pressure of the drug is removed, the mutant population may shrink below the level of detection. Nevertheless, these mutants persist in the patient and when the selective drug pressure is reapplied the mutants replicate and a resistant population quickly predominates.

5. Cross-resistance is common among protease inhibitors and non-nucleoside reverse transcriptase inhibitors. Multi-drug resistance mutations (MDR) have also been found for nucleoside analogs (see Drug Resistance Key).

6. This test was done by consensus sequencing of the patient’s HIV-1. The results reflect the predominate genotype of all viral variants in the patient. Variants comprising less than 30 percent of the sample may not be detected.

This test was developed and its performance characteristics determined by University of Washington Academic Medical Centers, Department of Laboratory Medicine. It has not been cleared or approved by the U.S. Food and Drug Administration.
**UW Clinical Virology Drug Resistance Key for HIV-1 Genotypic Analysis**

**Protease Inhibitors:** HIV-1 resistance to PI increases with the number of mutations in the gene encoding protease; *unshaded mutations confer resistance, and shaded mutations increase viral replication capacity*.

### Nucleoside/Nucleotide Reverse Transcriptase Inhibitors

**A.A. Wild Type Mutation**

<table>
<thead>
<tr>
<th>A.A.</th>
<th>Wild Type Mutation</th>
<th>10 L&gt; F/ I/ V</th>
<th>20 K&gt; M/R</th>
<th>23 L&gt; I</th>
<th>24 D&gt; N</th>
<th>30 V&gt; I</th>
<th>32 L&gt; V/F</th>
<th>33 M&gt; I/L</th>
<th>36 V&gt; I</th>
<th>46 G&gt; V</th>
<th>48 I&gt; V</th>
<th>50 I&gt; L</th>
<th>54 I&gt; L/M/V</th>
<th>71 V&gt; T</th>
<th>73 G&gt; A</th>
<th>82 V&gt; A/F</th>
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### Non-Nucleoside Reverse Transcriptase Inhibitors

**A.A. Wild Type Mutation**

<table>
<thead>
<tr>
<th>A.A.</th>
<th>Wild Type Mutation</th>
<th>41 L&gt; V</th>
<th>44 E&gt; D/K</th>
<th>62 K&gt; R</th>
<th>65 A&gt; D&gt; N</th>
<th>67 T&gt; Sxx</th>
<th>69 V&gt; I</th>
<th>70 K&gt; R</th>
<th>74 V&gt; T/I</th>
<th>75 V&gt; Y&gt; F</th>
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<td>Tenofovir</td>
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<td>Zalcitabine</td>
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<td>Zidovudine</td>
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<td></td>
</tr>
</tbody>
</table>

### Entry Inhibitors

**A.A. Wild Type Mutation**

<table>
<thead>
<tr>
<th>A.A.</th>
<th>Wild Type Mutation</th>
<th>36 G&gt; V</th>
<th>37 V&gt; A/M</th>
<th>38 R&gt; N&gt; T</th>
<th>42 D&gt; N</th>
<th>43 V&gt; T</th>
<th>89 D/S/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Delavirdine</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Efavirenz</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Nevirapine</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

### Legend:

- **+++** Indicates major mutation, frequently observed with high-level resistance to the drug.
- **++** Indicates mutation that is frequently observed with low-level resistance to the drug.
- **+** Indicates mutation which may cause resistance to the drug, usually in combination with other resistance mutations.
- **^** Any combination of 3 the following mutations likely confer multi-drug PI resistance: 46I/L; 54I/M; 82F/A/T/S; 84V; 90M
- ***** Mutation of T69S followed by the insertion of any two amino acids confers low-level resistance to the NRTI; w/ ZDV-resistance mutations confers moderate to high level resistance.
- **^^** M41L + L210W + T215Y/F facilitate resistance to tenofovir
- **Q151M** is the pivotal mutation in a multi-drug complex which includes amino acids (AA) 62, 75, 77, and 116, that confers high-level resistance to all nucleoside RT inhibitors
- **^** M41L + L210W + T215Y/F facilitate resistance to tenofovir

These tables are based on data from the Los Alamos Database (http://hiv-web.lanl.gov), the Stanford HIV Database (http://hivdb.stanford.edu), and available presented or published clinical and laboratory studies.
Viral Serologies

Cytomegalovirus (CMV) Serologies

**Cytomegalovirus (CMV) antibody screen** is a qualitative assay that detects antibody of all classes to CMV. The CMV antibody screen is run 5 times a week.

Specimen: 5 mL red top tube
Performed: M - F

The **Cytomegalovirus IgM** assay detects both IgM and IgG to CMV. The CMV IgM assay can distinguish acute primary infection from reactivation and distinguish passively transferred maternal IgG from neonatal IgM in infants. The CMV IgM assay is run once each week.

Specimen: 5 mL red top tube
Performed: once per week, day can vary

Epstein-Barr (EBV) Battery

**Epstein Barr Virus (EBV) antibody** assays include IgG and IgM antibodies to EBV viral capsid antigen (VCA) and antibodies to EBV nuclear antigen (EBNA). Testing is performed twice a week. An interpretive report is included.

