Clinical Virology Laboratory
Department of Laboratory Medicine, Yale New Haven Hospital

Director: Marie L. Landry, M.D. (office 203-688-3475) Laboratory Manager: David Ferguson, M.T., A.S.C.P. (beeper 412-0981)
Location: 55 Park St. Clinical Laboratory Building 6th floor Lab phone number: 688-3524
Standard Hours of Operation: Mon-Fri 7:00 a.m.-to 12:30 a.m.; Sat and Sun 8:00 a.m.-4:30 p.m.
During Winter Respiratory Season (December-March), extended hours are in effect. Call Laboratory for schedule.

TABLE OF CONTENTS **PAGES**

I. GUIDELINES FOR SPECIMEN COLLECTION **1-2**
- A. Test ordering
- B. Viral antibody studies
- C. Specimen collection for viral culture: labeling, timing, collection devices, and holding temperature
- D. Specimen collection instructions for selected specimens

II. VIROLOGY TEST SELECTION ORGANIZED BY VIRUS (alphabetical listing): **3-9**
Virus suspected, Clinical Syndromes, Test Selection and Special Instructions and Time to Result

III. CLINICAL SYNDROMES: Viruses Associated, Specimens to Collect and Test Method of Choice **10-13**

IV. INTERPRETATION OF TEST RESULTS **14-17**

V. SERVICES OFFERED AT THE VIROLOGY REFERENCE LABORATORY, VA-CT **18**

********** ********** **********

I. GUIDELINES FOR SPECIMEN COLLECTION

A. Specimen collection

NOTE: SPECIMENS THAT ARE NOT PROPERLY LABELLED WILL BE REJECTED
Collect specimens for antigen, PCR or culture early in illness when viral shedding is maximal.
If you have questions, about tests to order, 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 for IgG 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 required to confirm an antibody rise or a seroconversion. Virus infections whose clinical symptoms are immune-mediated are exceptions and antibody is usually present at onset of clinical symptoms (e.g. EBV, HBV, parvovirus B19). *Note: False positive IgM results commonly mislead clinicians*. A seroconversion of IgG is more reliable.

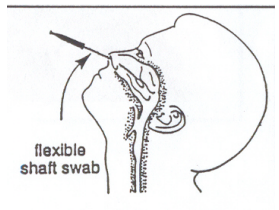
Reactivation of latent or persistent viruses may or may not be associated with rise in IgM and/or IgG.

C. Collection devices and holding temperature

Sample	Collection device*	Holding temperature	Comments
Virus isolation, PCR or antigen test:			
Swabs	Use viral transport medium	Refrigerate	Viral transport medium with swabs can be obtained from hospital storeroom.
NP aspirate	Use sterile trap	Refrigerate	
Body fluids, BAL, stool	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 Collection time required	Room temperature	Sample must be processed within 4-6 hrs of collection.
Viral antibody test:			
Blood (serum)	Collect 1 red top tube	Room temperature	

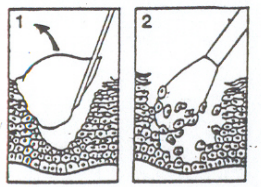
D. Specimen collection instructions for selected specimens

