

# LABORATORY BULLETIN

2009 – July - 9

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**To: Alberta Health and Wellness, Brenda Hannah, Infectious Disease Physicians, Laboratory Managers and Directors, Medical Officers of Health, and All Physicians**

**Re: Testing and Interpretation of Laboratory Results for Influenza A (seasonal and swine origin influenza virus) and other Respiratory Viruses**

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**Background:**

Testing for influenza A and the other respiratory viruses is achieved through a combination of DFA (Direct Fluorescent Antigen) and various molecular assays for detection and confirmation. Combinations of these assays are structured within the testing algorithm based upon; acuity of the clinical needs related to patient care, outbreak investigations, incidence of circulating viral pathogens and turn-around time to test results.

Influenza A viruses are defined by their subtype; namely the hemagglutinin (HA) and the neuraminidase (NA). The seasonal subtypes, H1N1 and H3N2, are included in this years' 2008/09 seasonal vaccine, whereas the novel swine-origin influenza virus (S-OIV) which is also an H1N1 subtype, is genetically different from its seasonal H1N1 counterpart.

Below is a brief description of the various assays and the interpretation of the results that arise.

**Direct Fluorescent Antigen (DFA):**

Only nasopharyngeal swabs and aspirates are suitable for testing by the DFA, which detects influenza A & B, RSV (Respiratory Syncytial Virus) and the parainfluenza viruses. Although the DFA detects S-OIV, a sufficient sample size has not been tested to evaluate the sensitivity and specificity with certainty. Table 1 gives the relative sensitivity of the DFA for influenza A (both S-OIV and seasonal subtypes combined) and the other respiratory viruses compared to the molecular Respiratory Virus Panel (RVP) assay based upon data from the Provincial Laboratory DIAL (Data Integration for Alberta Laboratories) database.

Table 1

| Virus Target     | Sensitivity (%)* |
|------------------|------------------|
| Influenza A      | 46               |
| Influenza B      | 53               |
| RSV              | 82               |
| Parainfluenza gp | 51               |

\*% positive of total DFA positive tests/RVP+DFA Positive tests for 1 yr period for specified viral target (data from DIAL)

A negative DFA cannot be used to rule out infection by one or more of the above viruses. Some common explanations for a negative result include:

- Poorly collected sample – usually reflected by few columnar respiratory cells or numerous squamous epithelial cells. These samples will have a comment in the laboratory report indicating a less than optimal sample.

- Sample collected in convalescent phase – generally the yield is very poor if sampling is five or more days after the onset of symptoms.
- Past exposure – those patients who were immunized with the current seasonal vaccine or with cross-immunity to the infecting subtype have a much lower viral titre and can test negative.
- Lower sensitivity relative to molecular assays.
- Respiratory infection due to another agent not detected by the DFA test.

When positive, the DFA is definitive for that agent. The turn-around time for the DFA (positive or negative to a reported result) is the same day. Although molecular assays such as the RVP assay (*described below*) have a much higher level of sensitivity than the DFA, the DFA has a shorter turn-around-time and can be used to triage critical cases. Currently, the DFA is performed on patients who are: (a) hospitalized, (b) one year or under in age, and (c) for selected respiratory outbreaks in institutions. Samples positive for influenza A by the DFA are prioritized to determine whether they are seasonal or S-OIV subtypes. For completeness, samples testing negative by the DFA or those that are not suitable for this assay, such as throat swabs, are tested by the RVP to establish if there is a viral agent.

**Molecular Assays for respiratory virus detection:**

The following assays detect the presence of influenza A and other respiratory viruses, and where appropriate, subtype influenza A as either, seasonal H1N1 or H3N2 or S-OIV.