Specimen: 5 mL red top
Performed: 2x/week, days can vary

Hepatitis Serologies

**Hepatitis A** is a fecal-oral transmitted infection often acquired through contaminated food. Hepatitis A Virus or antigen detection is not done because viral shedding occurs in the weeks before clinical symptoms appear. Hepatitis A antibody testing is performed daily, Monday through Friday; IgM antibody tests are performed on all reactive specimens. Hepatitis A IgM indicates acute infection. All positive Hepatitis A IgM results are called to the requesting physician or laboratory and the local King County Dept. of Health. The physician must also report acute Hepatitis A infections to the local County Department of Public Health (in King County, telephone the Communicable Disease Department at (206) 296-4774). For vaccine recipients a Hepatitis A vaccine screen can be ordered. This assay does not include IgG/IgM differentiation if positive.

Specimen: 7 mL red top tube
Performed: M-F, negative results same day; Positive results next day

**Hepatitis B** is transmitted through blood or secretions of infected patients. Infectivity of a patient is determined by enzyme immunoassay for Hepatitis B surface antigen (HBsAg), which is run Monday through Friday. Reports of reactive HBsAg are called to the ordering physician or laboratory and are automatically run for Hepatitis B viral DNA by PCR. Patients with needle sticks and other parenteral exposures need to receive Hepatitis B Immune Globulin within 72 hours of exposure. The routine HBsAg run can accommodate most specimens generated by accidental parenteral exposure. The physician must also report acute Hepatitis B infections to the local County Department of Public Health (in King County, telephone the Communicable Disease Department at (206) 296-4774).

Specimen: 7 mL red top tube
Performed: M-F, results same day, confirmation results next day.
Note: Positive HBsAg will reflex to a Hepatitis B DNA by PCR at an additional charge.

**Hepatitis B surface antibody** (anti-HBs) and **Hepatitis B core antibody** (anti-HBc) assays are useful for identifying persons susceptible to Hepatitis B infection (i.e. needle stick exposures), for documenting persons with past infection with Hepatitis B virus, for vaccine screening, and for determining response to vaccination. The Hepatitis B surface antibody result is reported international units (I.U.) of Hepatitis B surface antibody. Values above a test standard containing 10 I.U. of Hepatitis B surface antibody are considered protective levels. Vaccination/re-vaccination is warranted with values below this level. Anti-HBs assays and anti-HBc assays are performed daily Monday through Friday.

**Hepatitis B Core Antibody & Hepatitis B Surface Antibody**

Specimen: 7 mL red top tube
Performed: M-F, results same day

Presence of **Hepatitis B core IgM antibodies** helps to distinguish chronic carriers of HBsAg from acute cases of Hepatitis B. Persons with recent exposure to Hepatitis B will be reactive for core IgM, while chronic carriers of HBsAg will not.

Specimen: 7 mL red top
Performed: once/week, test day varies

The **Hepatitis A & B Battery** consists of the HBsAg, anti-HBs, anti-HBc and anti-Hep A tests. The laboratory requires 3-5 mL clotted blood or 1-2 mL of sera for the full battery.

Specimen: 10 mL red top; Refrigerate.
Performed: See individual tests.
Note: A positive HBsAg reflexes to a Hepatitis B by PCR, quant. at an additional charge.

**Hepatitis C virus (HCV)** is the leading cause of post transfusion hepatitis and of “non-A, non-B” hepatitis. It may be acquired by blood products or by non-parenteral routes. Most HCV infections are sub-clinical, and chronic hepatitis is common. All positive Hepatitis C antibodies are confirmed by PCR at an additional charge. Hepatitis C Antibody screens are run daily, M-Sat.

Specimen: 10 mL red top - preferred, (Lavender or Blue - ok).
Performed: M-Sat., results same day. For PCR confirmation add 2-3 days.

**Herpes Simplex Virus (HSV) types 1 and 2 (Western Blot Serology)**

Viral isolation and subsequent subtyping is generally the best way to document an acute HSV infection. However, serologies for HSV are useful in determining whether a person has had a past infection with HSV-1 or HSV-2, and is the best way to detect “silent carriers” of HSV-2. Our laboratory detects HSV Antibodies by Western blot assay, which is not only highly sensitive for detecting HSV antibodies, but also is highly accurate in differentiating past HSV-1 from HSV-2 infections and determining whether someone has antibodies to both viruses.

The specificity of the Western blot assay provides an accurate distinction in 99% of patients between antibody to HSV-1 and antibodies to HSV-2. In addition, Western blot readily documents seroconversion. Since fewer than 5% of patients demonstrate a detectable rise in antibody titer during recurrent HSV episodes, an interpretive report is given instead of numerical values. HSV Western blot serologies are run three times a week. Most results are available within five days. If the antibody subtype is unclear (about 20% of specimens), the serum will be adsorbed against HSV-1 and HSV-2 proteins and re-tested. This requires an additional week. Call Community Services if you have further questions, (800) 713-5198.
This Western blot assay detects IgG antibody. HSV IgM antibody is present during both primary and recurrent infections making result interpretation difficult. For infants with suspected HSV disease, viral culture and PCR tests should be performed.

