

Clinical Virology Laboratory
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During Winter Respiratory Season (December-March), extended hours are in effect. Call Laboratory for schedule.

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I. GUIDELINES FOR SPECIMEN COLLECTION

A. Specimen collection

NOTE: SPECIMENS THAT ARE NOT LABELLED WILL BE REJECTED

Collect specimens for culture, PCR or antigen early in illness when viral shedding is maximal.

Provide clinical information and/or virus suspected so that the laboratory can select the proper test systems.

If you have questions, call the laboratory and ask for the Laboratory Manager or Director.

B. Viral antibody studies

For immune status testing (past infection), a single serum sample is sufficient.

PLEASE NOTE: During acute infection, virus is present but antibody is often negative.

To detect acute infection, both acute and convalescent sera are generally required to detect an antibody rise or a seroconversion. Virus infections whose clinical symptoms are immune-mediated are exceptions and antibody is usually present (e.g. EBV, HBV, parvovirus B19).

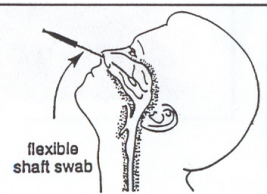
Reactivation of latent or persistent viruses may or may not be associated with rise in IgM and/or IgG.

C. Collection devices and holding temperature

Sample	Collection device*	Holding temperature	Comments
Virus isolation, PCR or antigen test:			
Swabs	Use viral transport medium Large swabs for NP, throat, lesion, etc. Small wire shaft swab for urethral samples	Refrigerate	Viral transport medium with swabs can be obtained from hospital storeroom.* (Stockroom #108145)
NP aspirate Body fluids, BAL, stool	Use sterile trap Use sterile leakproof containers	Refrigerate	Do not dilute body fluids or BAL in transport medium.
Tissues	Place in tubes containing liquid viral transport media to keep tissue moist	Refrigerate	Viral transport medium can be obtained from hospital storeroom.
Blood (plasma)	Collect 2 lavender top tubes <u>Collection time required</u>	Room temperature	Sample must be processed within 4-6 hrs of collection.
Viral antibody test:			
Blood (serum)	Collect 1 red top tube	Room temperature	

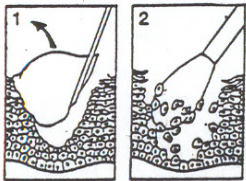
D. Specimen collection instructions for selected specimens

Nasopharynx swab Insert large swab into nasopharynx, just past point of resistance. Gently rub nasal turbinate or rotate to dislodge respiratory epithelial cells; remove and place in transport medium. For infants and small children NP aspirate preferred.



Nasopharynx aspirate Use suction pump connected to a catheter through a mucus trap; catheter should be French gauge 8 for infants, French gauge 12 for adults. Insert catheter as far into nose as possible. Specimen should be taken from posterior part of nasopharyngeal mucosa which is lined with respiratory epithelium, and not from anterior part which is lined with squamous epithelium. Collect as much of NP secretions as possible; do not dilute sample with saline unless necessary.

Throat swab Swab posterior pharyngeal wall, not buccal mucosa, tonsils, tongue or palate. Swab firmly and thoroughly. Throat swabs are suboptimal for DFA testing due to predominance of squamous instead of respiratory epithelial cells obtained.



Lesion swab Clean lesion with sterile saline soaked gauze pad. Unroof vesicles or remove crusts. Firmly swab base and margins of the lesion, obtaining fluid and cells. *After* sample collection, clean lesion thoroughly with betadine. If culture desired, do not use disinfectant prior to sample collection since virus may be inactivated.

Rectal swab Stool specimen required for enteric pathogens; swab of rectal mucosa can be done for proctitis.

II. VIROLOGY TEST SELECTION ORGANIZED BY VIRUS

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Adenovirus types 1-51	URI, pharyngitis, pneumonia, conjunctivitis, keratoconjunctivitis, hepatitis, hemorrhagic cystitis, gastroenteritis, intussusception, genital infections	Throat swab eye swab, urine, BAL, tissue, stool	Virus isolation or PCR	Virus isolation can detect the "unexpected". PCR is more sensitive and more rapid	1-14 days
		NP aspirate or swab; eye swab	Immunostain (DFA) or PCR	<i>DFA detects only 60% of culture positives</i>	2 hrs
	Disseminated (severely compromised host)	Plasma (lavender)	Quantitative PCR	Viral load in plasma can be monitored; absolute quantification varies for different serotypes	1-3 days
Enteric adenovirus types 40,41	Gastroenteritis	Stool	PCR		1-3 days
Arboviruses (EEE, WEE, St. Louis encephalitis, LaCrosse, Jamestown Canyon, West Nile, POW) <i>See also Dengue</i>	Encephalitis, aseptic meningitis, paralytic disease, febrile illness in summertime <i>Note: Different arboviruses are prevalent in other parts of the world. Travel history is key.</i>	CSF Serum (red top), acute and convalescent	IgM and IgG antibody in CSF and serum	WNV done in-house. <u>WNV IgM positives and all other arbovirus requests sent out.</u> <i>Early samples can be falsely negative. Cross-reactions can give false positives.</i>	1-4 days WNV; 7-10 days for others
		CSF, Brain biopsy	PCR, Virus isolation	Special arrangements may be possible; call laboratory.	<i>WNV IgM positives confirmed by State Lab</i>
BK virus (Polyomavirus)	Hemorrhagic cystitis in bone marrow transplants, ureteral stenosis post kidney transplant; tubulointerstitial nephritis; URI, tonsillitis	Urine Plasma, to monitor renal transplants	PCR, qualitative or quantitative PCR, quantitative	<u><i>Note: Mutations can lead to falsely low or negative results.</i></u> Plasma levels of >10,000 copies/ml associated with risk of nephropathy	1-3 days
Coronavirus SARS CoV	SARS CoV: fever, cough, diarrhea, dyspnea, pneumonia, ARDS	Deep respiratory samples, stool <i>AND</i>	RT-PCR Virus isolation (BSL3)	Sent to State Laboratory; <u>needs approval</u> ; epidemiologic risk factors must be present. For most recent CDC recommendations, see www.cdc.gov	Days to weeks
Human CoV OC43, 229E, NL63, HKU1	Common cold viruses; pneumonia	Paired sera collected early and at ≥ 28 days NP swab or aspirate	Antibody RT-PCR, Virus isolation	Note: <i>Acute and convalescent antibody most sensitive test</i> Not offered outside research setting at present	