Nasopharynx swab — Insert swab deep into nasopharynx, past point of resistance. Gently rotate to dislodge respiratory epithelial cells, and rub nasal turbinate; remove and place in transport medium. For infants and small children NP aspirate often 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 (not swab) is 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, eye swabs, urine, BAL, tissue, stool NP swab or aspirate	PCR (included in Respiratory Virus PCR Panel), or virus isolation. PCR or DFA	PCR is more sensitive and more rapid. Virus isolation can detect the "unexpected". *DFA detects only 60% of culture positives and 40% of PCR positives*	1-2 d (PCR) 1-14 days (culture) 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 adeno-virus types 40,41	Gastroenteritis	Stool	PCR		1-3 days
Arboviruses causing neurologic disease: EEE, WEE, St. Louis encepha-litis, LaCrosse, Jamestown Canyon, West Nile, POW) *See also Dengue, CHIK*	Encephalitis, aseptic meningitis, paralytic disease, febrile illness in summertime *Note: Different arboviruses are prevalent in other parts of the world. Travel history is key.*	CSF Serum (red top), acute and convalescent CSF*	IgM and IgG antibody in CSF and serum PCR*, Virus isolation* *Order PCR if patient cannot make antibody*	WNV done in-house. <u>All other arbovirus requests sent out</u>. *Early samples can be falsely negative. Cross-reactions often give false positives*. *Special arrangements with CDC may be necessary; call laboratory. Only WNV and dengue PCRs available commercially.	1-4 days WNV; 7-10 days for others
BK virus (Polyomavirus)	Tubulointerstitial nephritis Hemorrhagic cystitis in bone marrow transplants, ureteral stenosis post kidney transplant	Plasma, to monitor renal transplants Urine	PCR, quantitative PCR, quantitative	Plasma levels of >10,000 copies/ml associated with risk of nephropathy *<u>Note: Mutations can lead to falsely low or negative results</u>.*	1-3 days
Coronavirus OC43, 229E, NL63, HKU1 MERS-CoV	Common cold viruses; pneumonia Pneumonia, ARDS after travel to Arabian Peninsula	NP swab or aspirate Lower respiratory samples, sputum, NP/OP swabs, paired sera	RT-PCR RT-PCR and serology	Not currently offered at YNHH; Testing at State Lab or CDC. Needs approval. Contact Hospital Epi. <u>Notify lab, then hand carry to lab. Special Pathogen protocol.</u>	Not available <1 day
Chikungunya	Fever, myalgia, arthralgia, rash, edema	Serum	Antibody >day 5 of illness PCR for <u>≤</u>5 days of illness	Serum sent out to ARUP	7 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Cytomegalovirus (CMV) *Roche assay implemented July 2015.*	Fever, leukopenia, mononucleosis, hepatitis, pneumonia, oral, esophageal and gastrointestinal ulcerations, neurologic syndromes	BAL, tissue biopsies; urine, saliva	PCR, Virus isolation	Note: PCR on tissue or BAL can detect positives that are not clinically relevant.	1-21 days
		Plasma (lavender tube)	Quantitative PCR	*Roche provides no interpretive guidelines.*	1-2 days
		Blood (red top), acute and convalescent	Antibody (IgM and/or IgG)	Use antibody tests to confirm primary infection or to determine immune status; *do NOT use to follow seropositive patients.*	1-2 days
	Encephalitis	CSF (1 ml)	PCR	*Note: CMV in blood can contam-inate CSF and give positive PCR*	1-2 days
	Congenital CMV	Saliva or urine collected at birth	PCR, CMV rapid culture	To diagnose <u>congenital</u> infection *samples must be obtained within 2-3 wks of birth. Positives after 3 wks can reflect perinatal infection.*	1-7 days
Dengue	Fever, myalgias, rash; hemorrhagic fever	Serum	Antibody >day 5 of illness PCR for ≤5 days of illness	Serum sent out to ARUP	7 days
Ebola	Fever, myalgia, prostration, diarrhea, vomiting, ARDS, multiorgan failure, hemorrhage	Blood	PCR	See department and YNHH Viral Hemorrhagic Fever Protocol for details. **Special Pathogen alert.**	1-2 days
Enterovirus	Summer rashes, herpangina, hand-foot-mouth disease, myocarditis, pleurodynia	Throat swab, stool, skin vesicle swab, biopsy tissue	PCR preferred; virus isolation (Note: Coxsackie A viruses may not grow in routine cell cultures.)	Collect <u>stool</u>, not rectal swab, for best results *Note: Diagnosis by antibody titer is not practical or reliable.*	1-14 days
	Aseptic meningitis, encephalitis, paralytic disease, rhomboencephalitis	CSF (1 mL)	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. Order 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	1-2 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)	Plasma (lavender tube) or tissue	Quantitative PCR	*Tissue in situ hybridization done by Molecular Diagnostics Lab*	1-3 days
	Nasopharyngeal carcinoma (NPC)	Blood (red top)	EBV antibody panel and VCA IgA antibody	EBV VCA IgA available 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 day
	Immune status, for travelers to HAV endemic areas		Antibody (anti-HAV IgG)	Specify "immune status"	1 day
Hepatitis B *See test interpretation on page 16*	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 day
		Blood (red top)	PCR	Quantitation of HBV DNA should be done prior to therapy and to monitor response. *PCR may help clarify atypical serology results.*	1-4 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Hepatitis C *See test interpretation on pages 16, 17*	Acute hepatitis, chronic hepatitis, hepatocellular carcinoma, cirrhosis, essential mixed cryoglobulinemia, porphyria	Blood (red top)	Antibody (anti-HCV CLIA) anti-HCV RIBA no longer available HCV RNA (RT-PCR) HCV genotype (LiPA)	Can be ordered as a single test, as part of acute hepatitis or chronic hepatitis panels HCV RNA test can be used to confirm CLIA results instead of RIBA. Quantitation of HCV RNA should be done when therapy is initiated, and to monitor response Used to guide therapy	1 day 1-3 days 2-7 days
