

Virology

(Specimen Handling, Mailing, and Test Interpretation)

A. *Virus Culture:*

1. *Culture transport swabs:* Swabs should be mailed on foam refrigerant but not frozen. Place **coolant** in the bottom of a large Styrofoam® container. Next, enclose swab in tightly-sealing plastic bag, and place this bag on top of refrigerant. **DO NOT BEND OR BREAK SWAB.** Pack vacant space with any type of packing material. Mark “REFRIGERATE ONLY” on the side of the outside mailing carton to prevent it from being placed in a freezer if received during off hours or weekends.
2. *Urine and cerebrospinal fluid:* Send 1 mL in a sterile vial. Specimens should be handled under sterile conditions to avoid contamination. Specimens should be considered infectious. See “Infectious Material” in Special Instructions for shipping instructions.
3. *Miscellaneous (sputum, brain biopsy):* Specimen should be in a sterile container and handled under sterile conditions to avoid contamination. Send tissue refrigerated in a sterile container containing 1 mL to 2 mL sterile saline or multi-microbe medium (M5). Specimens should be considered infectious. See “Infectious Material” in Special Instructions for shipping instructions.
4. M4 or M5 medium is an optimal transport medium.

B. *Viral Serology*—Indirect immunofluorescence assay (IFA) and enzyme immunoassay (EIA) comprise almost all serologic assays in diagnostic virology. For serologic testing, indicate specific tests desired.

1. See individual test descriptions for specimen collection and transport instructions.
2. *Test interpretation IgG antibodies:*
 - a. *Congenital infections:* Serial sera from both the mother and infant are recommended for laboratory diagnosis. IgG antibody levels in the infant which are passively acquired from the mother will decrease markedly within 2 to 3 months. Active infection, however, is indicated by antibody levels that are unchanged or increased in serial sera over 2 to 3 months. The absence of antibody to a particular virus in the mother rules out intrauterine infection. In addition, for cytomegalovirus and herpes simplex virus, IgM antibodies may be determined on a serum specimen from the infant (see below).
 - b. *Other infections:* A 4-fold or greater rise in antibody titers between the acute and convalescent phase sera or the presence of IgM-class antibody is indicative of acute infection. However, IgM class antibodies to some viruses (eg, West Nile virus) may persist for months following exposure and do not always suggest active infection. The timing for obtaining the sera is of utmost importance. In most instances, a serologic test for IgG-class antibody performed on a single serum specimen will not differentiate recent infection from one that occurred sometime in the past.
3. *Test interpretation IgM antibodies:* The detection of IgM-class antibodies may indicate acute-phase infection but may also occur in individuals who were exposed months prior to specimen collection.
 - a. *Congenital infections:* IgM is the first class of antibody produced after exposure to microbial agents. Detection of IgM is especially useful in the diagnosis of congenital and neonatal infections since, unlike IgG, this immunoglobulin does not cross the placental barrier, and so any IgM detected in a neonate represents actively produced antibody.
 - b. *Other infections:* Except in congenital diseases, IgM antibodies can be detected for approximately 3 to 4 weeks after primary infections. Although, in some cases, detectable IgM antibodies can persist for several months. IgM antibodies may also reach detectable levels following reinfections (rather than primary infections) or immunization and may persist for a period of several months.
 - c. For our assays, IgM is physically separated from whole serum to avoid interference of rheumatoid factor-containing specimens in the test procedures.
4. *Immune status:* Detectable IgG antibody is evidence of previous infection with the following viruses:
 - a. Varicella-zoster virus (VZV) (chickenpox)
 - b. Measles virus (rubeola)
 - c. Mumps virus
 - d. Rubella

C. *Specimen Selection:*

The 2 tables which follow are meant to guide you in selecting the proper specimens for viral studies.

Table I is used if only the clinical picture is known and the physician does not indicate a specific organism. Please be sure to provide the clinical information requested.

Table II lists the most suitable sources for culture and serologic testing and is used when the physician has a specific virus in mind.