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Cytomegalovirus (CMV) <i>See test interpretation on page 14.</i>	Fever, leukopenia, mononucleosis, hepatitis, pneumonia, oral, esophageal and gastrointestinal ulcerations, neurologic syndromes	BAL, tissue biopsies; urine, saliva	Virus isolation (both conventional and rapid centrifugation culture); PCR	Note: PCR on tissue or BAL can detect positives that are not clinically relevant.	1-21 days
		Plasma (lavender tube)	Quantitative PCR	<i>See p.14 for interpretive guidelines</i>	1-3 days
	Encephalitis Congenital CMV	Blood (red top), acute and convalescent	Antibody (IgM and/or IgG)	Use antibody tests to confirm primary infection or to determine immune status; <i>do NOT use to follow seropositive patients.</i>	1-3 days
		CSF (1-2 ml)	PCR	<i>Note: CMV in blood can contaminate CSF and give positive PCR</i>	1-3 days
		Urine collected at birth	Virus isolation	To diagnose <u>congenital</u> infection <i>urine must be obtained within 3 wks of birth. Positives after 3 wks can reflect perinatal infection.</i>	1-21 days
Dengue	Fever, myalgias, rash; hemorrhagic fever	Serum	Antibody Virus isolation	Serum sent to State Laboratory; virus isolation and PCR done at CDC	7-14 days
Enterovirus	Summer rashes, herpangina, hand-foot-mouth disease, myocarditis, pleurodynia	Throat swab, stool, skin vesicle swab, biopsy tissue	Virus isolation or PCR (Coxsackie A viruses may not grow in routine cell cultures.)	Collect <u>stool</u> , not rectal swab, for best results <i>Note: Diagnosis by antibody titer is not practical or reliable.</i>	1-14 days
	Aseptic meningitis, encephalitis, paralytic disease, rhomboencephalitis	CSF (1-2 mL)	RT-PCR (Virus isolation can be used as back-up)	Preferred test for CSF; however, parechovirus and cardiovirus are not detected. EV71 may be detected only in stool despite CNS disease.	1 -2 days
	Neonatal "sepsis"	Blood, CSF and urine	RT-PCR	Parechoviruses not detected. Need separate PCR.	1- 2 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Epstein-Barr virus (EBV)	Infectious mononucleosis (I.M.)	Blood (red top)	Heterophile antibody (monospot)	Positive in 90% of adults and <50% of children with I.M.	< 1 day
	Also hepatitis, pneumonitis, neurologic syndromes, hemolytic anemia, thrombocytopenia, hemophagocytic syndrome	Blood (red top)	EBV antibody panel: VCA IgG, VCA IgM, and EBNA antibodies	Request if heterophile antibody is negative or if unusual clinical presentation	1-3 days
		CSF (CNS lymphoma) plasma; tissue	PCR	Note: EBV in lymphocytes can give positive CSF PCR in absence of EBV-associated disease	1-3 days
	Lymphoproliferative disease (PTLD)	Blood-PBMC or tissue	Quantitative PCR for blood, in situ hybridization for tissue biopsy	Send to Molecular Diagnostics Lab, Dept. Lab Medicine	7 days
Nasopharyngeal carcinoma (NPC)	Blood (red top)	EBV antibody panel <u>and</u> VCA IgA antibody	EBV VCA IgA available by special request (sent out); Elevated VCA IgA antibody useful in early NPC detection and monitoring for recurrence	3-7 days	
Hantavirus Pulmonary Syndrome (HPS)	Pneumonia, ARDS in previously healthy individual	Blood (red top)	Antibody	Sent to State Health Dept; form, available in the virology laboratory, must be filled out.	3-7 days
		Biopsy tissues	Virus isolation and PCR	Sent to CDC via State Health Dept	Weeks to months
Hepatitis A	Acute hepatitis, relapsing hepatitis	Blood (red top)	Antibody (anti-HAV IgM)	Order only in cases of acute infectious hepatitis	1-3 days
	Immune status, for travelers to HAV endemic areas		Antibody (anti-HAV total)	Specify "immune status"	1-3 days
Hepatitis B <i>See test interpretation on page 16</i>	Acute hepatitis, chronic hepatitis, hepatocellular carcinoma, cirrhosis, polyarteritis nodosa	Blood (red top)	HBsAg, anti-HBc total and IgM, anti-HBs, HBeAg and anti-HBe	Can be ordered as single tests, as part of acute hepatitis, chronic hepatitis or hepatitis B virus panels	1-3 days
		Blood (red top)	PCR	Quantitation of HBV DNA should be done prior to therapy and to monitor response. <i>PCR may help clarify atypical serology results.</i>	1-7 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Hepatitis C <i>See test interpretation on pages 16, 17</i>	Acute hepatitis, chronic hepatitis, hepatocellular carcinoma, cirrhosis, essential mixed cryoglobulinemia, porphyria	Blood (red top)	Antibody (anti-HCV CLIA)	Can be ordered as a single test, as part of acute hepatitis or chronic hepatitis panels	1-3 days
			Antibody (anti-HCV RIBA)	HCV RIBA is reflexively performed only for samples with low-positive CLIA values. <u>If CLIA value is high, RIBA or PCR is done only if requested.</u>	2-7 days
			HCV RNA (RT-PCR)	HCV RNA test should be used to confirm high positive CLIA results instead of RIBA. Quantitation of HCV RNA should be done when therapy is initiated, and at recommended time points to monitor response	3-7 days
			HCV genotype (LiPA)	Used to guide therapy	1 week
Hepatitis D (Delta)	Acute hepatitis, chronic hepatitis, fulminant hepatitis, deterioration of chronic HBsAg carrier	Blood (red top)	Antibody (anti-HDV or anti-Delta)	Patient must be HBsAg positive to be infected with HDV.	7 days
			Delta antigen	Rarely detectable in serum. Tests sent out.	
Hepatitis E	Acute hepatitis, with pruritus, joint pain, cholestasis; 20% mortality in pregnant women	Blood (red top)	Antibody (anti-HEV IgM, IgG)	Test sent out.	1-2 weeks
Herpes simplex virus (HSV) types 1 and 2	Cold sore, gingivostomatitis, skin lesions, genital lesions, meningitis, esophagitis, proctitis, hepatitis, pneumonia	Lesion swab, biopsy tissue, mucosal swab, BAL	Virus isolation; PCR	PCR can be used for tissues, BAL, and asymptomatic shedding.	1-7 days
		Lesion swab, BAL, tissue (touch prep)	HSV/VZV DFA	Must have adequate cells for valid result; routine for all lesion swabs; if DFA positive, culture or PCR not done	2 hrs
	Encephalitis, recurrent meningitis; neonatal HSV; retinitis; hepatitis; disseminated HSV	CSF (1-2 mL); ocular fluid; swabs for neonatal HSV surveillance; blood	PCR	Viral load can be done in disseminated HSV or HSV hepatitis	1-2 days
		Serum	Type-specific IgG antibody	Immune status (carrier state)	1-3 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Human immunodeficiency virus (HIV) types 1 and 2 (3 rd generation) <i>If HIV-2 suspected, please notify laboratory</i>	No symptoms, mononucleosis, acute retrovirus syndrome, AIDS, failure to thrive To determine viral load and diagnose HIV infection in antibody-negative window. To guide antiretroviral therapy. Neonatal infection	Serum (red top)	Antibody (CLIA)	Note: HIV tests require notification of patient, but not signed consent. 3 rd generation test detects IgM.	1 day
		Plasma (lavender) allows faster result	Rapid HIV-1/2 antibody	<u>Call lab to facilitate rapid result</u>	30 min
		Whole blood (2 lavender top tubes)	HIV-1 Western blot	<i>All positives require Western blot confirmation. HIV-2 sent out.</i>	1-7 days
		Whole blood (2 lavender top)	Quantitative plasma RNA by RT-PCR (Roche TaqMan)		1-3 days
		Whole blood (2 lavender top)	Resistance genotype	Requires 400-1,000 HIV copies/mL	1-2 weeks
		Same as above	DNA PCR	HIV-1 DNA detected in PBMCs	2-14 days
Human herpesvirus type 6 (HHV-6A and B)	Roseola infantum, febrile seizures, infectious mono, hepatitis, pneumonitis, encephalitis in HSCT To confirm primary infection	CSF, plasma	PCR (quantify plasma)	Some patients have stable high levels of HHV-6 DNA in blood; due to universal infection by age 2, serology is rarely helpful	1-3 days
		Blood (red top)	Antibody, IgM and IgG	HHV-6 antibody tests sent out	1-2 wks
Human metapneumovirus	URI, pneumonia, bronchiolitis	NP aspirate, swab, BAL	HMPV DFA RT-PCR if hospitalized	Consider if respiratory screen DFA negative (peak late winter, early spring); submit separate sample	2 hrs 1-3 days
HTLV I/II	Tropical spastic paraparesis or HTLV associated myelopathy; human T cell leukemia/lymphoma	Blood (red top)	Antibody (EIA screen; all positives need confirmation by Western blot)	EIA does not distinguish HTLV-I from II; (sent out)	3-7 days
		Blood (2 lavender)	PCR on PBMCs	PCR useful if antibody tests are indeterminate (sent out)	7-14 days
Influenza A, B	Influenza syndrome, URI, bronchitis, bronchiolitis in infants, pneumonia, myopericarditis, myositis	Nasopharynx (NP) swab, wash, or aspirate, endotracheal aspirate, BAL	Respiratory screen DFA PCR if hospitalized DFA-negative	Sufficient respiratory epithelial cells required (NP >>> throat) Collect specimens <u>first 2-4 days of illness</u> ; <i>samples collected in first 24 hrs can be falsely negative</i>	2-14 hrs 1-3 days
H1N1 (swine)	Influenza syndrome	Same as above	Request Subtype by PCR for novel viruses	<i>Subtype also for drug resistance</i>	1-3 days
Avian influenza (H5N1)	Pneumonia, ARDS, diarrhea, neurologic disease	BAL, endotracheal aspirate, <u>throat swab</u> (not NP swab)	H5N1-specific RT-PCR	Notify lab; requires BSL3 safety precautions ; initial tests may be negative; repeat testing needed	4-48 hrs