Hepatitis D (Delta)	Acute hepatitis, chronic hepatitis, fulminant hepatitis, deterioration of chronic HBsAg carrier	Blood (red top)	Antibody (anti-HDV or anti-Delta) Delta antigen	Patient must be HBsAg positive to be infected with HDV. Sent out. Used to follow antibody positive patients.	7 days
Hepatitis E	Acute hepatitis, cholestasis; 20% mortality in pregnant women; acute and chronic infection post-transplant, and in compromised hosts	Blood (red top) Serum, stool	Antibody (anti-HEV IgM, IgG) RT-PCR for definitive diagnosis	Tests on travelers sent to ARUP. Tests on transplant patients sent to CDC.	1-4 weeks
Herpes simplex virus (HSV) types 1 and 2	Cold sore, gingivostomatitis, skin lesions, genital lesions, meningitis, esophagitis, proctitis, hepatitis, pneumonia Encephalitis, recurrent meningitis; neonatal HSV; retinitis; hepatitis; disseminated HSV	Lesion swab, biopsy tissue, mucosal swab, BAL Lesion swab CSF (1 mL); ocular fluid; swabs for neonatal HSV surveillance; blood Serum	PCR, Virus isolation HSV PCR, Direct PCR Type-specific IgG antibody	PCR most sensitive. *DFA discontinued July 2015.* Viral load can be done in disseminated HSV or HSV hepatitis Immune status (carrier state); *IgM is not useful*	1-7 days 1 day <1 day 1-2 days 1-2 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Human immuno-deficiency virus (HIV) type 1 & 2 (4th generation: IgM, IgG, p24 Ag)	No symptoms, mononucleosis, acute retrovirus syndrome, AIDS, failure to thrive	Serum (red top)	Antibody (CLIA)	Note: HIV tests require notification of patient, but not signed consent. 4th generation test detects IgM and p24 antigen.	1 day
		Plasma (lavender) allows faster result	Rapid HIV-1/2 antibody	Call lab to facilitate rapid result	30 min
	To determine viral load and diagnose HIV infection in antibody-negative window.	Serum or plasma	HIV-1,-2 multispot	All positives require Multispot antibody differentiation. If multisport negative, do PCR on separately collected sample to rule out acute HIV infection.	1 day
		Whole blood (2 lavender top tubes)	Quantitative plasma RNA by RT-PCR (Roche TaqMan)		1-3 days
	To guide antiretroviral therapy.	Whole blood (2 lavender top)	Resistance genotype	Requires 400-1,000 copies/mL	1-2 weeks
	Neonatal infection	Same as above	RNA PCR on plasma	DNA PCR (provirus) discontinued	2-7 days
Human herpesvirus type 6 (HHV-6A and B)	Roseola infantum, febrile seizures, infectious mono, hepatitis, pneumonitis, encephalitis in HSCT	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
	To confirm primary infection	Blood (red top)	Antibody, IgM and IgG	HHV-6 antibody tests rarely useful	1-2 wks
Human meta-pneumovirus	URI, pneumonia, brnochiolitis	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 confirma-tion 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, myoperi-carditis, myositis	Nasopharynx (NP) swab, wash, or aspirate, endotrach-eal aspirate, BAL	Respiratory screen DFA PCR if hospitalized (in Resp Virus PCR Panel) Request Subtype by PCR for novel viruses	Sufficient respiratory epithelial cells required (NP >>> throat) Collect specimens first 2-4 days of illness; *samples collected in first 24 hrs can be falsely negative*	2-14 hrs 1-2 days
Avian influenza (A/ H5N1, H7N9, etc)	Pneumonia, ARDS, diarrhea, neurologic disease	BAL, endotracheal aspirate, sputum (not NP swab)	Not subtypable by seasonal virus 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 mulitfocal leukoencephalopathy (PML)	CSF Brain biopsy	PCR Histopathology; EM to detect viral particles	Sensitive NIH assay used. Done by Pathology	1-3 days 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	NP swab, urine Blood (red top), acute and convalescent	PCR, Virus isolation Antibody, IgM and IgG	Collect early in disease; *notify Hospital Epidemiology and the laboratory prior to sample collection; requires approval from State Epi and CDC;* ***sent to CDC*** IgM sent out (acute infection); beware false positive IgM!	2-7 days 1-4 days
Mumps	Parotitis, orchitis, meningitis, encephalitis	Saliva, CSF, urine Blood (red top), acute and convalescent	PCR, Virus isolation Antibody IgG and IgM	Requires special test; *please notify the laboratory* IgM sent to CDC (acute infection)	3-14 days 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)	*Genetic variation in virus strains can lead to falsely negative results*; samples should be collected early in illness for best results	1-3 days
Papillomavirus (over 150 types)	Warts, cervical dysplasia	Cervical swab or biopsy	PCR	Tests only available for genital HPV Sent to Yale Pathology Lab	2-7 days
Parainfluenza types 1-4	URI, croup, bronchitis, penumonia	NP swab or aspirate, tracheal aspirate, BAL, lung tissue	Respiratory screen DFA PCR for inpatients (in Resp Virus PCR Panel) Virus isolation	DFA for types 1-3 only PCR for 1-3 only Can detect type 4	2 hrs 1-2 days 3-14 days
Parechovirus types 1-16	Similar to enterovirus; especially neonatal sepsis	NP, blood, CSF, stool, urine	RT-PCR	Order Parechovirus RT-PCR; not detected by enterovirus PCR	1-2 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) Serum; bone marrow; amniotic fluid	Antibody, IgG and IgM PCR	Immunocompromised hosts may not develop antibody PCR can be positive for months after infection; infection may be persistent, especially in compromised hosts	1-3 days 1-3 days

Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Rabies	Clinical rabies, ascending paralysis, rapidly progressive encephalitis *Note: In U.S., half of rabies cases report no history of animal bite. Bats are main source in U.S. cases.*	Brain biopsy, skin biopsy from nape of neck (to include hair follicles), corneal scrapings ; saliva Blood (red top) and CSF	Rabies antigen (immunostain of tissue) RT-PCR, culture Antibody	Sent to CDC Rabies Lab (www.cdc.gov/ncidod/dvrd/rabies/Professional/professi.htm) Note: Serum test invalid if rabies immune globulin has been given	2-14 days 7-14 days
Respiratory syncytial virus	Bronchiolitis, pneumonia, URI	NP aspirate or NP swab, BAL, lung tissue	Respiratory screen DFA RT-PCR if hospitalized (in Resp Virus PCR Panel)	Need adequate respiratory epithelial cells or test invalid; NP aspirate gives best results *RT-PCR and DFA >> RSV culture*	2 hrs 1-3 days 3-14 days
Rhinovirus	URI; lower tract disease in infants, asthma, COPD, ICH	NP swab or aspirate; BAL	RT-PCR if hospitalized (in Resp Virus PCR Panel)	RT-PCR much more sensitive than culture; group C does not grow in culture	1-2 days 3-14 days
Rotavirus groups A, B	Infantile gastroenteritis (group A); diarrhea in adults (group B)	Stool	Rotavirus antigen (ELISA)	ELISA detects only group A and may yield false positive results in neonates	4 hrs
Rubella	Rubelliform rash, post-auricular adenopathy, arthralgias, congenital rubella syndrome	Blood (red top), acute and convalescent Tissue, throat swab and urine	Antibody Virus isolation	IgG done in-house, IgM sent out if rubella high risk- *notify laboratory* Special request; *please notify laboratory*	1-7 days 3-14 days
Varicella-zoster virus (VZV)	Chicken pox, herpes zoster, pneumonia, neurologic syndromes, retinal necrosis Pneumonia Meningitis, encephalitis, uveitis Immune status CNS vasculopathy	Skin lesion swabs BAL, tissues CSF (1 ml); ocular fluid Blood (red top) CSF	VZV Direct PCR PCR PCR Antibody, IgG	Need vigorous swab of lesion to dislodge cells; PCR most sensitive *DFA discontinued July 2015.* *Note: VZV PCR of CSF can be positive in uncomplicated zoster* Collect serum promptly after exposure to determine immune status; *IgM is not useful* IgG useful if tested delayed and PCR now negative	<1 day 1-2 days 1-2 days
West Nile virus (WNV)				**See Arboviruses**	

