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

Director: Marie L. Landry, M.D. (office 203-688-3475) Laboratory Manager: Maureen Owen, M.T., A.S.C.P. (office 688-1102)
Location: 55 Park St. Clinical Laboratory Building 6th floor, PS619. Lab phone number: 203-688-3524
Standard Hours of Operation: Mon-Fri 7:00 a.m.-to 11:30 a.m.; Sat and Sun 8:00 a.m.-4:30 p.m.
During Winter Respiratory Season (December-March), extended weekend 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 PROPERLY LABELLED WILL BE REJECTED
Collect specimens for PCR, antigen, 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 generally sufficient.

PLEASE NOTE: During acute infection, virus is present, but antibody is often negative.
To **detect acute infection** by antibody response, both acute and convalescent sera are recommended to show an antibody rise or a seroconversion from negative to positive antibody. Exceptions are virus infections whose clinical symptoms are immune-mediated and thus 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 than IgM for diagnosis of acute infection. **Reactivation of latent or persistent viruses, or secondary mumps or measles vaccine failures,** may or may not be associated with a positive IgM or rise in IgG. Rather, PCR is recommended.

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 specified hrs of collection.
Viral antibody test:			
Blood (serum)	Collect 1 **gold** top tube	Room temperature	

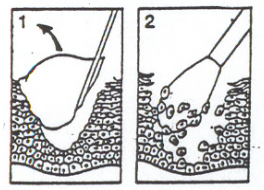
D. Specimen collection instructions for selected specimens

Nasopharynx swab — Insert swab deep into nasopharynx, past point of resistance, to posterior pharynx. Gently rotate to dislodge respiratory epithelial cells. Alternatively, rub nasal turbinate. Remove and place in transport medium. For infants and small children NP aspirate may be preferred.

Throat swab — Swab posterior pharyngeal wall, not buccal mucosa, tonsils, tongue or palate. Swab thoroughly.
Throat swabs can be used for lab developed PCR tests, especially for enterovirus, but are not acceptable for DFA testing or for rapid influenza PCR (GeneXpert).

Saliva swab — Neonatal CMV: Place sterile swab in baby's mouth under the edge of the tongue until it is completely saturated with saliva. Insert saliva-saturated swab into VTM tube. Collect immediately before or 90 minutes after breast feeding to avoid contamination. For older patients, for mumps or CMV testing, saliva can be spit into sterile cup.

Buccal swab — For mumps, collect saliva by swabbing mid-buccal mucosa by Stensen's duct to collect saliva from parotid glands



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 infectious virus may be inactivated.

Rectal swab — **Stool** specimen (not rectal swab) is required for enteric pathogens; swab of rectal mucosa can be done for proctitis.

BAL CSF, Stool — Collect each in sterile cup.

*see List of Virology Tests Performed, on website https://medicine.yale.edu/labmed/sections/virology/

II. VIROLOGY TEST SELECTION ORGANIZED BY VIRUS [Note – Test schedule varies with virus. Contact Lab for details.]

Virus suspected	Clinical symptoms	Specimens	Tests*	Special instructions and comments	Time to result
Adenovirus types 1-51	URI, pharyngitis, pneumonia, conjunctivitis, keratoconjunc-tivitis, hepatitis, hemorrhagic cystitis, gastroenteritis, intussusception, genital infections	NP swab or aspirate Throat, eye swabs, urine, BAL, tissue, stool	Respiratory Virus PCR Panel (RVP) Virus isolation, if lower respiratory tract or tissue. Respiratory virus DFA screen (NP swab only)	PCR is more sensitive and more rapid. Virus isolation can detect the "unexpected". *DFA detects only <60% of culture and <40% of PCR positives*	1 d (PCR) 1-14 days (culture) 4 hrs DFA
	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 encephalitis, LaCrosse, Jamestown Canyon, West Nile, POW) *See also Dengue, Chikungunya*	Encephalitis, aseptic meningitis, paralytic disease, febrile illness in summertime *Note: Different arboviruses are found 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 for WNV *if patient cannot make antibody*	WNV done in-house. <u>All other arbovirus requests sent out</u>. POW done only at CDC. *Early samples can be falsely negative.* *Cross-reactions often give false positives. May need PRNT to confirm specificity.* Only WNV and dengue PCRs available commercially. If traveled, special testing at CDC may be necessary; call lab.	1-4 days WNV 4-7 days for others 2-4 wks if sent to CDC
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, included in RVP. RT-PCR and serology	Peaks mid-winter Testing at State Lab or CDC. Needs approval. Contact Hospital Epi. <u>Notify lab, then hand carry to lab. Special Pathogen protocol.</u>	< 1 day 1-3 days
Chikungunya	Fever, myalgia, arthralgia, rash, edema	Serum	Antibody >day 5 of illness PCR for <u>≤</u>5 days of illness	Serum sent out to Quest	7 days