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
JC virus (Polyomavirus)	Progressive multifocal leukoencephalopathy (PML)	CSF	PCR	Sensitive NIH assay used.	1-3 days
		Brain biopsy	Histopathology; EM to detect viral particles	Sent to Pathology	4-14 days
Measles	Coryza, conjunctivitis, rash, Koplik's spots; giant cell pneumonia or respiratory symptoms without rash in compromised hosts; encephalitis; atypical measles in previously immunized	Throat swab, NP swab, urine	Virus isolation	Samples must be collected early in disease; <i>requires special test-please notify the laboratory prior to sample collection</i>	2-14 days
		Blood (red top), acute and convalescent	Antibody, IgM and IgG	IgM sent out (acute infection)	1-7 days
Mumps	Parotitis, orchitis, meningitis, encephalitis	Saliva, CSF, urine	Virus isolation	Requires special test; <i>please notify the laboratory</i>	3-14 days
		Blood (red top), acute and convalescent	Antibody IgG and IgM	IgM sent out (acute infection)	1-7 days
Norovirus	Gastroenteritis, common source outbreaks (food, shellfish, contaminated water or ice)	Stool collected within 48 hrs of onset of symptoms	RT-PCR (detects genogroups I and II)	<i>Genetic variation in virus strains can lead to falsely negative results</i> ; samples should be collected early in illness for best results	1-3 days
Papillomavirus (over 70 types)	Warts, cervical dysplasia	Cervical swab or biopsy	PCR or Hybridization	Tests only available for genital HPV Sent to Yale Pathology Lab	7 days
Parainfluenza types 1-4	URI, croup, bronchitis, pneumonia	NP swab or aspirate, tracheal aspirate, BAL, lung tissue	Respiratory screen DFA PCR option for inpatients	DFA for types 1-3 only PCR can miss some DFA positives	2 hrs 1-3 days
			Virus isolation	Can detect type 4	3-14 days
Parvovirus B19	Erythema infectiosum (fifth disease), arthralgias, various exanthems and enanthems, aplastic crisis, chronic anemia in compromised hosts, nonimmune hydrops fetalis	Blood (red top)	Antibody, IgG and IgM	Immunocompromised hosts may not develop antibody	1-3 days
		Serum; bone marrow; amniotic fluid	PCR	PCR can be positive for months after infection; infection may be persistent, especially in compromised hosts	1-3 days