1. Respiratory Viral Panel (RVP): detects influenza A and other respiratory agents
2. Influenza A PCR (Matrix gene): specific for influenza A virus alone, detects seasonal subtypes and S-OIV
3. Influenza A subtype (HA gene): for subtyping influenza A, discriminates between seasonal and S-OIV subtypes
4. Influenza A S-OIV confirmatory PCR: for confirmation of influenza A as S-OIV from the above screening or typing assays

**1) Respiratory Viral Panel:**

This molecular-based assay detects influenza A & B, RSV, parainfluenza, human metapneumovirus, adenovirus, respiratory coronaviruses and rhino/enterovirus. Samples testing positive for influenza A are directly subtyped based upon the hemagglutinin gene and reported as one of the following (see Table 2). Turnaround time to a result is generally 48 hours.

Table 2:

| Respiratory Virus Panel (NAT)  | Result                 | Interpretation   |
|--|------------------------|--|
| Influenza A virus Screen (RNA)   | Negative               | Influenza A (seasonal or S-OIV) NOT detected – can be negative for both at low viral titre                     |
| Influenza A virus Screen (RNA)   | Positive               | Influenza A (seasonal or S-OIV) Detected   |
| Influenza A virus H1 subtype (RNA)                                       | Positive               | Seasonal H1N1 subtype  |
| Influenza A virus H3 subtype (RNA)                                       | Positive               | Seasonal H3N2 subtype  |
| Influenza A virus H1 subtype (RNA)<br>Influenza A virus H3 subtype (RNA) | To Follow<br>To Follow | Suggests S-OIV especially in combination with “Non-Typeable” result by “Influenza A Subtype PCR” (see Table 3) |

In some circumstances where an equivocal result is obtained in the RVP assay for influenza A, additional molecular assays will be performed, namely influenza A matrix (M) gene PCR, followed by subtyping and/or S-OIV confirmatory assay as required.

2) Influenza (RNA) PCR (Matrix gene):

This assay is sensitive and specific for influenza A viruses (both seasonal and S-OIV). It can be used as a rapid screen for S-OIV, and there may be circumstances when this assay is positive whereas the RVP assay is negative for influenza A (Table 3).

3) Influenza A Subtype PCR (Hemagglutinin (HA) gene):

This assay differentiates between seasonal subtypes of influenza A as H1N1 or H3N2, based upon the HA gene, whereas S-OIV is genetically different and therefore “Non-Typeable” (Table 3).

4) S-OIV Confirmatory Assay:

This is a confirmatory assay specific for this subtype, based upon unique genetic sequences in the hemagglutinin (HA) gene (Table 3).

Table 3:

| Additional PCR Assays |  | Result                                   | Interpretation   |
|-----------------------|--|--|--|
| 2                     | <i>Influenza (RNA) PCR</i><br>(Matrix gene)                  | Positive<br>Negative                     | Influenza A (seasonal or S-OIV) Detected<br>Definitively negative for BOTH seasonal influenza and S-OIV  |
| 3                     | <i>Influenza A Subtype PCR</i><br>(hemagglutinin (HA) gene)  | H1<br>H3<br>Non-Typeable<br>Unresolvable | Seasonal H1N1 subtype<br>Seasonal H3N2 subtype<br>Highly suggestive of S-OIV, and will be followed by confirmatory result<br>Low viral titre than does not allow discrimination between seasonal & S-OIV |
| 4                     | <i>Influenza A Subtype PCR</i><br>(S-OIV confirmatory assay) | Influenza A (H1N1)<br>Swine              | Confirms S-OIV either from a previously reported “Non-Typeable” result or positive influenza A screen  |

**Questions:**

Contact the Virologist-on-call:  
Edmonton Laboratory at (780) 407 7121  
Calgary Laboratory at (403) 944 1200





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Marie Louie, MD FRCP(C)  
Associate Medical Director and  
Program Leader  
ProvLab  
3030, Hospital Drive NW  
Calgary, AB T2N 4W4  
[M.Louie@provlab.ab.ca](mailto:M.Louie@provlab.ab.ca)  
Tel: 403 944 1263

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Kevin Fonseca, PhD, ABMM  
Clinical Virologist and  
Program Leader  
ProvLab  
3030, Hospital Drive NW  
Calgary, AB T2N 4W4  
[K.Fonseca@provlab.ab.ca](mailto:K.Fonseca@provlab.ab.ca)  
Tel: 403 944 1263

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