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

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Papillomas, papules	Papillomavirus Molluscum contagiosum	Biopsy Biopsy	Contact Pathology Dept for options *(Note: Clinical diagnosis usually sufficient)*
Exanthematous	Measles Rubella Enterovirus Parvovirus B19 Human herpesvirus type 6 Dengue West Nile Epstein-Barr virus Adenovirus Cytomegalovirus	NP swab or TS, serum Serum; NP swab or TS, urine, tissue TS, stool Serum Serum Serum Serum, CSF Serum NP swab or TS, urine; stool Blood, urine, saliva	PCR; rapid culture; serology PCR, Serology RT-PCR Serology; PCR PCR; Serology Serology; PCR Serology; PCR 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	 Stool Stool Stool Stool Stool GI biopsy, blood Lesion swab, rectal swab	 ELISA, PCR RT-PCR PCR RT-PCR PCR PCR PCR, culture
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, blood	Serology Serology; PCR Serology; RT-PCR Serology, Antigen Serology, PCR Serology; PCR PCR, Culture PCR, Culture PCR; Culture *Note: PCR blood very high viral load in HSV hepatitis; allows rapid diagnosis*

Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Hematologic			
Bone marrow suppression	EBV	Serum, bone marrow	Serology, PCR
	Cytomegalovirus	Blood, bone marrow	PCR
	Human herpesvirus type 6	Serum, bone marrow	PCR, serology
	Hepatitis A, B, C	Serum	Serology, PCR
	Parvovirus B19	Serum, bone marrow	Serology, PCR
	Influenza	NP aspirate or swab	DFA, RT-PCR, culture
	Adenovirus	Throat, stool, blood, bone marrow	PCR, DFA, culture
	HIV	Serum; plasma	Serology, RT-PCR
Virus associated hemophagocytic syndrome	EBV	Serum, bone marrow	Serology, PCR
	Cytomegalovirus	Blood, bone marrow	PCR, culture
	Varicella-zoster	Skin lesions, bone marrow	PCR, culture
	Herpes simplex	Skin lesions, bone marrow	PCR, culture
	Adenovirus	Throat, stool, bone marrow	PCR, Culture, DFA
	Human herpesvirus type 6	Serum, bone marrow	PCR, serology
	Parvovirus B19	Serum, bone marrow	Serology, PCR
Hemolytic anemia	EBV	Serum, bone marrow	Serology, PCR
	Cytomegalovirus	Blood, bone marrow	PCR, culture
	Hepatitis B	Serum	Serology
	Measles	Serum, throat and urine	RT-PCR, Serology, culture
	Mumps	Serum, throat and urine	RT-PCR, serology, Culture
	Rubella	Serum, throat and urine	RT-PCR, Serology, culture
Atypical lymphocytes	EBV	Serum, bone marrow	Serology, PCR
	Cytomegalovirus	Blood, bone marrow	PCR, culture
	Hepatitis A, B, C	Serum	Serology
	Measles	Serum, throat and urine	RT-PCR, Serology, culture
	Mumps	Serum, throat and urine	RT-PCR, serology, Culture
	Rubella	Serum, throat and urine	RT-PCR, Serology, culture
	Respiratory syncytial	NP aspirate or swab	RT-PCR, DFA, culture
	Parvovirus B19	Serum	Serology, PCR
	HIV	Serum; plasma	Serology, RT-PCR
Neutrophilia	Mumps	Serum, throat and urine	RT-PCR, Serology, culture
	Hepatitis B	Serum	Serology
	Viral hemorrhagic fevers**	Serum (biosafety precautions)**	RT-PCR, Serology (BSL 3 or 4)
Aplastic anemia	Hepatitis C	Serum, bone marrow	Serology, PCR
Pure red cell aplasia	Parvovirus B19	Serum, bone marrow	Serology, PCR
	Hepatitis C	Serum, bone marrow	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	CSF; brain biopsy CSF, blood	PCR; culture PCR
	Cytomegalovirus	CSF, autopsy tissue	PCR, culture
	Varicella-zoster	CSF, lesion swab	PCR
	EBV	Serum, CSF	Serology; PCR
	Arbovirus (EEE, WEE, SLE, West Nile, POW, etc)*	CSF and serum	Serology; RT-PCR
	Adenovirus	CSF, TS, stool	PCR, culture
	Measles, Rubella	NP swab, urine, serum	RT-PCR, Serology; culture
	Mumps	CSF, urine; serum	RT-PCR, Serology; culture
	Influenza	NP swab or TS, CSF	RT-PCR, DFA, Culture
	Enterovirus	CSF, TS, stool (serum in neonates)	RT-PCR
	Parechovirus	CSF, TS, stool (serum in neonates)	RT-PCR
	HIV	CSF; serum	PCR; serology
	BKV	CSF; urine	PCR
	HHV-6	CSF	PCR
	Rabies*	Brain biopsy; Skin biopsy Saliva; serum, CSF	DFA, (for antigen); PCR Culture; serology
	LCMV (transplant)	Serum , CSF	Serology; RT-PCR
Meningitis	Enterovirus, parechovirus	CSF, stool, TS (CSF, serum in neonates)	RT-PCR
	Herpes simplex virus type 2>> 1	CSF, lesion swab	PCR; Culture
	Varicella-zoster	CSF, lesion swab	PCR
	EBV	Serum, CSF	Serology; PCR
	HIV (acute infection)	Plasma, CSF	RT-PCR, serology
	Mumps	CSF, urine; serum	RT-PCR, serology; culture
	WNV, Jamestown Canyon*	CSF, Serum	Serology
	Lymphocytic choriomeningitis virus (LCMV)	Serum, CSF	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 microsopy; 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. Cultures of CSF and stool discontinued July 2015; PCR preferred test.