Specimen: 10 mL red top tube  
Performed: M, W, Th

**Human T-Cell Leukemia Virus**

**Human T-cell Leukemia Virus 1 (HTLV-1)** is a retrovirus which may cause Adult T-cell Leukemia, Tropical Spastic Paraparesis, or HTLV-1-associated Myelopathy. HTLV-1 does NOT cause Acquired Immunodeficiency Syndrome and should not be confused with Human Immunodeficiency Virus (HIV).

The HTLV-1&2 antibody screen is an enzyme immunoassay (EIA). Antibody to HTLV-2 is also detected but not differentiated from HTLV-1 by this screening assay. Specimens which are repeatedly reactive in the EIA are forwarded to a reference laboratory for confirmation by Western blot at an additional charge.

Specimen: 5 mL red top tube  
Performed: 2x/month, test day varies.

**Mumps**

**Mumps Immune Status** is determined on a single serum by IFA. Mumps titers require acute & convalescent paired serum and are forwarded to the appropriate reference laboratory.

Specimen: 5 mL red top tube  
Frequency: once/week, test day varies

**Pericarditis - CNS Syndrome Serologies**

**Coxsackie B** related myocarditis or pericarditis occurs after viral shedding from throat or rectum has ceased. Therefore, the best way to diagnose this infection is with acute and convalescent sera drawn 2 weeks apart. Our test assays neutralizing antibodies to Coxsackie B1 through B6. The test requires 0.5-1 mL each of paired sera only. Acute serum will be held until convalescent blood is received. There is an additional serum storage charge. Coxsackie B serologies are not performed on single serum, since a result from a single serum is not interpretable. The test is run as needed and results are ready 5-7 days after set-up.

Lymphochoriomeningitis virus and encephalitis viral serologies require paired acute and convalescent sera. These serologies are not performed locally, but forwarded to the appropriate reference laboratory.

**Respiratory Syndrome Serologies**

**Influenza A and B, Adenovirus, Respiratory Syncytial Virus and Parainfluenza virus serologies** require paired acute and convalescent sera. These serologies are not performed locally, but forwarded to the appropriate reference laboratory. Culture and DFA for these community acquired respiratory viruses are available and preferred for acute cases; see preceding section for details.

**Rubella/Rubeola (measles)**

**Rubella or Rubeola Immune Status** is an EIA screen for Rubella or Rubeola antibodies, and is done 2 times a week. A positive result indicates a past infection or immunization. For acute infections, an IgM is recommended. This is the preferred method because these viruses are very unstable and difficult to culture. For questions on IgM and testing please contact the State Health Department.

Specimen: 5 mL red top tube  
Performed: 2x/week, test days vary
Varicella Zoster Virus (VZV) Antibody

The Varicella Zoster Virus (VZV) antibody screen is a qualitative assay to determine the immune status of individual with respect to VZV. It is particularly useful for immunocompromised or pregnant patients who are at risk of developing severe Varicella disease and who, if negative, may be candidates for Varicella Zoster Immune Globulin (VZIG). It is also useful for screening health care providers who may be exposed to the disease. VZIG must be given within 72 hours of exposure. The VZV antibody screen is run 5 times a week and is offered on a STAT (24-hour turn-around) basis on weekends. All STAT requests for VZV antibody determinations in candidates for VZIG must be approved by the Laboratory director, Virology physician on call, or Laboratory Medicine resident.

Specimen: 5 mL red top tube (For spinal fluids, see Varicella Zoster by PCR)
Performed: M-F

A VZV antibody titer can be performed on paired acute and convalescent sera specimens to document a recent past infection. A four-fold rise in titer is seen in both primary infection (chicken pox) and reactivation episodes (Herpes Zoster or Shingles).

Specimen: 5 mL red top tube for each acute & convalescent specimen. Blood must be drawn 14 days apart.
Performed: M-F

West Nile Virus, IgG & IgM Serologies

Serological testing for West Nile Virus is done by enzyme immunoassay (EIA). Presence of IgM antibody indicates recent infection. This assay is used to monitor patients with suspected West Nile Virus exposure. For measure of infectivity, PCR testing is recommended. We run the serology test in the summer months only. In the off season we request all serology testing to be sent to the State Lab.

Specimen: 10 mL plain red or Gold top tube. Separate serum from cells ASAP. If parallel testing is preferred, convalescent samples must be received within 30 days from receipt of the acute samples. Please mark sample plainly as "acute" or "convalescent."
Performed: Once a week or as needed

Serological Tests Not Performed by the Virology Division Laboratory

- “Acute and/or convalescent viral titers” or “viral studies” requests are too vague. Sera with only this information will be held for 6 weeks and if no other information is called to the laboratory, the sera will be discarded.
- “TORCH” screens are not available through the Virology Laboratory. Viral serologies on babies are not generally useful unless they measure IgM antibodies; IgG assays will only reflect maternal serostatus. For the “TORCH” organisms, reliable IgM assays are available for rubella and CMV and Toxoplasma (Microbiology section). Herpes, CMV and enteroviruses are best diagnosed by culture of babies’ urine, throat, rectum or conjunctiva.
- Enterovirus serologies (except Coxsackie B) are not performed because there are no common antigens among the 31 serotypes. Throat, rectal and occasionally CSF cultures are recommended. Most enteroviruses are shed for 2 to 4 weeks following onset of disease. Enterovirus PCR is also available for CSF samples.
- Chlamydia psittaci serologies are performed at the Seattle-King County Health Department and Washington State Department of Health Laboratories. Chlamydia psittaci cultures are also performed at the Washington State Department of Health laboratories.
Hepatitis Serological Profiles