III. CLINICAL SYNDROMES: Most Commonly Associated Viruses, Specimens to Collect and Diagnostic Test of Choice

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Respiratory Pneumonia	Influenza A, B Adenovirus Respiratory syncytial virus Human metapneumovirus Parainfluenza Rhinovirus Cytomegalovirus Varicella-zoster Herpes simplex Hantavirus* SARS CoV*	NP aspirate or swab, BAL, lung tissue NP aspirate or swab, BAL, lung tissue NP aspirate or swab, BAL, lung tissue NP aspirate or swab, BAL, lung tissue NP aspirate or swab, BAL, lung tissue NP aspirate or swab, BAL, lung tissue BAL, lung tissue; blood BAL, lung tissue BAL, lung tissue Lung tissue, serum Deep respiratory sample, stool, paired sera	DFA, RT-PCR, Culture DFA, PCR, Culture DFA, RT-PCR, Culture DFA, RT-PCR DFA, RT-PCR, Culture RT-PCR, Culture Culture; PCR DFA; PCR; Culture DFA; PCR; Culture Serology, PCR, Culture Serology, RT-PCR, Culture
URI/pharyngitis	Rhinovirus Respiratory syncytial virus Adenovirus Parainfluenza Influenza A,B Enterovirus EB virus	NP aspirate or swab NP aspirate or swab NP aspirate or swab NP aspirate or swab NP aspirate or swab Throat and/or NP swab Serum	RT-PCR; Culture DFA, RT-PCR, Culture DFA, PCR, Culture DFA, PCR, Culture DFA; RT-PCR; Culture Culture; RT-PCR Serology
Pleurodynia	Enterovirus	TS, NP swab	Culture
Ocular Conjunctivitis/ keratitis/ retinitis	Enterovirus Adenovirus Herpes simplex virus Varicella-zoster virus Cytomegalovirus Vaccinia Measles	Conjunctival/ corneal swab, TS Conjunctival/ corneal swab, NP Conjunctival/ corneal swab; ocular fluid Conjunctival/ corneal swab, ocular fluid Ocular fluid Conjunctival/ corneal swab, lesion swab Conjunctival/ corneal swab, NP, serum	Culture, RT-PCR Culture, DFA; PCR Culture, DFA, PCR DFA; PCR PCR Culture Culture, DFA, serology
Infectious mononucleosis	EB virus Cytomegalovirus Adenovirus HIV HHV-6	Serum Blood, urine, saliva; serum NP swab, TS, urine Serum; blood Serum	Serology PCR, Culture; serology Culture, DFA; PCR Serology; PCR Serology; PCR
Cutaneous and mucous membrane Vesicular/ ulcerative	Herpes simplex virus Varicella-zoster virus Enterovirus Vaccinia Cytomegalovirus (ICH) Adenovirus (ICH)	Lesion swab Lesion swab TS, stool, lesion swab Lesion swab Lesion swab; blood Lesion swab; throat, stool	DFA, Culture DFA, Culture Culture; RT-PCR Culture Culture; PCR Culture, DFA; PCR