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Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Cytomegalovirus (CMV)	Fever, leukopenia, mononucleosis, hepatitis, pneumonia, oral, esophageal and gastrointestinal ulcerations, neurologic syndromes, retinitis	BAL, tissue biopsies; ocular fluid	PCR (lab developed, LDT) Virus isolation	Note: PCR of tissue or BAL can detect positives that are not clinically relevant.	1-21 days
Roche Cobas Ampliprep PCR		Plasma (lavender tube)	Quantitative PCR (plasma viral load)		1-2 days
		Serum, acute and convalescent	Antibody (IgM and IgG); Seroconversion to document primary infection	Use antibody tests to confirm primary infection or to determine immune status; *do NOT use to follow seropositive patients.*	1-2 days
		Serum	Immune status IgG only		1-2 days
	Encephalitis	CSF (1 ml)	PCR (LDT)	*Note: CMV in blood can contaminate CSF and give positive PCR*	1-2 days
	Congenital CMV	Saliva or urine collected at birth Amniotic fluid	PCR (LDT) CMV rapid culture	To diagnose <u>congenital</u> infection *samples must be obtained within 2-3 wks of birth. Positives after 3 wks can be perinatal infection.*	1-2 days
Dengue	Fever, myalgias, rash; hemorrhagic fever	Serum	Antibody >day 5 of illness PCR for <u>≤</u>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 (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	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 (plasma), CSF and urine	RT-PCR	Parechoviruses order separate PCR.	1- 2 days

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Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
Epstein-Barr virus (EBV)	Infectious mononucleosis (I.M.)	Serum	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	Serum	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 Pathology*	1-3 days
	Nasopharyngeal carcinoma (NPC)	Blood (red top)	EBV antibody panel <u>and</u> 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	Serum	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	Serum	Antibody (anti-HAV IgM)	**Order only in cases of acute infectious hepatitis;** *false positives> true positives if low risk*	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	Serum	HBsAg, anti-HBc total and IgM, anti-HBs, HBeAg and anti-HBe	Can be ordered as single tests, as part of acute hepatitis, hepatitis general, or hepatitis B virus panels	1 day
		Serum	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	Serum	Antibody (anti-HCV) HCV RNA (RT-PCR) (*anti-HCV RIBA not available since 2013*) HCV genotype (LiPA)	Can be ordered as a single test, as part of acute hepatitis or chronic hepatitis panels HCV RNA test is used to confirm antibody 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	Serum	Antibody (anti-HDV or anti-Delta) HDV RT-PCR	Patient must be HBsAg positive to be infected with HDV. Sent out. Used to monitor 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	Serum Serum, stool	Antibody (anti-HEV IgM, IgG) RT-PCR for definitive diagnosis	Tests on travelers sent to Quest. Tests on transplant patients sent to CDC.	1-4 weeks
Herpes simplex virus (HSV) types 1 and 2	Esophagitis, proctitis, hepatitis, pneumonia, retinitis; corneal ulcer Cold sore, gingivostomatitis, skin lesions, genital lesions Encephalitis, meningitis Neonatal HSV Hepatitis; disseminated HSV Immune status	Biopsy tissue, mucosal swab, BAL, ocular swab or fluid Lesion swab CSF (1 mL) Swabs for neonatal HSV; blood, CSF Plasma Serum	PCR Virus isolation HSV PCR, Direct HSV culture PCR PCR PCR Type-specific IgG antibody	PCR most sensitive. Direct PCR done twice a day. Not for ocular or neonatal swabs. PCR on CSF done once a day Viral load can be done in disseminated HSV or hepatitis; low level HSV viremia can be seen with simple cold sores and does not indicate dissemination Immune status (carrier state); *HSV IgM is not useful*	1 day 1-7 days <1 day 1-7 days 1 day 1 day 1-2 days

