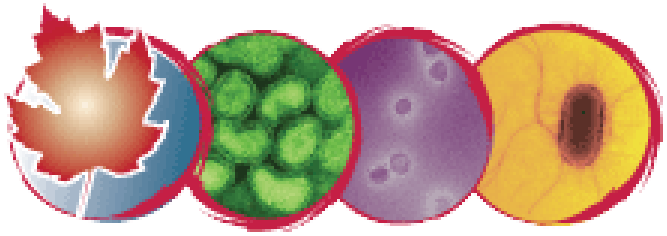
2020

Canadian Public Health Laboratory Network (CPHLN) Protocol for Microbiological Investigations of Emerging Respiratory Pathogens, Including Severe Acute Respiratory Infections (SARI)

MAY 1, 2020

RESPIRATORY VIRUS INFECTIONS WORKING GROUP



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Introduction

Although a protocol for Severe Acute Respiratory Infections (SARI) was initially developed as a response to the 2003 SARS outbreak, its intended use is to facilitate the diagnosis of novel and emerging respiratory infections, including SARI, due to both unknown and known respiratory pathogens that have the potential for large-scale epidemics. With both the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and influenza A(H7N9) virus, a key factor is the determination of risk based on epidemiologic factors, which is in turn related to exposure in an "area of concern". With the more highly transmissible Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus, the cause of the Coronavirus Disease 2019 (COVID-19) pandemic, testing guidelines change depending on the stage of the pandemic. Initial testing focused on those with travel-associated risk factors, with a shift to broader testing once more cases were locally acquired in Canada. The initial risk assessment must be done in concert with your local Ministry of Health (MOH). Signals of novel and emerging respiratory infections, including SARI alerts, should trigger clinicians to "Think, Tell and Test':

- **Think** about the possibility of an emerging respiratory infection (*e.g.* novel influenza A virus)
- **Tell** the local medical officer of health or local public health official; notify your local laboratory and provincial public health laboratory (PPHL) that you are suspecting a novel pathogen.
- **Test** for pathogen only after appropriate consultation and based on clinical symptoms

Laboratory Protocol (Figure 1)

When to Test: Guidance on when to test for novel or emerging pathogens is influenced by many epidemiological factors. At the time of the emergence of a novel pathogen, before wide spread human infection, the probability that a SARI is due to this novel pathogen is extremely low. Therefore, in patients with no epidemiological risk factors the most common pathogens should be ruled out before considering an unusual or more highly virulent pathogen. However, when appropriate risk factors exist, novel pathogens should still be ruled out regardless of whether another pathogen is detected, as viral co-infections have been well documented with novel viral respiratory pathogens (e.g. MERS-CoV, influenza A(H7N9) and SARS-CoV-2)

Although testing is initially focused on those with epidemiologic links, such as travel to a region where the pathogen is circulating, once there is wide spread activity such as in the COVID-19 pandemic, healthcare providers should have a low threshold to consider testing for the novel pathogen when reviewing patients with acute respiratory illness (ARI). However, this will continue to be influenced by the epidemiology of the infection such as the stage of the pandemic wave that the jurisdiction is in; whether there is local or widespread activity; and whether they are in a containment or mitigation phase of response. Specific testing guidelines are developed at the provincial level, and will vary across Canada. Other factors that may influence approaches to testing include the availability of testing supplies and reagents, which may be in short supply at various times during a pandemic. This may be done at the local laboratory or the PPHL depending on local capacity and expertise.

Specimens to Collect: Until the ideal specimen to detect an emerging pathogen has been identified, a broad range of specimens should be collected including nasopharyngeal swab (NPS), throat or combined throat/nares swab, bronchoalveolar lavage (BAL), endotracheal secretions, and sputum. For pediatric patients, a nasopharyngeal aspirate is a suitable replacement to a NPS, although it is an aerosol generating medical procedure which requires airborne precautions whereas NPS does not. Although saliva has been suggested for detection of some emerging pathogens like SARS-CoV-2, it requires more validation prior to being recommended as the sole, specimen for collection.