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Papillomas, papules	Papillomavirus Molluscum contagiosum	Biopsy Biopsy	Hybridization, EM EM <i>(Note: Clinical diagnosis usually sufficient)</i>
Exanthematous	Measles Rubella Enterovirus Parvovirus B19 Human herpesvirus type 6 Dengue, West Nile Epstein-Barr virus Adenovirus Cytomegalovirus	NP swab or TS, urine; serum Serum; NP swab or TS, urine, tissue TS, stool Serum Serum Serum Serum NP swab or TS, urine; stool Blood, urine, saliva	Culture; serology Serology; culture (special request) Culture; RT-PCR Serology; PCR Serology Serology Serology Culture, DFA; PCR PCR (blood), Culture; serology
Cardiovascular Myocarditis/ Pericarditis	Enterovirus Cytomegalovirus Influenza Adenovirus Rhinovirus group C	TS, stool, endocardial biopsy Blood, urine, endocardial biopsy NP swab, endocardial biopsy NP swab or TS, urine; stool NP swab or TS; pericardial fluid	RT-PCR; Culture PCR, Culture Culture, DFA; RT-PCR Culture, DFA; PCR PCR
Digestive tract Gastroenteritis Colitis Proctitis	Rotavirus Norovirus Adenovirus Parechovirus Enterovirus (not common) Cytomegalovirus Herpes simplex virus Papillomavirus	Stool Stool Stool Stool Stool GI biopsy, stool; blood Lesion swab, rectal swab Lesion biopsy	ELISA, PCR RT-PCR PCR Culture Culture; PCR Culture; RT-PCR Culture; DFA (lesion swab only) Histology; hybridization
Hepatitis	Hepatitis A Hepatitis B Hepatitis C Hepatitis D Hepatitis E EB virus Cytomegalovirus Adenovirus Herpes simplex virus	Serum Serum Serum Serum Serum Serum Liver tissue, blood Liver tissue Liver tissue	Serology Serology; PCR in special cases Serology; RT-PCR Serology Serology Serology; PCR PCR, Culture Culture; DFA; PCR PCR; DFA; Culture

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Hematologic Bone marrow suppression	EBV Cytomegalovirus Human herpesvirus type 6 Hepatitis A, B, C Parvovirus B19 Influenza Adenovirus HIV	Serum, bone marrow Blood, bone marrow Serum, bone marrow Serum Serum, bone marrow NP aspirate or swab Throat, stool, blood, bone marrow Serum; plasma	Serology, PCR PCR, culture PCR, serology Serology, PCR Serology, PCR DFA, culture Culture, DFA, PCR Serology, RT-PCR
Virus associated hemophagocytic syndrome	EBV Cytomegalovirus Varicella-zoster Herpes simplex Adenovirus Human herpesvirus type 6 Parvovirus B19	Serum, bone marrow Blood, bone marrow Skin lesions, bone marrow Skin lesions, bone marrow Throat, stool, bone marrow Serum, bone marrow Serum, bone marrow	Serology, PCR PCR, culture DFA, PCR, culture DFA, PCR, culture PCR, Culture, DFA PCR, serology Serology, PCR
Hemolytic anemia	EBV Cytomegalovirus Hepatitis B Measles Mumps Rubella	Serum, bone marrow Blood, bone marrow Serum Serum, throat and urine Serum, throat and urine Serum, throat and urine	Serology, PCR PCR, culture Serology Serology, culture Culture, serology Serology, culture
Atypical lymphocytes	EBV Cytomegalovirus Hepatitis A, B, C Measles Mumps Rubella Respiratory syncytial Parvovirus B19 HIV	Serum, bone marrow Blood, bone marrow Serum Serum, throat and urine Serum, throat and urine Serum, throat and urine NP aspirate or swab Serum Serum; plasma	Serology, PCR PCR, culture Serology Serology, culture Culture, serology Serology, culture DFA, culture Serology, PCR Serology, RT-PCR
Neutrophilia	Mumps Hepatitis B Viral hemorrhagic fevers**	Serum, throat and urine Serum Serum (biosafety precautions)**	Culture, serology Serology Serology, culture, EM (BSL 3 or 4)
Aplastic anemia	Hepatitis C	Serum, bone marrow	Serology, PCR
Pure red cell aplasia	Parvovirus B19 Hepatitis C	Serum, bone marrow Serum, bone marrow	Serology, PCR Serology, PCR