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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	Antibody (CLIA)	Note: HIV tests require notification of patient, but not signed consent.	<1 day
		Plasma (lavender) allows faster result	Rapid HIV-1/2 antibody (OraQuick)	Call lab to facilitate rapid result; done by Core Lab when Virology is closed	30 min
		Serum or plasma	HIV-1,-2 IgG by Geenius	All positive 4th gen require IgG antibody differentiation. Do PCR if IgG negative to rule out acute HIV.	1 day
	To determine viral load, to diagnose acute HIV infection or neonatal infection.	Whole blood (2 lavender top tubes)	Quantitative plasma RNA by RT-PCR (Roche TaqMan)	In acute infection, viral load should be high	1-3 days
	To guide antiretroviral therapy.	Whole blood (2 lavender top tubes)	Resistance genotype	Requires 400-1,000 copies/mL	1-2 weeks (sent out)
Human herpesvirus type 6 (HHV-6A and B)	Roseola infantum, febrile seizures, infectious mono, hepatitis, pneumonitis, encephalitis in HSCT	CSF, plasma	PCR (quantify plasma)	Patients with chromosomally integrated HHV-6 have high levels of HHV-6 DNA in blood and CSF	1-3 days
	To confirm primary infection	Serum	Antibody, IgM and IgG	HHV-6 antibody rarely useful since infection is universal by age 2	1-2 wks
Human meta-pneumovirus	URI, pneumonia, bronchiolitis	NP aspirate, swab, BAL	RT-PCR, in RVP;	Peaks in late winter, early spring	1 day
HTLV I/II	Tropical spastic paraparesis or HTLV associated myelopathy; human T cell leukemia/lymphoma	Serum	Antibody (EIA screen; all positives need confirma-tion by Western blot)	EIA does not distinguish HTLV-I from II; (W. blot sent out). Positive serology indicates carrier.	3-7 days
		Whole blood (2 lavender)	Qualitative PCR on PBMCs; not viral load	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	Rapid influenza PCR in ED, outpatients Included in RVP	Collect specimens first 2-4 days of illness; *samples collected in first 24 hrs can be falsely negative*	1-14 hrs
			A subtype or B lineage by PCR if hospitalized		
Avian influenza (A/ H5N1, H7N9, etc)	Pneumonia, ARDS, diarrhea, neurologic disease	BAL, endotracheal aspirate, sputum (not NP swab)	Not subtypeable by seasonal virus PCRs.	**Notify lab; requires BSL3 safety precautions**; initial tests may be negative; repeat testing needed	4-48 hrs

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Virus suspected	Clinical symptoms	Specimens	Tests	Special instructions and comments	Time to result
JC virus (Polyomavirus)	Progressive multifocal 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 Serum, acute and convalescent	PCR Antibody, IgM and IgG IgG avidity testing at CDC	Collect early in disease; *notify Hospital Epidemiology and the laboratory prior to sample collection; requires approval from State Epi; done at CT DPH Lab* IgM, IgG avidity sent to CDC; IgM false positive and false negative	2-3 days 1-7 days
Mumps	Parotitis, orchitis, meningitis, encephalitis	Saliva, urine, CSF, Serum, acute and convalescent	PCR Antibody IgG and IgM IgG avidity testing at CDC	Sent to CT DPH; *please notify the laboratory* IgM sent to CDC; IgM prone to false positive and false negative	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, pneumonia	NP swab or aspirate, tracheal aspirate, BAL, lung tissue	PCR (in Resp Virus PCR Panel) DFA for outpatient pediatrics Virus isolation	PCR for types 1-4 DFA for types 1-3 only	1 day 4 hrs 3-10 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 3-14 days
Parvovirus B19	Erythema infectiosum (fifth disease), arthralgias, various exanthems and enanthems, aplastic crisis, chronic anemia in compromised hosts, nonimmune hydrops fetalis	Serum 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