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).

NOTE: Respiratory Virus PCR Panel is most sensitive option. Use for hospitalized patients when sensitivity is key. When a faster result is needed, DFA can be ordered, but results are less sensitive. Comparative performance of antigen tests vs culture and PCR is given below.

VIRAL ANTIGEN

Virus	Sample	Test	Sensitivity* vs. Culture	Sensitivity* vs. PCR	Interpretation of positive result
Influenza, **	NP aspirate or swab	DFA	85-90%	55-85%	Acute infection; detects types A and B; sensitivity compared to culture
RSV**	NP aspirate or swab	DFA	99%	55-80%	Acute infection; can remain positive longer than culture
Adenovirus**	NP aspirate or swab; eye swab	DFA	60%	25%	Acute infection; culture more sensitive for adenovirus
Parainfluenza types 1-3**	NP aspirate or swab	DFA	>90%	60-70%	Acute infection; parainfluenza type 4 not included
HMPV	NP aspirate or swab	DFA	>85%	55-75%	Acute infection
Rotavirus	Stool	EIA	>99%	NA	Acute infection; false positives reported in neonates; detects group A only

*Sensitivity compared with culture or PCR of the same sample. *Results better in young children than adults*. Specificity for DFA and PCR 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 TEST ALGORITHM

1. Perform Rapid GDH Ag & toxin EIA using C.diff QuikCheck Complete (4 times a day; 30 min test TAT)
2. Both negative: **Final Report - *C. difficile* not detected**
3. Both positive: **Final Report - Positive toxin in a patient with diarrhea is indication for therapy**
4. GDH Ag positive, rapid toxin negative, do cytotoxin neutralization in cell culture (4-48 hr TAT)
5. If GDH positive and cytotoxin negative; **Final Report interpretation is colonization**
6. If GDH positive and cytotoxin positive: **Final Report- A positive cytotoxin n a patient with diarrhea is an indication for therapy.**