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Neurologic Encephalitis	Herpes simplex virus type 1>>2, except neonatal HSV type 2> 1 Cytomegalovirus Varicella-zoster EBV Arbovirus (EEE, WEE, SLE, West Nile, POW, etc)* Adenovirus Measles, Rubella Mumps Influenza Enterovirus Parechovirus HIV BKV HHV-6 Rabies* LCMV (transplant)	CSF; brain biopsy CSF, blood CSF, autopsy tissue CSF, lesion swab Serum, CSF CSF and serum CSF, TS, stool NP swab, urine, serum CSF, urine; serum NP swab or TS, CSF CSF, TS, stool (serum in neonates) CSF, TS, stool (serum in neonates) CSF; serum CSF; urine CSF Brain biopsy; Skin biopsy Saliva; serum, CSF Serum , CSF	PCR; DFA; culture PCR PCR, culture PCR, DFA Serology; PCR Serology; RT-PCR PCR, culture Serology; DFA, culture Culture; serology DFA, Culture; RT-PCR RT-PCR; Culture RT-PCR PCR; serology PCR; Culture PCR DFA, (for antigen); PCR Culture; serology Serology; RT-PCR
Meningitis	Enterovirus, parechovirus Herpes simplex virus type 2>> 1 Varicella-zoster EBV HIV (acute infection) Mumps WNV, Jamestown Canyon* Lymphocytic choriomeningitis virus (LCMV)	CSF, stool, TS (CSF, serum in neonates) CSF, lesion swab CSF, lesion swab Serum, CSF Plasma, CSF CSF, urine; serum CSF, Serum Serum, CSF	RT-PCR; Culture PCR; Culture; DFA if skin lesions PCR, DFA Serology; PCR RT-PCR, serology Culture; serology; PCR (sent out) Serology Serology
Progressive multifocal leukoencephalopathy	Polyomavirus (JC)	CSF; Brain tissue	PCR; histopathology; EM

Abbreviations:

Specimens: NP, nasopharyngeal swab or aspirate (provides results superior to TS for respiratory viruses); TS, throat swab;

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

Test Methods: ELISA, enzyme linked immunosorbent assay; DFA, direct fluorescence assay; EM, electron microscopy; PCR, polymerase chain reaction
 RT-PCR, reverse transcriptase polymerase chain reaction

Please Note:

Acute and convalescent serum should be collected for antibody studies. Serologic testing is not practical for enteroviruses, rhinoviruses, papillomaviruses. and polyomaviruses.

* Testing is done at the State Laboratory and/or CDC; call the Virology Laboratory for details and to fill out required forms

**Notify Health Department and CDC.

IV. INTERPRETATION OF TEST RESULTS

VIRUS ISOLATION

Please Note: Clinical information and/or virus suspected are needed to select proper culture systems.
EBV is diagnosed by serology, NOT culture.

Viruses isolated in routine cell cultures	Special request required	Interpretation of positive culture
Adenovirus, cytomegalovirus, enteroviruses, herpes simplex, influenza A and B, parainfluenza types 1-4, rhinoviruses, RSV, vaccinia, varicella-zoster virus	Arboviruses, BK virus, measles, mumps, rubella	Varies with virus, specimen source and clinical setting. For example, latent viruses can reactivate with or without symptoms (e.g. CMV, HSV, adenovirus). Isolation of other viruses occurs only with acute infection (e.g. measles, influenza).

CMV VIRAL LOAD by real-time TaqMan PCR

In June 2009, YNHH transitioned from antigenemia to PCR as the main viral load test. The relationship between CMV antigen-positive neutrophils and genome copies free in plasma is inconsistent. DNA copies/ml are MUCH higher. It is important that patients are not over-treated due to misinterpretation of results. Treatment varies with risk factors. Significant DNA copy numbers can vary from 1,000 for bone marrow/stem cell transplant, to 4,000 for D+/R-solid organ transplant, to 10,000 for low risk transplant or HIV+ patients. Rapidly rising titers may be more predictive of disease than a single point in time.

Greatly simplified correlation:

0-1 antigen positive cell = 100-200 DNA copies/ml plasma (very low)

10 antigen positive cells = 1,000-2,000 DNA copies/ml plasma (considered low, except in hematopoietic transplant patients)

50 antigen positive cells = 5,000-10,000 DNA copies/ml plasma (high)

VIRAL ANTIGEN

Virus	Sample	Test	Sensitivity vs. culture*	Interpretation of positive result
Influenza, novel H1N1**	NP aspirate or swab	DFA	~80%	Acute infection; sensitivity compared to PCR for children <5 yrs old is >92%; PCR most sensitive test; PCR subtype differentiates novel (swine) from seasonal H1N1
Influenza, seasonal **	NP aspirate or swab	DFA	~90%	Acute infection; detects types A and B; sensitivity compared to culture
RSV**	NP aspirate or swab	DFA	99%	Acute infection; can remain positive longer than culture
Adenovirus**	NP aspirate or swab; eye swab	DFA	60%	Acute infection; culture more sensitive for adenovirus
Paraflu 1-3**	NP aspirate or swab	DFA	>90%	Acute infection; parainfluenza type 4 not included
HMPV	NP aspirate or swab	DFA	>85%	Acute infection
Rotavirus	Stool	EIA	>99%	Acute infection; false positives reported in neonates; detects group A only
VZV	Skin lesions	DFA	95%	Active infection
HSV	Skin lesions Mucosal lesions	DFA	95% 80%	Active infection. DFA only 80% sensitive for mucosal lesions (mouth, vulva, eye) <i>Use culture to detect asymptomatic shedding.</i>

*Sensitivity compared with **culture**, or PCR for Novel H1N1 influenza and HMPV, of the same sample. Sensitivity compared to PCR will be lower. Specificity for DFA is >99% for all tests.