Hepatitis A

Hepatitis B

Hepatitis B Chronic Carrier Serological Profile: No Seroconversion

Hepatitis B Chronic Carrier Serological Profile: Late Seroconversion
<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Seasonal Prevalence</th>
<th>Appropriate Specimens</th>
<th>Stability In Transport Media</th>
<th>Serologies</th>
<th>Tests to Order</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Conjunctivitis Kerato-conjunctivitis Pneumonitis Hemolytic uremia Acute respiratory disease</td>
<td>Year round</td>
<td>Eye Throat Urine Rectal Swab</td>
<td>48 hrs @ 4°C Titer stable @ -70°C</td>
<td>Adenovirus antibodies</td>
<td>Virus Culture Screen, Respiratory FA Adenovirus serology (acute and cx sera required) Adenovirus Shell Vial Adenovirus PCR, Serum/CSF/Urine - Enteric Adeno EIA</td>
<td>FA or Shell Vial for rapid detection (shed in stool up to 1 year after infection).</td>
</tr>
<tr>
<td>Adenovirus 40,41</td>
<td></td>
<td>CSF, Serum, Stool, Tissue</td>
<td>Stable @ 4°C</td>
<td>Not available</td>
<td>Enteric Adenovirus EIA</td>
<td></td>
<td>Second major cause of viral infant diarrhea.</td>
</tr>
<tr>
<td>Coxsackie A</td>
<td>Herpangina, FUO Hand, foot and mouth disease Aseptic meningitis Paralytic syndromes Exanthem URI</td>
<td>Summer Fall</td>
<td>Throat Rectal CSF</td>
<td>48 hrs @ 4°C</td>
<td>Not Available</td>
<td>Virus Culture Screen Enterovirus by PCR, CSF</td>
<td>Some of the 24 Coxsackie A viruses grow only in suckling mice; special requests &amp; consultations through lab director.</td>
</tr>
<tr>
<td>Coxsackie B</td>
<td>Aseptic meningitis Exanthem Hemolytic uremia</td>
<td>Summer Fall</td>
<td>Throat Rectal CSF, Urine</td>
<td>48 hrs @ 4°C</td>
<td>Paired (acute &amp; convalescent) neutralization titers</td>
<td>Virus Culture: Screen Enterovirus PCR, CSF Enterovirus PCR, CSF</td>
<td>Separate specimens required for PCR and culture.</td>
</tr>
<tr>
<td>Coxsackie B</td>
<td>Myocarditis Pericarditis Pleurodynia Bornholm disease</td>
<td>Year round</td>
<td>Serum</td>
<td>Paired neutralization titers for Coxsackie B</td>
<td>Coxsackie B1-B6 serology</td>
<td></td>
<td>Virus shedding ceases before clinical pericarditis develops. Draw acute and convalescent sera to do paired neutralization titers.</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>Non-gonococcal urethritis (NGU) Cervicitis Conjunctivitis Epididymitis Pneumonia -infants Lymphogranuloma venereum Proctitis Salpingitis Epididymitis</td>
<td>Year round</td>
<td>Urethral swab Cervical swab Body Fluids N-P (not throat) Lower eyelid Exudates or tissue Urine</td>
<td>72 hrs @ 4°C Stability in transport media reflects cultures not NAAT testing.</td>
<td>IgM, IgG</td>
<td>Chlamydia trachomatis culture Chlamydia trachomatis FA Chlamydia trachomatis serology (IgG &amp; IgM) Chlamydia trachomatis NAAT</td>
<td>Chlamydia trachomatis is not a virus.</td>
</tr>
</tbody>
</table>
## Virology Diagnostic Chart

