

LABORATORY BULLETIN

2009-October-26

Bulletin #2009 - 15

To: Alberta Health & Wellness, CDC Nurses, Infection Prevention and Control, Infectious Disease Physicians, ICU Physicians, Laboratory Managers & Directors, Laboratory Physicians, Medical Officers of Health, Respiratory Physicians, Transplant Coordinators & Managers, Transplant Program Directors

Re: Guidelines for monitoring viral shedding in ICU patients infected with Pandemic (H1N1) 2009.

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Purpose and summary:

This document provides guidelines for the frequency and modality of testing to be used for monitoring viral shedding in patients infected with Pandemic (H1N1) 2009 admitted to the Intensive Care Unit (ICU). Testing for viral shedding will assist the decision-making by Infection Prevention and Control (IPC) regarding the discontinuation of isolation and infection control precautions.

Testing by RT-PCR is the testing modality of choice.

A review of the literature suggests that persistent positivity by RT-PCR implies ongoing viral replication and therefore it is prudent to assume the presence of infectious viruses.

Monitoring Protocol:

- Monitoring of viral shedding in patients with pandemic influenza in the ICU should be done by RT-PCR, once weekly until shedding stops as documented by negative RT-PCR. Request for higher frequency of testing should be discussed with the Virologist-On-Call (VOC) at ProvLab.
- Testing can be done on nasopharyngeal swab (NPS), or lower respiratory tract (LRT) samples such as broncho-alveolar lavages (BALs) or endotracheal aspirates (ETAs). Caution is advisable in the interpretation of a negative RT-PCR on a NPS in a patient with LRT involvement as testing of a sample from the LRT might be preferable.
- Viral isolation in cell culture is not deemed appropriate to monitor viral shedding, because of lesser sensitivity and longer turn around time.
- Viral isolation in cell culture should be reserved for cases where detection of resistance to oseltamivir (or detection of other mutations of interest) is indicated. Consultation with the VOC is required.
- Persistent shedding in patients treated with antivirals may be due to infection with resistant mutants. Contacting the VOC to arrange for resistance testing may be indicated.
- The decision to discontinue infection control precautions must be taken in consultation with IPC ⁽¹⁾

Background and short review of the literature:

Most infection control guidelines called for isolation of patients with influenza in hospitals for a period of 5-7 days⁽²⁾. This was based on a measured duration of virus shedding of 5-7 days, determined from observations on patients and from experimental infections in human volunteers. This shedding duration was however determined on immunocompetent adults; it has been known for a long time that in young children or in immunocompromised hosts the shedding can last up to 10 days or more^(3,4)

Recent studies to detect influenza A virus in clinical samples have demonstrated that in some patients, shedding occurs for considerably longer. Leekha et al recently showed that among elderly patients with chronic medical conditions, shedding of influenza viruses was observed by RT-PCR beyond 7 days in 54% of patients (29% by viral isolation on cell culture). The authors suggested that it might be prudent for such patients to prolong isolation for the duration of the hospital stay⁽²⁾.

Other studies have also noted prolonged influenza shedding, documented by RT-PCR, in many patients and especially among immunocompromised patients⁽⁵⁻⁸⁾, including an instance of shedding for 275 days⁽⁶⁾.

A positive RT-PCR assay on a patient's sample certainly documents the shedding of viral RNA. Given that the mucosa, mucous and lining fluid all contain RNases, the actual detection of viral RNA implies intact virions and/or intracellular RNA (the latter implies actively infected cells). The question is whether these virions are still infectious. The argument usually invoked at this juncture is that RT-PCR does not assess infectivity, and a positive assay does not guarantee the presence of *infectious* virions. This is certainly a valid point for a single isolated test. The situation is different if there are several positive RT-PCR results over the course of many days/weeks in the same patient: because of the clearance from the respiratory tract (ciliary motility, swallowing of mucus, action of macrophages etc), non-infectious virus would be cleared within days after a host stops producing virions. Persistence of RT-PCR positivity therefore implies ongoing viral replication. In the presence of ongoing viral replication, it would be wise to assume the presence of infectious viruses.

In their series of patients hospitalized with influenza, Lee et al⁽⁷⁾ noted that a large fraction of patients in fact eradicated the infection and no longer shed viruses at 5 or 7 days after the onset of symptoms, as measured by RT-PCR (31.5% and 67.3%, respectively). This demonstrates that when the infection is eradicated, viral particles are quickly cleared from the respiratory tract, even when measured by RT-PCR; thus, persistence of virus by RT-PCR in the respiratory tract implies ongoing viral replication. Additional published evidence for ongoing viral replication in persistent shedders include the persistence of symptoms correlated with detection of virus by RT-PCR^(6,7); viral clearance at the time of immune reconstitution^(5,6); decreasing viral load upon instigation of oseltamivir treatment⁽⁵⁻⁷⁾; and accumulation of mutations over time^(6,8).

Some have proposed the use of viral isolation in cell culture to decide whether the patient is in fact shedding *infectious* viruses. Such an approach would be misleading because viral culture is considerably less sensitive than RT-PCR. Khanna et al compared quantitative RT-PCR and culture, and established the threshold for positive culture (with the methodology in use at their laboratory) at approximately 1.25×10^5 RNA copies / mL⁽⁵⁾; this leaves a considerable margin for a sizeable amount of infectious viruses to be

present but undetected by culture. One TCID₅₀ (tissue culture infectious dose 50%) contains approximately 400 genome copies [range, 150 to 675; ⁽⁹⁻¹¹⁾], so 1.25×10^5 RNA copies / mL corresponds approximately to 5×10^7 TCID₅₀ per mL; the infectious dose 50% for humans by the aerosol route is about one TCID₅₀, and by the large droplet route, 167 to 302 TCID₅₀ [reviewed in ⁽¹²⁾].

It would therefore be prudent to assume that a patient with a positive RT-PCR test for influenza is still infected and still infectious. The possibility of shedding for more than 7 days is implicitly acknowledged in the interim infection control guidelines of the CDC for SOIV A (H1N1) which advised isolation for 7 days or until resolution of symptoms, whichever is longer ⁽¹³⁾.

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Questions:

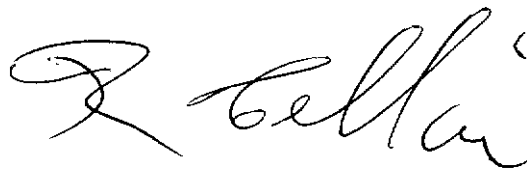
Contact the Virologist-on-Call:

Edmonton Laboratory – Phone: 780 407 7121 (ask for VOC) or 780 407 8822 UAH Switchboard (ask for VOC)	Calgary Laboratory – Phone: 403 944 1200 (ask for VOC)
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