LAB NEWS

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Clinical Virology Laboratory Newsletter

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Diagnosis of Influenza at YNHH by DFA, Culture and Real-time PCR

Annual epidemics of influenza virus lead to excess deaths and hospitalizations in the winter months in temperate zones. A rapid diagnosis can influence patient management, including reductions in tests ordered and antibiotics prescribed, institution of antiviral therapy, and of proper infection control practices in hospitalized patients. At YNHH, Respiratory Screen direct immunofluorescence (DFA) is the mainstay of diagnosis because DFA is equivalent in sensitivity to culture in a well-collected sample; DFA is reported within 2 hrs of arrival of the sample in the Virology Lab; and Respiratory Screen DFA detects not only influenza, but RSV, parainfluenza types 1,2,3, and adenovirus (1,2).

The Virology Laboratory is offering a new test for Influenza A and B this season, a multiplex Influenza A and B real-time TaqMan RT-PCR (3). NOTE: Influenza RT-PCR should be reserved for: 1) hospitalized patients who are DFA-negative and in whom influenza remains a likely diagnosis; 2) suspected avian flu cases (in addition to DFA). *The laboratory must be notified when avian flu is considered, to ensure that extra safety precautions are implemented in the lab and the sample is NOT placed into culture.*

Summary of Current Clinical Virology Laboratory Tests for Influenza

Test	Sensitivity	Detects	Time	Availability
Respiratory Screen DFA	Detects 98% of influenza culture-positive samples	Influenza A, B, RSV, parainfluenza 1,2,3, adenovirus	2 hrs	When Virology is open, 7 days a week
Viral culture	"Gold standard" by which other methods are judged	Viruses above plus CMV, HSV, VZV, rhinovirus, enterovirus, parainfluenza 4	1-14 days	When Virology is open, 7 days a week
Influenza PCR	≥ 1 $\log_{10}$ more sensitive than culture	Influenza A, B	6 hrs – 3 days	Testing performed once a day, Monday -Friday

Note: Binax Flu A + B is performed during peak flu season from 12-6 AM in the Core Laboratory for patients in the ED. Samples are re-tested by DFA when Virology opens. Sensitivity is 50% in adults and 75% in children when compared to DFA or culture (4).

Samples: Acceptable samples include nasopharyngeal (NP) swabs, NP aspirates, NP washes; sputum; BAL. NOTE: Submit a separate sample when requesting Influenza RT-PCR.

References

1. Landry ML, Ferguson D. SimulFluor Respiratory Screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. J Clin Microbiol 38:708-711, 2000.
2. Landry, ML, Ferguson D. Suboptimal detection of influenza in adults by Directigen Flu A + B and correlation with number of antigen-positive cells detected by cytospin immunofluorescence. J Clin Microbiol 41:3407-3409; 2003.
3. Ward CL et al. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. J Clin Virol 29:179-188, 2004.
4. Landry ML, Cohen S, Ferguson D. Comparison of Binax NOW and Directigen for Rapid Detection of Influenza A and B. J Clin Virol 31:113-114, 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
Polyoma BK virus	4	CMV antigenemia	295
Cytomegalovirus	41	Herpes simplex DFA	246
Enterovirus	63	Influenza A DFA	273
Herpes simplex type 1	56	Influenza B DFA	12
Herpes simplex type 2	53	Parainfluenza DFA	179
Herpes simplex, untyped	0	Respiratory syncytial DFA	638
Influenza A	1	Rotavirus (ELISA)	110
Influenza B	2	Varicella zoster DFA	66
Parainfluenza type 1	0	**Total antigen positive:**	**1876**
Parainfluenza type 2	2		
Parainfluenza type 3	11	**Molecular tests**	**No. positive**
Parainfluenza type 4	1	HIV RNA RT-PCR[b]	1319
Respiratory syncytial	1	Ultrasensitive HIV PCR[b]	1623
Rhinovirus	23	HIV DNA PCR[b]	1
Varicella zoster	2	Hepatitis C TaqMan RT-PCR[c]	523
Total virus isolates:	**287**	Hepatitis B DNA PCR[b]	125
		HSV DNA PCR[d]	14
	No. positive	VZV DNA PCR[d]	9
***C. difficile* cytotoxin**	**478**	CMV DNA PCR[d]	6
		Enterovirus RNA NASBA[e]	59
		Parvovirus B19 TaqMan PCR[d]	1
		HMPV Taqman RT-PCR[d]	2
		Total molecular positive	**3682**

a, Direct immunofluorescence (DFA) is used to detect all viral antigens except rotavirus.
b, Roche Amplicor Monitor assays
d, Roche TaqMan
d, In-house methods
e, BioMerieux NASBA Basic Kit used in setting up assay

Other molecular tests performed in the Clinical Virology Laboratory:
HCV genotyping by Invader (Third Ware Technologies) replaced LiPA in 2004.

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