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Seasonal Prevalence</th>
<th>Appropriate Specimens</th>
<th>Stability In Transport Media</th>
<th>Serologies</th>
<th>Tests to Order</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium difficile</em> toxin B</td>
<td>Pseudomembranous colitis</td>
<td>Year round</td>
<td>Stool (not in Viral Transport Media)</td>
<td>48hrs @ 4°C</td>
<td>Not available</td>
<td><em>C. difficile</em> toxin B</td>
<td>For bacterial culture &amp;/or antigen detection, send stool to Microbiology.</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Congenital infection</td>
<td>Year round</td>
<td>Urine Throat Tissue Serum Nasopharynx Buffy Coat CSF</td>
<td>12-18 hrs @ 4°C</td>
<td>CMV antibody screen CMV IgG/IgM</td>
<td>Herpes Group Culture CMV Rapid Assay CMV Immune Status CMV Antigenemia CMV by PCR CMV IgG/IgM CMV Drug Resistance UL97</td>
<td>Specific assay depends on sample</td>
</tr>
<tr>
<td>Echovirus</td>
<td>Aseptic meningitis Exanthem FUO, URI</td>
<td>Summer Fall</td>
<td>Throat Rectal swab CSF</td>
<td>48 hrs @ 4°C</td>
<td>Not available as there are over 31 serotypes</td>
<td>Virus Culture: Screen Enterovirus PCR, CSF</td>
<td>Virus is detectable by rectal swab up to 4 weeks post onset of infection.</td>
</tr>
<tr>
<td>Enterovirus (including 68-71)</td>
<td>Hand, Foot and Mouth Disease, Meningoencephalitis, Aseptic Meningitis, Paralysis, Pneumonia and Bronchiolitis</td>
<td>Summer Fall</td>
<td>CSF Serum</td>
<td>48 hrs @ 4°C</td>
<td>See specific Cox A, Cox B, Echovirus</td>
<td>Enterovirus by PCR, CSF or serum</td>
<td>Test cross reacts with some Rhinovirus serotypes, do not submit respiratory samples for PCR testing</td>
</tr>
<tr>
<td>Epstein-Barr (EBV)</td>
<td>Mononucleosis (Heterophile + generally, unless patient is &lt; 6 yrs) Post transplant Lympho-proliferative disease</td>
<td>Year round</td>
<td>Serum</td>
<td>N/A</td>
<td>EBV serology by EIA</td>
<td>Epstein-Barr antibody battery</td>
<td>Convalescent specimen not necessary.</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Hepatitis</td>
<td>Year round</td>
<td>Serum</td>
<td>N/A</td>
<td>Hepatitis A anitbody (IgG/IgM)</td>
<td>Hepatitis A antibody</td>
<td>Differentiate between IgM &amp; IgG (Reportable disease in Washington State).</td>
</tr>
<tr>
<td>Agent</td>
<td>Disease</td>
<td>Seasonal Prevalence</td>
<td>Appropriate Specimens</td>
<td>Stability In Transport Media</td>
<td>Serologies</td>
<td>Tests to Order</td>
<td>Comments</td>
</tr>
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<td>--------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Hepatitis, Acute and Chronic</td>
<td>Year round</td>
<td>Serum (not plasma)</td>
<td>Stable @ room temp</td>
<td>Hepatitis B surface Ag, Hepatitis B surface Ab, Hepatitis B core Ab “e” Ag/Ab *Hepatitis A &amp; B Battery includes HBsAg, Anti-HBs, Anti-HBc, &amp; Anti-HA</td>
<td>*Hepatitis B Battery includes HBsAG, Anti-Hbs, &amp; Anti-HBc *Hepatitis Panel or screen includes Hepatitis A, B &amp; C *Hepatitis B PCR</td>
<td>Hepatitis viruses do not grow in tissue culture. All positive HBsAg are run for HBV DNA by PCR. Presence of HBV DNA indicates infectious state. HBV DNA levels may be used to follow response to antiviral therapy. Presence of HBe Ag indicates highly infectious state. Acute Hep B. reportable disease in Washington state</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Hepatitis; Acute, Chronic and Fulminant, Liver Cirrhosis, Hepatocellular Carcinoma, Glomerulonephritis, Polyarteritis nodosa, Cryoglobulinemia</td>
<td>Year round</td>
<td>Serum</td>
<td>PCR assay, freeze @ -70°C within 2 hours or refrigerate.</td>
<td>Hepatitis C Ab.</td>
<td>Hepatitis C PCR Hepatitis C Antibody Hepatitis C Genotyping</td>
<td>All positive HCV antibodies are confirmed by PCR for HCV RNA</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Gingivostomatitis Genital Infection Proctitis Keratitis Neonatal Infection Encephalitis Aseptic Meningitis Pneumonia Hepatitis</td>
<td>Year round</td>
<td>Lesion sites Genital-Cervical Swab Throat Swab Rectal Swab (proctitis CSF (meningitis) Urine (urethritis) Brain biopsy (encephalitis) Blood</td>
<td>72 hrs @ 4°C</td>
<td>Herpes simplex 1 &amp; 2 Western blot</td>
<td>Virus Culture Screen, Plus Herpes Group FA; HSV Western blot HSV by PCR HSV I, II, Subtyping by PCR</td>
<td>Direct FA available for rapid diagnosis. Most isolations are positive by 3 days. PCR for encephalitis (CSF) HSV Western blot can distinguish type 1 from type 2 antibody. If women pregnant, please specify</td>
</tr>
<tr>
<td>Human herpes virus-6</td>
<td>Roseola Infant febrile seizures Encephalitis in transplant patients</td>
<td>Year round</td>
<td>Serum CSF</td>
<td>N/A</td>
<td>N/A</td>
<td>HHV-6 by PCR</td>
<td></td>
</tr>
<tr>
<td>Human Immunodeficiency Virus (HIV-1 or 2)</td>
<td>HIV Infection Opportunistic infections</td>
<td>None</td>
<td>Serum, plasma for serologies Whole blood for PCR and culture Plasma for viral load</td>
<td>(Do not place specimen in transport media)</td>
<td>HIV 1 &amp; 2 EIA with confirmatory Western blot</td>
<td>HIV 1 &amp; 2 EIA HIV 1 DNA PCR HIV 1 Culture HIV 1 Genotypic Resistance Assay HIV 1 realtime rtPCR HIV 1 p24 antigen</td>
<td>All HIV antibody EIA reactive specimens are confirmed by western blot.</td>
</tr>
<tr>
<td>Agent</td>
<td>Disease</td>
<td>Seasonal Prevalence</td>
<td>Appropriate Specimens</td>