Table I. Symptom-Oriented Guide to Viral Studies	
Clinical Manifestation	Suggested Specimen
Respiratory—pharyngitis, croup, bronchitis, pneumonia	<ol style="list-style-type: none"> 1. Throat swab, sputum, nasopharyngeal aspirate (respiratory syncytial virus) 2. Serum—please specify particular virus (see Table II for list of specific agents)
Rash—maculopapular exanthem	<ol style="list-style-type: none"> 1. Throat swab, rectal swab (enteric virus only) 2. Serum—please specify particular virus (see Table II for list of specific agents)
Rash—vesicular exanthem	<ol style="list-style-type: none"> 1. Vesicle fluid on culture transport swab (throat swab, rectal swab) 2. Serum—please specify particular virus (see Table II for list of specific agents)
Aseptic meningitis, encephalitis	<ol style="list-style-type: none"> 1. Throat swab, rectal swab, urine (mumps), spinal fluid for PCR for herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus, enterovirus, JC virus (please specify) 2. Serum—please specify particular virus (see Table II for list of specific agents)
Congenital	<ol style="list-style-type: none"> 1. Throat swab for culture or PCR, urine (fresh morning)-PCR for CMV, vesicle fluid on culture transport swab-PCR for HSV 2. Serum—please specify particular virus (see Table II for list of specific agents)
Ocular	<ol style="list-style-type: none"> 1. Conjunctival swab 2. Serum—please specify particular virus (see Table II for list of specific agents)
Infectious mononucleosis-like syndrome	<ol style="list-style-type: none"> 1. Throat swab for culture or PCR, urine-PCR for CMV 2. Serum (heterophile-negative)—EBV enzyme immunoassay (EIA)—CMV enzyme-linked fluorescent immunoassay (ELFA)
Immunosuppressed or immunodeficient	<ol style="list-style-type: none"> 1. Urine-PCR for CMV, plasma-quantitative PCR for CMV, throat swab, blood (5 mL, EDTA)-quantitative PCR for EBV 2. Serum—please specify particular virus (see Table II for list of specific agents)

Table II. Specimen Guide for Specific Viruses

Common Pathogenic Viruses	For Culture or PCR*	Serologic Tests**
Respiratory:		
Adenovirus	Throat (PCR), fresh tissue	Serum for IF
Cytomegalovirus	Throat (PCR), urine specimen (PCR)	Serum for ELFA
Enterovirus	Throat, rectal	No test
Herpes simplex virus	Throat (PCR)	Serum for EIA/IF
Influenza virus	Throat (PCR)	Serum for IF
Mumps virus	Throat, urine specimen	Serum for ELFA/IF
Parainfluenza virus	Throat	No test
Respiratory syncytial virus	Nasopharyngeal aspirate, throat	Serum for IF
Rhinovirus	Throat	No test
Rash—maculopapular:		
Adenovirus	Throat (PCR), rectal (PCR)	No test
Enterovirus	Throat, rectal	No test
Measles virus (rubeola)	Not cultured	Serum for EIA/IF
Parainfluenza virus	Throat	No test
Respiratory syncytial virus	Nasopharyngeal aspirate, throat	Serum for IF
Rubella virus	Not cultured	Serum for ELFA
Rash—vesicular:		
Coxsackieviruses A-9, A-16	Vesicle fluid, throat, rectal	No test
Herpes simplex virus	Vesicle fluid (PCR), throat (PCR)	Serum for EIA/IF
Varicella-zoster virus	Vesicle fluid (PCR), throat (PCR)	Serum for EIA/IF
Aseptic meningitis and encephalitis:		
California virus	Not cultured	Serum for IF
Cytomegalovirus	Spinal fluid (PCR)	Serum for ELFA
Enterovirus	Spinal fluid (PCR), throat, rectal	No test
Epstein-Barr virus	Spinal fluid (PCR)	Serum for MFI
Herpes simplex virus	Spinal fluid (PCR)	Not recommended
JC virus	Spinal fluid (PCR)	No test
Mumps	Throat, urine specimen	CSF and serum for ELFA/IF
St. Louis encephalitis	Not cultured	Serum for IF
Varicella-zoster virus	Spinal fluid (PCR)	Serum for EIA/IF
Western equine encephalitis	Not cultured	Serum for IF
Congenital:		
Cytomegalovirus	Urine specimen (PCR), throat (PCR)	Serum for ELFA
Herpes simplex virus	Vesicle fluid (PCR), throat (PCR)	Serum for EIA/IF
Rubella virus	Not cultured	Serum for ELFA
Ocular:		
Adenovirus	Conjunctival (PCR)	Serum for IF
Enterovirus	Conjunctival (PCR)	No test
Herpes simplex virus	Conjunctival (PCR)	Serum for EIA/IF
Infectious mononucleosis-like syndrome:		
Cytomegalovirus	Urine specimen (PCR), throat (PCR)	Serum for ELFA
Epstein-Barr virus (heterophile-negative)	Not cultured	Serum for MFI
Immunosuppressed or immunodeficient:		
Cytomegalovirus	Urine specimen (PCR), throat (PCR), plasma (2 mL, EDTA) (QN PCR)	Serum for ELFA
Epstein-Barr virus	Blood (5 mL, EDTA) (QN PCR)	Serum for MFI
Herpes simplex virus	Vesicle fluid (PCR), throat (PCR)	Serum for EIA/IF
Varicella-zoster virus	Vesicle fluid (PCR)	Serum for EIA/IF
Gastrointestinal:		
Rotavirus	Stool specimen (EIA)	No test
*Use culture transport swabs or submit fluid specimen		
**CSF, cerebrospinal fluid		
ELFA, enzyme-linked fluorescent immunoassay		
EIA, enzyme immunoassay		
IF, immunofluorescence		
MFI, multiplex flow immunoassay		
PCR, polymerase chain reaction		
QN PCR, quantitative PCR		