Note: If sample poorly collected or collected late in illness, results of all tests will be poor.

NP = nasopharynx DFA= direct fluorescent antibody (immunostain) EIA= enzyme immunoassay

**Order Respiratory Screen DFA (Influenza A and B, RSV, parainfluenza types 1-3 and adenovirus included). Note: HMPV DFA is separate test.

CLOSTRIDIUM DIFFICILE BACTERIAL GDH ANTIGEN SCREEN by ELISA; IF POSITIVE DO CYTOTOXIN

For Bacterial Antigen Positives:	Toxin detected	Result	Interpretation
Cytotoxicity in cell culture, followed by neutralization with specific antitoxin	Toxin B (cytotoxin)	Negative	Toxin not detected. False negatives can occur. Repeat test. If patient severely ill, continue therapy. Toxin present. Treat.
		Positive	

VIRAL ANTIBODY

Please note: Administration of blood products or immunoglobulin may result in passive transfer of antibody and transiently positive antibody test results. False negative antibody results may occur in immunocompromised hosts or agammaglobulinemic patients.

IMMUNE STATUS TESTING

Sensitivity and specificity of these assays ranges from 97-99% in various studies.

Virus	Method	Result	Interpretation
Cytomegalovirus, Herpes simplex, Measles, Rubella, Varicella-zoster	CLIA	Negative Positive Equivocal	-No antibody detected -Antibody present -Non-specific reaction or low level antibody. Submit second sample.

CLIA= chemiluminescence immunoassay

DIAGNOSIS OF ACUTE INFECTION

Virus	Method	IgG Result	IgM Result	Interpretation
Cytomegalovirus	CLIA	-	+	Primary infection (or false positive)
		+	+	Primary or reactivation infection (Note: CMV IgM rise can be due to EBV, and vice versa)
		+	-	Past infection
		-	-	No antibody detected
Parvovirus	EIA	+ or -	+	Acute infection
		+	-	Past infection
		-	-	No antibody detected
West Nile Virus	EIA	- or +	+	Acute infection (must be confirmed by State Lab); or false positive
		+	-	Past infection with flavivirus; cross reaction with CMV or enterovirus
		-	-	Uninfected or early in infection; positive IgM make take 8 days
		-	-	

PATTERNS OF EBV-SPECIFIC ANTIBODY RESULTS AT DIFFERENT STAGES OF INFECTION:

Antibody to EBV antigens:	Uninfected	Primary	Past	Reactivation ^b
Viral capsid antigen (VCA)-IgG	-	++	+ ^a	++
Viral capsid antigen (VCA)-IgM	-	+	-	+ or -
Epstein-Barr nuclear antigen (EBNA)	-	-	+	+

- a, High titers to EBV VCA IgG may persist for years after primary infection in healthy individuals.
 b, To link EBV serologic reactivation with clinical disease requires tissue EBV PCR or hybridization; high viral load by PCR in blood may also be helpful



HEPATITIS TEST RESULTS

Positive Result	Interpretation
HBsAg	Active hepatitis B infection. Detectable during incubation period, acute hepatitis, and chronic HBV infection. Patient is considered infectious. Persistence beyond 6 months indicates chronic infection.
Anti-HBs	Marker of recovery and immunity. Detectable 1-3 months after HBsAg disappears. Indicates previous hepatitis B, immunization with HBV vaccine, or passive antibody via hepatitis B immune globulin.
Anti-HBc (total antibody)	Detects both IgG and IgM. Indicates current or past hepatitis B infection. Present during the "window" period when HBsAg has disappeared, but anti-HBs is not yet detectable. May persist longer than anti-HBs and be the only marker for past HBV infection. Not associated with recovery or immunity. If anti-HBc is the only positive HBV test, it may indicate past infection, non-specific result, or low-level chronic HBV infection.
Anti-HBc IgM	Consistent with recent hepatitis B infection. Antibody usually persists 4-6 months after acute stage. Occasionally present in chronic active hepatitis. Test done routinely on all samples positive for anti-HBc but negative for anti-HBs.
HBeAg	Serum contains HBV e antigen. This suggests that the patient is highly infectious. Persistence beyond 10 weeks suggests chronic liver disease.
Anti-HBe	Anti-HBe appears prior to loss of HBsAg and signals reduced level of infectious virus. Suggests early convalescence or past infection with HBV, but may also be seen in HBsAg carrier state.
Anti-Delta	Delta is a defective virus causing hepatitis only in association with HBV. Delta can be acquired simultaneously with HBV (coinfection) or as a superinfection in HBV carriers. Patients with delta virus infection have anti-delta antibody in their serum.
Anti-HAV total	Detects both IgG and IgM. Positive result is consistent with current or past hepatitis A, immunization or passive antibody from immune globulin. Patients with anti-HAV are usually immune to further HAV infection and are not infectious.
Anti-HAV IgM	Positive test indicates recent infection with HAV. IgM anti-HAV persists for about 4-6 months after acute infection. Low positive results can be non-specific. Test should only be ordered in cases of acute infectious hepatitis.
Anti-HCV	Indicates infection with hepatitis C. Negative results do not exclude infection with HCV, since antibody levels may be below assay detection limits. Detection or quantitation of HCV RNA in serum can be helpful in assessing disease activity.

