LAB NEWS

From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

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Virology Testing Update

I. Diagnosis of Norovirus by Real-time PCR ****NEW TEST****

Norovirus is the leading cause of outbreaks of nonbacterial gastroenteritis in industrialized countries, and is a frequent cause of sporadic gastroenteritis in all ages. Detection of norovirus, however, has been limited because 1) it does not grow in cell culture, and 2) antigenic variation impedes detection by ELISA. The advent of molecular testing for norovirus has been a significant improvement. Although norovirus is currently divided into 5 genogroups, genogroup I (with seven clusters) and genogroup II (with 16 clusters) contain *most* of the norovirus strains infecting humans. The Clinical Virology Laboratory is now offering a multiplex Norovirus RT-PCR for genogroups I and II.

Tests available at YNHH: Norovirus Real-time TaqMan RT-PCR (genogroups I and II)
Samples: Stool collected early in illness.
***References*:** Kageyama T et al, J Clin Microbiol 41:1548-1557, 2003.
Pang XL et al. J Clin Virol 33:168-171, 2005.

II. Quantitative CMV PCR in Plasma for Leukopenia and Reference Testing ****NEW TEST****

CMV pp65 antigenemia has been used at YNHH since 1992 to determine CMV viral load in peripheral blood. As performed in the Virology Laboratory at YNHH, CMV antigenemia 1) is slightly more sensitive than the commercially available Roche Amplicor CMV PCR in plasma, 2) is cheaper, and 3) has a faster time to result. However, antigenemia has limitations, namely, requirements for adequate peripheral blood neutrophils and for processing within 6 hrs of blood collection. Thus, although CMV antigenemia will remain the standard assay used at YNHH, the Virology Laboratory is now offering a quantitative real-time PCR assay for use in severely leukopenic patients (<1000 WBC/µL) and for samples with delayed processing (e.g. shipped a distance). *Please note that the CMV DNA copy numbers per ml of plasma are much higher (100-5,000 times) than the number of CMV positive leukocytes in the same specimen.*

Tests available at YNHH: CMV Real-time TaqMan PCR, quantitative
Samples: Plasma (lavender top)
***References*:** Li H et al. J Clin Microbiol 41:187-191, 2003.

III. Updated Recommendations for BKV Nephropathy Surveillance in Renal Transplants

Polyomavirus BK-associated nephropathy is a potential cause of kidney transplant failure, affecting 1-10% of patients in various studies. In May 2005, the Clinical Virology Laboratory began offering BKV PCR to aid in the early diagnosis of BKV-associated nephropathy and thus allow for timely intervention. Recommendations have since been published by Hirsch et al, and others, that urine should be screened for BKV at specified intervals in the first 1-2 years posttransplant, and if positive, quantitative plasma BKV PCR should be obtained. A BKV DNA level in plasma of >10,000 copies/ml (or 10^7copies/ml of urine) is an indication for concern. Definitive diagnosis is by renal biopsy.

Tests available at YNHH: BKV Real-time TaqMan PCR, qualitative and quantitative
Samples: Urine (qualitative or quantitative PCR); plasma (lavender top; quantitative PCR)
***References*:** Hirsh HH et al. Transplantation 79:1277-1286, 2005.

Clinical Virology Laboratory: Summary of Viruses Detected, Jan-Dec 2005

Viruses Cultured[a]	No. positive	Viral Antigen Tests[c]	No. positive
Adenovirus	14	Adenovirus DFA	74
Polyoma BK virus	5	CMV antigenemia	287
Cytomegalovirus	42	Herpes simplex DFA	216
Enterovirus	19	Influenza A DFA	653
Herpes simplex type 1	63	Influenza B DFA	267
Herpes simplex type 2	36	Parainfluenza DFA	299
Herpes simplex, untyped	0	Respiratory syncytial DFA	686
Influenza A	7	Rotavirus (ELISA)	165
Influenza B	3	Varicella zoster DFA	60
Parainfluenza type 1	4	**Total antigen positive:**	**2707**
Parainfluenza type 2	0		
Parainfluenza type 3	2	**Molecular tests**	**No. positive**
Parainfluenza type 4	0	HIV RNA RT-PCR[d]	1242
Respiratory syncytial	1	Ultrasensitive HIV PCR[d]	1550
Rhinovirus	18	HIV DNA PCR[d]	2
Varicella zoster	1	Hepatitis C TaqMan RT-PCR[e]	608
Total virus isolates:	**215**	Hepatitis B DNA PCR[d]	134
		HSV DNA Taqman PCR[f]	21
	No. positive	VZV DNA Taqman PCR[f]	18
***C. difficile* cytotoxin[b]**	**717 (15.8%)**	CMV DNA Taqman PCR[f]	22
		EBV DNA Taqman PCR[f]	16
HCV genotyping by Invader		JCV DNA Taqman PCR[f]	5
Genotype 1	222	BKV DNA PCR-quantitative[f]	20
Genotype 2	21	Enterovirus Taqman RT-PCR[f]	63
Genotype 3	17	Parvovirus B19 TaqMan PCR[f]	12
Genotype 4	10	HMPV Taqman RT-PCR[f]	39
Genotype 5	0	**Total molecular positive**	**3,752**
Genotype 6	0		

a, If rapid direct immunofluorescence (DFA) tests are positive on the same sample, cultures are usually cancelled.

b, The *C. difficile* cytotoxicity test remains the gold standard for toxin detection and is more sensitive than rapid ELISA tests. Results are reported at 4, 24 and 48 hrs. It requires inoculation of cell culture, and therefore is done in the Virology Laboratory.

c, DFA is used to detect all viral antigens except rotavirus.

d, Roche Amplicor Monitor assays

e, Roche TaqMan assay

f, In-house real-time TaqMan methods (In 2006 to date, influenza A and B, adenovirus, norovirus and quantitative CMV PCR have been added to the menu. Influenza H5N1 is available in special cases.)

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