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</tr>
<tr>
<td>Human T Lymphotropic virus (HTLV-1)</td>
<td>T Cell Leukemia Lymphoma (Adult)</td>
<td></td>
<td></td>
<td></td>
<td>EIA</td>
<td>HTLV 1/2 EIA</td>
<td>Confirmatory immunoblot sent to reference lab at additional charge</td>
</tr>
<tr>
<td>Influenza</td>
<td>Influenza (headache, fever, myalgia, myositis)</td>
<td>Winter Spring</td>
<td>Throat, Tracheal aspirate, Sputum, Nasal wash, BAL Fluid</td>
<td>Unstable @ 4°C forward to lab within 12 hours</td>
<td>State Health Lab</td>
<td>Virus Culture Screen plus respiratory FA, *Respiratory FA alone during &quot;Flu season&quot; (Jan./Feb.)</td>
<td>FA for rapid diagnosis; may be more sensitive than culture. Culture average 5-7 days to preliminary positive; FA is recommended as well as culture.</td>
</tr>
<tr>
<td>Mumps</td>
<td>Mumps</td>
<td>Winter Spring</td>
<td>Throat swab, Urine, CSF, Serum</td>
<td>Unstable @ 4°C forward to Lab within 12 hours</td>
<td>State Health Dept.</td>
<td>Mumps immune status</td>
<td>Virus Culture Screen plus Mumps Immune Status</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Bronchitis, Croup, Bronchopneumonia</td>
<td>Winter Spring, Parainfluenza 3 - all year</td>
<td>Nasal wash, Tracheal aspirate, Sputum, N-P swab, Throat swab, BAL Fluid</td>
<td>Unstable @ 4°C forward to lab within 12 hours</td>
<td>State Health Dept.</td>
<td>Virus Culture: Screen plus respiratory FA</td>
<td>FA for rapid diagnosis. Culture average 7 days for preliminary positive.</td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>Erythema infectiosum</td>
<td>Winter Spring</td>
<td>Serum, Bone Marrow</td>
<td></td>
<td>IgG, IgM</td>
<td>PCR for Parvovirus DNA</td>
<td></td>
</tr>
<tr>
<td>Polio</td>
<td>Polymyelitis</td>
<td>Summer</td>
<td>Throat, Rectal, CSF</td>
<td>48 hrs @ 4°C</td>
<td>State Health Dept.</td>
<td>Virus Culture Screen plus Enterovirus by PCR, CSF</td>
<td>Isolation often following vaccination with live vaccine (not currently used).</td>
</tr>
<tr>
<td>Respiratory syncytial virus (RSV)</td>
<td>Bronchiolitis, Bronchopneumonia, Croup</td>
<td>Winter Spring</td>
<td>Nasal wash, Tracheal aspirate, N-P swab</td>
<td>Labile agent: keep @ 4°C, forward within 12 hours</td>
<td>State Health Dept.</td>
<td>Virus Culture: Screen plus respiratory FA, RSV FA (recommend Nov-April only)</td>
<td>Nosocomial spread common; very severe in compromised children - Direct FA for rapid diagnosis and is often more sensitive than isolation.</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Common Colds</td>
<td>Year round</td>
<td>N-P swab</td>
<td>Keep @ 4°C, transport within 12 hours</td>
<td>Not available</td>
<td>Virus Culture: Screen</td>
<td></td>
</tr>
<tr>
<td>Agent</td>
<td>Disease</td>
<td>Seasonal Prevalence</td>
<td>Appropriate Specimens</td>
<td>Stability In Transport Media</td>
<td>Serologies</td>
<td>Tests to Order</td>
<td>Comments</td>
</tr>
<tr>
<td>------------------</td>
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<td>---------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Rubella</td>
<td>Rubella Congenital infection</td>
<td>Winter Spring</td>
<td>Throat, Nasal secretions, Urine Placental or fetal tissue</td>
<td>Keep @ 4°C, forward ASAP</td>
<td>IgM-more reliable than culture Immune status screen Rubella titer (State Lab)</td>
<td>Virus Culture: Rubella IgM Rubella Immune status</td>
<td>Difficult to culture: please Call lab first and specify you want Rubella isolation - Special procedures required. IgM serology is best option.</td>
</tr>
<tr>
<td>Rubeola</td>
<td>Measles Subacute (SSPE) sclerosing panencephalitis</td>
<td>Winter Spring</td>
<td>Throat NP swab</td>
<td>Unstable @ 4°C, forward ASAP</td>
<td>State Health Dept. immune status or paired sera for titers</td>
<td>Virus Culture: Screen Rubeola Immune Status</td>
<td>Must inform lab if Rubeola suspected; special ID procedures are required.</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Non-bacterial gastroenteritis</td>
<td>Winter Spring</td>
<td>Stool (not in transport media) Rectal swab not acceptable</td>
<td>72 hrs @ 4°C</td>
<td>Not available</td>
<td>Rotavirus antigen by EIA</td>
<td>Nonscomial spread common, cannot be cultured.</td>
</tr>
<tr>
<td>SARS</td>
<td>Severe acute respiratory syndrome</td>
<td></td>
<td>Nasal wash NPT Swab</td>
<td>24 hours</td>
<td>Not available</td>
<td>Contact State Health Dept. SARS PCR SARS Viral culture</td>
<td>Must indicate R/O SARS, special BL3 precautions required for processing</td>
</tr>
<tr>
<td>Varicella Zoster</td>
<td>Chickenpox Shingles Aseptic Meningitis Encephalitis</td>
<td></td>
<td>Lesion swab Throat swab Serum</td>
<td>Unstable Virus keep @ 4°C Do not freeze @ -70°C Transfer to lab ASAP</td>
<td>VZV immune status VZV antibody titer</td>
<td>Virus Culture: Screen Plus Herpes Group FA VZV Rapid Assay VZV immune status VZV Antibody titer (paired only) VZV by PCR</td>
<td>FA- for VZ antigen - more sensitive than culture. Culture and FA are recommended for VZ detection. Paired serology for titer change</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>West Nile Encephalitis, WNV Fever</td>
<td>Summer, Fall</td>
<td>CSF, Plasma</td>
<td>NA</td>
<td>IgM and IgG Serum, CSF</td>
<td>IgG &amp; IgM Serology West Nile Virus by PCR - CSF or Serum</td>
<td>Serologies currently performed at the state laboratory</td>
</tr>
</tbody>
</table>
CLINICAL LAB REQUEST
UW MEDICINE
LABORATORY MEDICINE COMMUNITY SERVICES