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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	RT-PCR (in Resp Virus PCR Panel) DFA outpatient pediatrics Virus isolation	*RT-PCR and DFA >> RSV culture*	1 day 4 hrs 3-14 days
Rhinovirus	URI; lower tract disease in infants, asthma, COPD, ICH	NP swab or aspirate; BAL	RT-PCR (in Resp Virus PCR Panel) Virus isolation	RT-PCR much more sensitive than culture; group C does not grow in culture	1 day 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	1-3 days
Rubella	Rubelliform rash, post-auricular adenopathy, arthralgias, congenital rubella syndrome	Serum, acute and convalescent Tissue, throat swab and urine	Antibody RT-PCR	IgG done in-house, IgM sent out if rubella high risk- *notify laboratory* Special request; *please notify laboratory; sent to CDC*	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 Serum 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* CSF IgG useful if testing delayed and PCR is 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	RT-PCR, Culture
Pneumonia	Adenovirus	NP aspirate or swab, BAL, lung tissue	PCR, Culture
	Respiratory syncytial virus	NP aspirate or swab, BAL, lung tissue	RT-PCR, Culture
	Human metapneumovirus	NP aspirate or swab, BAL, lung tissue	RT-PCR
	Parainfluenza	NP aspirate or swab, BAL, lung tissue	RT-PCR, Culture
	Rhinovirus	NP aspirate or swab, BAL, lung tissue	RT-PCR, Culture
	Coronavirus (229E, OC43, NL63, HKU1)	NP aspirate or swab, BAL, lung tissue	RT-PCR
	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, Coronaviruses	NP aspirate or swab	RT-PCR; Culture (RV)
	Respiratory syncytial virus	NP aspirate or swab	RT-PCR, DFA; Culture
	Adenovirus	NP aspirate or swab	PCR, DFA, Culture
	Parainfluenza	NP aspirate or swab	PCR, DFA, Culture
	Influenza A,B	NP aspirate or swab	RT-PCR; DFA, Culture
	Enterovirus, parechovirus	Throat and/or NP swab	RT-PCR; Culture
	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 (Special request)
	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

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Clinical Syndrome	Viruses associated	Specimens to collect	Test method of choice
Papillomas, papules	Papillomavirus	Biopsy	Contact Pathology Dept for options
	Molluscum contagiosum	Biopsy	*(Note: Clinical diagnosis usually sufficient)*
Exanthematous	Measles	NP swab or TS, serum	PCR; rapid culture; serology
	Rubella	Serum; NP swab or TS, urine, tissue	PCR, Serology
	Enterovirus	TS, stool	RT-PCR
	Parvovirus B19	Serum	Serology; PCR
	Human herpesvirus type 6	Serum	PCR; Serology
	Dengue	Serum	Serology; PCR
	West Nile	Serum, CSF	Serology; PCR
	Epstein-Barr virus	Serum	Serology
	Adenovirus	NP swab or TS, urine; stool	PCR, culture, DFA
	Cytomegalovirus	Blood, urine, saliva	PCR (blood), Culture; serology
Cardiovascular			
Myocarditis/ Pericarditis	Enterovirus	TS, stool, endocardial biopsy	RT-PCR; Culture
	Cytomegalovirus	Blood, urine, endocardial biopsy	PCR, Culture
	Influenza	NP swab, [endocardial biopsy-most negative]	RT-PCR, culture
	Adenovirus	NP swab or TS, urine; stool	PCR culture, DFA
	Rhinovirus group C	NP swab or TS; pericardial fluid	RT-PCR
	EBV	Serum	Serology
Digestive tract			
Gastroenteritis	Rotavirus	Stool	ELISA, PCR
	Norovirus	Stool	RT-PCR
	Adenovirus	Stool	PCR
	Parechovirus	Stool	RT-PCR
	Enterovirus (not common)	Stool	PCR
Colitis	Cytomegalovirus	GI biopsy, blood	PCR
Proctitis	Herpes simplex virus	Lesion swab, rectal swab	PCR, culture
Hepatitis	Hepatitis A	Serum	Serology
	Hepatitis B	Serum	Serology; PCR
	Hepatitis C	Serum	Serology; RT-PCR
	Hepatitis D	Serum	Serology, RT-PCR
	Hepatitis E	Serum	Serology, PCR
	EB virus	Serum	Serology; PCR
	Cytomegalovirus	Liver tissue, blood	PCR, Culture
	Adenovirus	Liver tissue	PCR, Culture
	Herpes simplex virus	Liver tissue, blood	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	RT-PCR, culture
	Adenovirus	Throat, stool, blood, bone marrow	PCR, 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
	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
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 (beware ciHHV6)	CSF	PCR
	Rabies*	Brain biopsy; Skin biopsy, Saliva; serum, CSF	DFA, (for antigen); PCR PCR, serology, Culture
	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; ciHHV6= chromosomally integrated HHV-6 in 1% of population.

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: Comprehensive culture now used only for lower respiratory tract samples. HSV culture for genital samples. Rapid CMV culture for selected samples. PCR preferred test. Cultures of CSF and stool discontinued July 2015.

Viruses isolated in routine cell cultures	Interpretation of positive culture
Adenovirus, cytomegalovirus, enteroviruses, herpes simplex, influenza A and B, parainfluenza types 1-4, rhinoviruses, RSV, vaccinia, varicella-zoster virus	Indicates infectious virus present. Significance 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. influenza).