COMMON HEPATITIS B SEROLOGY PATTERNS

	HBsAg	Anti-HBc IgM	Total anti-HBc	Anti-HBs
No evidence of HBV infection	-	-	-	-
Acute HBV infection	+	+	+	-
Chronic HBV (if HBsAg+ for > 6 months)	+	-	+	-
"Window" period during acute HBV	-	+	+	-
Previous HBV infection	-	-	+	+
HBV vaccine response	-	-	-	+

Note: HBV DNA PCR may aid in clarifying atypical serologic patterns.

HEPATITIS C VIRUS (HCV) ANTIBODY TEST INTERPRETATION

CLIA	RIBA-3	Interpretation
-	N.D.	A negative CLIA result does not absolutely exclude HCV infection. Antibodies are not detectable for 6-7 weeks after initial infection or may not develop in compromised hosts. In high risk individuals, repeat antibody testing in 2 months and/or HCV RNA PCR should be considered.
Strong positive	N.D.	This sample was strongly positive for HCV antibodies by CLIA and was not tested by RIBA. Over 95% of samples strongly positive by CLIA are RIBA positive. <u>However, if this patient has no evidence of hepatitis and does not have risk factors for HCV, this could be a false positive result. If you would like a RIBA or RT-PCR performed on this sample, please notify the laboratory.</u> PCR is recommended in high risk patients. RIBA is recommended in low risk patients.
Low positive	-	A negative RIBA does not exclude the possibility of HCV infection. This result could represent a false positive CLIA or an early seroconversion. Recommend repeat antibody testing in 2-6 months and/or HCV RNA PCR.
Low positive	Indeterminate	This sample is indeterminate for HCV antibodies. This result can be a nonspecific reaction or can indicate early seroconversion. The patient should be followed for at least 6-12 months for increased RIBA reactivity and/or tested by PCR for HCV RNA.
Low positive	+	Positive for HCV antibodies and indicative of past or present infection with HCV. If this result is not compatible with the patient's clinical picture, repeat antibody testing and/or HCV RNA PCR should be considered.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY TEST INTERPRETATION

CLIA	W. blot	Interpretation
-	N.D.	This specimen is antibody negative. A negative test does not exclude the possibility of infection with HIV. Negative results may be seen early during the course of infection, in advanced AIDS and in agammaglobulinemic patients.
+	-	This result can be indicative of a nonspecific reaction, but can also be associated with early HIV infection, passive transfer of antibodies, immunosuppression or infection with other human retroviruses. Another sample should be submitted in 2-6 weeks. Please notify the laboratory if patient has risk factors for HIV-2 or HTLV-I/II.
+	Indeterminate	This result can be indicative of a nonspecific reaction, but can also be associated with early HIV infection, passive transfer of antibodies, immunosuppression or infection with other human retroviruses. Another sample should be submitted in 2-6 weeks. Please notify the laboratory if patient has risk factors for HIV-2 or HTLV-I/II. If this patient has had serial samples with indeterminate W. blot results, direct detection of HIV-1 by PCR may be useful.
+	+	This specimen is HIV-1 antibody positive. This is indicative of HIV-1 infection. Another specimen should be submitted for repeat testing if clinical findings in this patient are contrary to these laboratory results. Repeat testing should also be considered if this is the first positive result in this patient.

V. SERVICES OFFERED AT THE VIROLOGY REFERENCE LABORATORY, VA-CT

Virology Reference Laboratory, VA Connecticut Health System

Acting Director: Sheldon Campbell, M.D., Ph.D, 932-5711, ext 2908

Hours of Operation: 7:30 a.m. to 4:30 p.m. Mon-Fri

Location: Building 5, 2nd floor, Room C-202

Lab Phone numbers: 937-3441 (outside direct dial); or 932-5711, ext. 3379, 3380

SERVICES OFFERED

Conventional and rapid culture assays

- Comprehensive viral cultures for the detection of adenovirus, cytomegalovirus (CMV), enterovirus, herpes simplex virus (HSV), influenza viruses (order PCR in season), measles, mumps, parainfluenza viruses, respiratory syncytial virus (RSV), rhinovirus, and varicella-zoster virus (VZV).
- Respiratory viral culture (for adenovirus, influenza A and B, parainfluenza viruses 1, 2, and 3, and RSV)

Viral serology

- HIV-1/2
- Hepatitis viruses (A, B, C)

Molecular assays

CMV PCR, quantitative
 Influenza/RSV PCR
 Human metapneumovirus PCR
 HIV-1 RNA RT-PCR
 HIV-1 antiviral resistance genotyping
 Herpesvirus multiplex PCR for HSV, VZV, EBV, CMV and HHV-6 in CSF
 HSV-1, HSV-2 and VZV Real-time PCR for detection in lesions
 HCV RNA RT-PCR (qualitative and quantitative)
 HCV genotyping
 PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoea*