Virology
(206) 598-6066 Billing/Specimen Pick-up
(206) 897-2000 Virology Physician on 24-hour Call.

1. Chlamydia, viral and routine microbiology transport media MAY NOT be used interchangeably.
2. Dacroswab (type 1) recommended for viral cultures.
3. Culturette recommended for PCR detection from mucosal surfaces.
4. Pur-Wrap swab recommended for chlamydia cultures.
5. Reflex tests § instructions can be found on back. Additional charges will be incurred for added tests if reflex testing occurs. Please see fee schedule for pricing.

VIRAL CULTURE & ANTIGEN DETECTION [206 987-2088]

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Serum</th>
<th>Plasma</th>
<th>Whole Blood</th>
<th>Urine</th>
<th>CSF</th>
<th>Stool</th>
<th>Other</th>
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</thead>
</table>

Acute Serum ☐ Convalescent Serum ☐ Follow-Up Convalescent (requested by Virology) ☐

ICD/DIAGNOSIS

SEND REPORT TO (Hospital, Clinic, Physician)

ADDRESS

CITY STATE ZIP

TELEPHONE

PATIENT ADDRESS

CITY STATE ZIP

TELEPHONE

PATIENT SOC. SEC. #

SUBSCRIBER NAME

SUBSCRIBER ID. #

GROUP# Blue Cross of WA Regence DSHS (attach current coupon)

Medicare (answer required question below)

Is this either a hospital outpatient or inpatient? Yes ☐ No ☐ (see reverse for additional information)

Other Insurance Name/Address

OTHER REQUESTS / COMMENTS

MEDICAL NECESSITY INFORMATION

When ordering tests for which Medicare reimbursement will be sought, physicians should only order tests which are medically necessary for diagnosis or treatment of the patient. You should be aware that Medicare generally does not cover routine screening tests, and will only pay for tests that are covered by the program and are reasonable and necessary to treat or diagnose the patient.
HCFA MEDICAL NECESSITY INFORMATION

It is our policy to provide health care providers with the ability to order only those lab tests medically necessary for the individual patient and to ensure that the convenience of ordering standard panels and custom profiles does not impact this ability. While we recognize the value of this convenience, indiscriminate use of panels and profiles can lead to ordering tests that are not medically necessary. Therefore, all tests offered in our panels and profiles can be ordered individually as well. If a component test is not listed individually on the request form, it may be written in the “OTHER REQUESTS” box. We encourage you to order individual tests or a less inclusive profile when not all of the tests included in the panel or profile are medically necessary for the individual patient.

Medicare Billing Information

Medicare billing policy prevents us from submitting a Medicare claim for laboratory testing referred to us on hospital inpatients or hospital outpatients. For these samples, we will bill the sending location.

Reflexive Test Descriptions

HIV-1&2 EIA With Reflexive Western Blot Confirmation
If HIV 1&2 EIA is reactive, HIV1 Western blot is performed. If HIV1 Western blot does not confirm EIA reactivity, further supplemental assays will be performed, and the sample may be forwarded to a reference laboratory for HIV2 antibody confirmation.

