LAB NEWS

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Clinical Virology Laboratory Newsletter

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JC Virus DNA PCR for Diagnosis of PML

Progressive multifocal leukoencephalopathy. Progressive multifocal leukoencephalopathy (PML) is a progressive demyelinating disease that is almost exclusively associated with underlying cellular immunodeficiency (1). It was first described in the 1950s in a patient with chronic lymphocytic leukemia (CLL). The causative virus was isolated in 1971 and was named JC after the initials of the patient. Originally associated with CLL, Hodgkin's disease and sarcoidosis, AIDS is now the most common predisposing condition. PML occurs in 4-5% of all AIDS patients and has been the AIDS defining illness in 1%. PML is characterized by progressive deterioration of visual, motor and cognitive functions.

JC polyomavirus: JC virus is a 45 nm, non-enveloped DNA virus belonging to the polyomavirus family. Infection is usually sub-clinical, is almost universal, occurs in childhood and persists for life.

Pathology and pathogenesis of PML: The pathology of PML is characterized by enlarged oligodendrocyte nuclei with "ground glass" appearance, and bizarre, giant astrocytes. Electron microscopy (EM) reveals crystalline or filamentous arrays of polyomavirus particles in oligodendrocyte nuclei. Oligodendroyctes, which maintain the myelin sheath, undergo lytic infection with JC virus, and their destruction leads to loss of the myelin sheath. Foci of demyelination are randomly distributed and most commonly occur in the subcortical white matter near the gray-white matter junction. Diagnosis has traditionally been made by characteristic histopathology and EM of affected brain tissue.

CT and MRI: MRI is more sensitive than CT in detecting lesions, however radiographic imaging alone is not specific enough to diagnose PML. CT scan reveals multifocal subcortical areas of decreased signal intensity; contrast enhancement is rarely seen. T2 weighted MRI shows patchy areas of increased signal intensity, while T1 weighted images show a decrease in signal intensity, consistent with demyelination.

Laboratory diagnosis: Detection of JC virus DNA in spinal fluid can provide a non-invasive diagnosis of PML (2, 3). While sensitivity may be >90%, false-negative CSF PCRs can occur and brain biopsy may be required. As with all laboratory tests, clinical correlation is essential.
Sample requirement: 0.5-1.0 mL CSF
Test method: Real-time TaqMan PCR protocol, developed at NIH (3) and validated at YNHH.
Test Availability: Test performed in the Clinical Virology Laboratory once a day, Monday-Friday, if sample received by 8 AM.
Time to result: Generally within one working day, excluding weekends and holidays (when staffing is limited and molecular tests are not performed).

References

1. Johnson RT and Major EO. Infectious demyelinating diseases. In Myelin Biology and Disorders, Volume 2, Elsevier 2004.
2. Hammarin AL et al. Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. J Clin Microbiol 34:2929-2932, 1996.
3. Ryschkewitsch C, et al. Comparison of PCR-Southern hybridization and quantitative real time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods 121:217-21, 2004.

Clinical Virology Laboratory: Summary of Viruses Detected, Jan-Dec 2004

Viruses Cultured	No. positive	Viral Antigen Tests[a]	No. (%) positive
Adenovirus	27	Adenovirus DFA	57 (0.9%)
Polyoma BK virus	0	CMV antigenemia	295 (12.7%)
Cytomegalovirus	33	Herpes simplex DFA	246 (22.3%)
Enterovirus	63	Influenza A DFA	273 (4.4%)
Herpes simplex type 1	35	Influenza B DFA	12 (0.2%)
Herpes simplex type 2	32	Parainfluenza DFA	179 (2.9%)
Herpes simplex, untyped	0	Respiratory syncytial DFA	638 (10.2%)
Influenza A	1	Rotavirus (ELISA)	110 (20.1%)
Influenza B	2	Varicella zoster DFA	66 (6.0%)
Parainfluenza type 1	0		
Parainfluenza type 2	2	**Molecular tests**	**No. (%) positive**
Parainfluenza type 3	11	HIV RNA RT-PCR[b]	1391 (72.4%)
Parainfluenza type 4	1	Ultrasensitive HIV PCR[b]	1623 (59.1%)
Respiratory syncytial	1	HIV DNA PCR[b]	1 (1.6%)
Rhinovirus	23	Hepatitis C RT-PCR[c]	523 (65.1%)
Varicella zoster	2	Hepatitis B DNA PCR[b]	125 (48.1%)
Total virus isolates (%):	**233 (7%)**	HSV DNA PCR[d]	14 (2.3%)
		VZV DNA PCR[d]	9 (3.4%)
	No. (%) positive	CMV DNA PCR[d]	6 (2.0%)
***C. difficile* cytotoxin**	478 (12.3%)	EBV DNA PCR[d]	6 (17.6%)
		Enterovirus RNA NASBA[e,d]	59 (18.8%)
		HMPV RNA RT-PCR[c,d]	2 (9.1%)
		Parvovirus B19 DNA PCR[c,d]	1 (4.3%)

a, Direct immunofluorescence (DFA) is used to detect all viral antigens except rotavirus.
b, Roche Amplicor Monitor assays
c, Real-time TaqMan assay
d, In-house methods
e, BioMerieux NASBA Basic Kit used in setting up assay
EBV, HMPV and Parvovirus B19 PCR were brought in-house in 2004

Other molecular tests performed in the Clinical Virology Laboratory:
HCV genotyping has been performed at YNHH Clinical Virology Laboratory since October 2001.

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