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

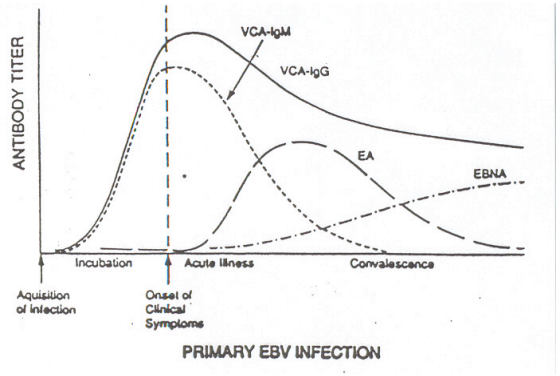
DIAGNOSIS OF ACUTE INFECTION

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 hybrdization; 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 IgG	Positive result is consistent with current or past hepatitis A, immunization or passive antibody from immune globulin. Patients with anti-HAV IgG 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 **Note: RIBA currently not available in US. Use PCR for confirmation.**

CLIA	RNA	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.
+ High	N.D.	A high level of antibody was detected in this specimen. If this patient is not known to have HCV infection, a confirmatory HCV RNA PCR should be ordered
+ Low	N.D.	A low level of reactivity was detected in this specimen. This may be a false-positive reaction, but may represent a low level of HCV-specific antibody. Depending on the clinical circumstances, HCV PCR to detect active viremia and/or repeat antibody testing in 4-6 weeks is recommended.
+	-	Not currently infected. Recovered or false positive screen.
+	+	Currently infected. Order HCV genotype.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY TEST INTERPRETATION

CLIA	Multispot differentiation	Interpretation
-	N.D.	This specimen is HIV antibody negative. A negative test does not exclude the possibility of infection with HIV. Negative results may be seen in early infection, advanced AIDS and agammaglobulinemic patients. If suspicion is high, submit a sample for HIV nucleic acid testing.
+	HIV-1	Positive for HIV-1 antibody. This sample was reactive by an HIV screening test and also reactive for HIV-1 by an HIV-1/HIV-2 differentiation immunoassay. If this is the first positive result from this patient, retesting using a separately drawn sample is recommended.
+	HIV-2	Positive for HIV-2 antibody. This sample was reactive by an HIV screening test and also reactive for HIV-2 by an HIV-1/HIV-2 IgG differentiation immunoassay. If this is the first positive result from this patient, retesting using a separately drawn sample is recommended.
+	HIV	**HIV positive (undifferentiated).** This sample was reactive by an HIV screening test and also reactive for both HIV-1 and HIV-2 by an HIV-1/HIV-2 IgG differentiation immunoassay. This sample should be considered positive for HIV antibody, however, additional tests are required to determine whether the patient is infected with HIV-1 and/or HIV-2. If this is the first positive result from this patient, retesting using a separately drawn sample is recommended.
+	Indet	**HIV indeterminate**. HIV NUCLEIC ACID TESTING ON A REPEAT SAMPLE IS RECOMMENDED. This sample was reactive by an HIV screening test, but nonreactive by an HIV-1/HIV-2 IgG differentiation immunoassay. This could be a false positive screening test result, or an early HIV infection, and should be evaluated by HIV nucleic acid testing using a new specimen.
+	Indet	**HIV indeterminate**. HIV NUCLEIC ACID TESTING ON A REPEAT SAMPLE IS RECOMMENDED. This sample was reactive by an HIV screening test, but indeterminate by an HIV-1/HIV-2 IgG differentiation immunoassay. This could be a false positive screening test result, or an early HIV infection, and should be evaluated by HIV nucleic acid testing using a new specimen.

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

Virology Reference Laboratory, VA Connecticut Health System
Director: David Peaper, M.D., Ph.D, 932-5711, ext 3544
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

Viral serology

- HIV-1/2 antibody + p24 Ag (4th Generation Assay)
- HIV-1/2 Differentiation Assay
- Hepatitis viruses (A, B, C)

Molecular assays

CMV PCR, quantitative and qualitative
Respiratory Pathogen panel using FilmArray: Influenza, RSV, Parainfluenza, Adenovirus, Rhinovirus/Enterovirus, hMPV, Coronaviruses, *B. pertussis, M. pneumoniae, and C. pneumoniae*
HIV-1 RNA Quantitative RT-PCR
HIV-1 antiviral resistance genotyping
HSV-1, HSV-2, VZV PCR from CSF
HSV-1, HSV-2 and VZV Real-time PCR for detection in lesions
HCV RNA Quantitative RT-PCR
HCV genotyping
High Risk Human Papilloma Virus PCR from ThinPrep specimens
Chlamydia trachomatis and *Neisseria gonorrhea* PCR from urine and genital specimens (Roche PCR Media)