NOTE: Respiratory Virus PCR Panel is more sensitive than culture or DFA. On outpatients, when a less expensive test is desired, respiratory screen DFA can be ordered, but results are best in young children. Performance of antigen tests vs culture and PCR is given below.

VIRAL ANTIGEN [DFA]

Virus	Sample	Test	Sensitivity DFA vs. Culture	PCR	Interpretation of positive result
Influenza A and B	NP swab	DFA	85-90%	55-85%	Culture and DFA associated with acute infection; PCR remains positive longer
RSV	NP swab	DFA	99%	55-80%	Culture and DFA associated with acute infection; PCR remains positive longer
Adenovirus	NP swab	DFA	60%	25%	Culture and DFA associated with acute infection; PCR remains positive longer
Parainfluenza types 1-3	NP swab	DFA	>90%	60-70%	Culture and DFA associated with acute infection; PCR remains positive longer
HMPV, Para-4, Rhinoviruses, Coronaviruses	NP swab	Not avail -able	Insensitive		For all respiratory viruses, low levels of nucleic acid by PCR can reflect recent past infection, poor sample collection, or possibly primary/probe mismatch

****DFA results are best in young children, who shed high titers of virus.*.** Specificity at YNHH 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 of respiratory epithelial cells)
Respiratory Screen DFA includes Influenza A and B, RSV, parainfluenza types 1-3 and adenovirus.
Respiratory virus PCR panel includes viruses in DFA screen plus HMPV, rhinovirus, parainfluenza 4, and coronaviruses 229E, OC43, NL63, HKU1.

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.**

For more details, see **C. difficile newsletter**, https://medicine.yale.edu/labmed/sections/virology/newsletter/ Volume 26 (1), Nov. 2017

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

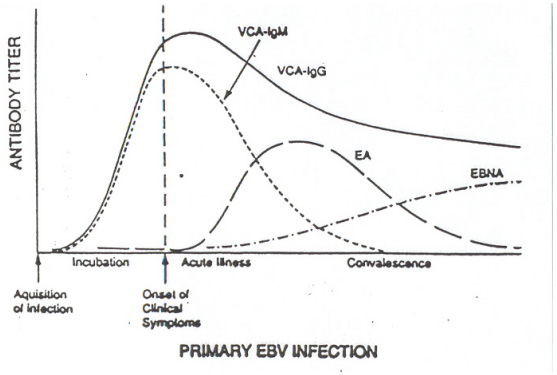
DIAGNOSIS OF ACUTE INFECTION

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 (or false positive) Past infection No antibody detected
West Nile Virus	EIA	- or + + -	+ - -	Acute infection 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



*see List of Virology Tests Performed, on website https://medicine.yale.edu/labmed/sections/virology/

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. Perform HBV DNA PCR to exclude 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 U.S. 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	HIV-1/2 IgG 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 IgG 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

Serology Assays
Viral Serology
- HIV-1/2 antibody + p24 Ag (4th Generation Assay)
- HIV-1/2 Differentiation Assay
- Hepatitis viruses (HAV IgM, HAV IgG, HBsAg, HBsAb, HBc IgM, HBc Total, HCV)
- MMRV (Measles, Mumps, Rubella, Varicella IgG)
- EBV (VCA IgM, VCA IgG, EBNA IgG)

Other Serology
- Celiac Antibodies (TTG IgA, TTG IgG, DPG IgG)

Molecular assays
Quantitative Viral Load Assays (Roche Cobas)
- HIV-1
- HCV
- HBV
- CMV

Highly Multiplexed PCR Panels (BioFire FilmArray)
- Respiratory Pathogen PCR panel: Influenza, RSV, Parainfluenza, Adenovirus, Rhinovirus/Enterovirus, hMPV, Coronaviruses, *B. pertussis, M. pneumoniae, and C. pneumoniae*
- Meningitis / Encephalitis PCR Panel: HSV-1, HSV-2, VZV, Enterovirus, Parechovirus, CMV, HHV-6, *E. coli K1, H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S. pneumoniae, C. neoformans/gattii*

Antiviral Drug Resistance
- HIV-1 Antiviral Resistance Genotyping
- HCV NS5a Antiviral Resistance Sequencing

Other PCR Assays
- CMV PCR (Qualitative)
- HSV-1, HSV-2 and VZV Real-time PCR for detection in lesions
- High Risk Human Papilloma Virus PCR from ThinPrep specimens
- *C. trachomatis* and *N. gonorrhea* PCR from urine and genital specimens (Roche PCR Media)