Hepatitis C Antibody Screen
If Hepatitis C antibody is positive by EIA, Hepatitis C RNA by PCR is performed.

Hepatitis B Surface Antigen
If Hepatitis B surface antigen is positive by EIA, Hepatitis B DNA Quantitation is performed.

Human Herpes 8
If EIA equivocal, HHV8 latent and lytic antibody testing is performed.

HTLV1 & HTLV2 Antibodies
If antibody testing is positive by EIA, confirmation serology is sent out to reference laboratory.

MEDICAL NECESSITY DOCUMENTATION - PARTIAL ICD9 LIST

For outpatient use only: This partial ICD9 code list is being provided only as informational assistance in documenting medical necessity. It is not an all-inclusive list of codes for conditions related to tests on this requisition. If the correct diagnosis, sign or symptom code is not found here or on a service-specific ICD9 code list at your location, please write the diagnosis, signs or symptoms in the Medical Necessity box located on the bottom front of this sheet. Do not circle codes here, please transcribe them to the front.

<table>
<thead>
<tr>
<th>CODE</th>
<th>DESCRIPTION</th>
<th>CODE</th>
<th>DESCRIPTION</th>
<th>CODE</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>793.9</td>
<td>Abnormal X-ray findings, NOS</td>
<td>054.9</td>
<td>Herpes Simplex</td>
<td>782.1</td>
<td>Rash</td>
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<tr>
<td>796.4</td>
<td>Abnormal lab findings, NOS</td>
<td>053.9</td>
<td>Herpes Zoster</td>
<td>519.8</td>
<td>Respiratory Tract Infection</td>
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<tr>
<td>719.40</td>
<td>Arthritis, site NOS</td>
<td>042</td>
<td>HIV symptomatic / AIDS</td>
<td>363.20</td>
<td>Retinitis</td>
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<tr>
<td>716.90</td>
<td>Arthritis, site NOS</td>
<td>V08</td>
<td>HIV, asymptomatic</td>
<td>789.2</td>
<td>Splenomegaly</td>
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<tr>
<td>616.0</td>
<td>Cervicitis, acute</td>
<td>487.1</td>
<td>Influenza</td>
<td>V42.81</td>
<td>Transplant, bone marrow, s/p</td>
</tr>
<tr>
<td>078.88</td>
<td>Chlamydia</td>
<td>464.0</td>
<td>Laryngitis, acute</td>
<td>V42.1</td>
<td>Transplant, heart, s/p</td>
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<tr>
<td>780.71</td>
<td>Chronic Fatigue Syndrome</td>
<td>785.6</td>
<td>Lymphadenopathy</td>
<td>V42.0</td>
<td>Transplant, kidney, s/p</td>
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<tr>
<td>707.9</td>
<td>Chronic Skin Ulcer, NOS</td>
<td>780.79</td>
<td>Malaise</td>
<td>V42.7</td>
<td>Transplant, liver, s/p</td>
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<tr>
<td>372.30</td>
<td>Conjunctivitis</td>
<td>322.9</td>
<td>Meningitis</td>
<td>V42.6</td>
<td>Transplant, lung, s/p</td>
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<tr>
<td>078.5</td>
<td>Cytomegalovirus Infection</td>
<td>075</td>
<td>Mononucleosis</td>
<td>V42.83</td>
<td>Transplant, pancreas, s/p</td>
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<tr>
<td>787.91</td>
<td>Diarrhea</td>
<td>729.1</td>
<td>Myalgia</td>
<td>V42.84</td>
<td>Transplant, peripheral stem cells, s/p</td>
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<tr>
<td>049.9</td>
<td>Encephalitis - Viral</td>
<td>429.0</td>
<td>Myocarditis</td>
<td>465.9</td>
<td>Upper Respiratory Infection</td>
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<tr>
<td>348.3</td>
<td>Encephalopathy, NOS</td>
<td>604.90</td>
<td>Orchitis, Epididymitis, NOS</td>
<td>597.80</td>
<td>Urethritis</td>
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<tr>
<td>008.8</td>
<td>Enteritis, viral</td>
<td>079.89</td>
<td>Parovirus Infection</td>
<td>364.3</td>
<td>Uveitis</td>
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<tr>
<td>530.10</td>
<td>Esophagitis</td>
<td>420.91</td>
<td>Pericarditis, acute</td>
<td>616.1</td>
<td>Vaginitis, NOS</td>
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<tr>
<td>780.6</td>
<td>Fever</td>
<td>462</td>
<td>Pharyngitis, acute</td>
<td>099.9</td>
<td>Veneral Disease, NOS</td>
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<tr>
<td>009.1</td>
<td>Gastroenteritis, infectious</td>
<td>480.9</td>
<td>Pneumonia, viral, NOS</td>
<td>780.4</td>
<td>Vertigo / Dizziness</td>
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<tr>
<td>571.9</td>
<td>Hepatic Disease, chronic, NOS</td>
<td>138</td>
<td>Poliomyelitis / Residuals</td>
<td>079.99</td>
<td>Viral Syndrome</td>
</tr>
<tr>
<td>078.10</td>
<td>Warts, NOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>