For patients not admitted to hospital (including those in emergency room settings), a single upper respiratory tract specimen is usually sufficient for testing emerging respiratory virial pathogens (such as SARS-CoV-2 or H7N9). Upper respiratory tract specimens include a NPS, throat OR combined throat/nares swab collected in universal transport medium (UTM). NPS is the preferred specimen due to possible increased sensitivity over a throat swab. A combined throat/nares specimen may also be collected provided the testing laboratory has approved submission of this combined specimen source type.

For hospitalized patients, in particular those with SARI, submission of both an upper and lower respiratory tract specimen is recommended when possible. As above, NPS is the preferred upper respiratory tract specimen. A throat or combined throat/nares swab collected in UTM may be submitted as an additional upper tract specimen. Lower respiratory tract specimens should also be submitted when possible. These include sputum, endotracheal aspirates BAL. For a number of emerging pathogens, including avian influenza and novel coronaviruses, there have been reports of patients who are negative on upper respiratory tract testing being positive when a lower respiratory tract specimen is tested.

Recommended Pathogens and Specimens to Test: At the time of the emergence of a novel pathogen, before wide spread human infection, the probability that a severe acute respiratory illness is due to the novel pathogen is extremely low. Therefore, in patients with no epidemiological risk factors the most common pathogens should be ruled out before considering an unusual or more highly virulent pathogen. This includes:

Conventional bacteria

- Sputum for routine bacterial Gram stain and culture.

Atypical bacteria

- Legionella –sputum, BAL, endotracheal aspirate, lung tissue for PCR and/or culture.
- Urine for Legionella urinary antigen testing.
- Mycoplasma/Chlamydia – NPS, throat swab, and/or lower tract specimen for PCR and/or culture.

Conventional respiratory viruses (*e.g.* human influenza, parainfluenza, respiratory syncytial virus, adenovirus, human metapneumovirus, rhinovirus/enterovirus, coronavirus)

Specimens: NPS, endotracheal secretions, BAL, +/- throat swab (or combined throat/nares swab) and sputum.

- NPS is the primary specimen type for respiratory viruses including seasonal influenza. However, based on our experience with pandemic H1N1, deeper specimens such as endotracheal

secretions or BAL must be collected in cases of severe respiratory infection with negative NPS.

- A number of avian influenza A viruses, including H7N9, have been detected in throat swabs. H7N9 was only detectable in sputum specimen in 1 of 4 patients. While sputum and throat swabs are not ideal for most influenza viruses, multiple specimens types should be considered in patients suspected of having avian influenza A viruses

Testing Methods:

Testing should be conducted using assays validated for the specific pathogen:

- SARS-CoV-2 by real-time RT-PCR (rRT-PCR) testing (see above).
- Influenza A and B by rRT-PCR with subtyping (H3N2 or H1N1) should be the primary method for detection of influenza (24 hour turnaround time). Preferred protocols for detection of novel influenza viruses are those developed by the United States Centers for Disease Control (CDC).
- Respiratory multiplex RT-PCR for parainfluenza, human metapneumovirus, coronavirus, rhinovirus/enterovirus, and adenovirus should be done on all specimens if possible, or on influenza-negative specimens when there is a clinical indication to detect non-influenza viruses.
- Rapid influenza diagnostic tests (RIDT) should not be used to rule out influenza A. The sensitivity of currently available RIDT for human influenza strains is suboptimal. The sensitivity of currently available commercial tests for detection of H7N9 is poor, and should not be used for clinical testing.
- SARS-CoV-2, novel influenza A viruses and the MERS-CoV are classified as RG 3 pathogens. Routine culturing of specimens from suspect patients should only be considered in PPHLs with containment level (CL) 3 facilities. Virus culture in a CL2 laboratory may be considered if the specimen has been tested for the relevant emerging pathogens and is negative by rRT-PCR.

- If more invasive samples are collected they should be processed for a wide range of pathogens:
  - BAL for testing for a broad range of pathogens (bacteria, viruses, mycobacteria, fungi)
  - Open lung biopsy –bacterial, mycobacterial and fungal, cultures, RT-PCR and histology (ensure specimen is NOT PUT IN FORMALIN)

When to Suspect Sars-Cov-2 Virus, the Cause of COVID-19

During the early phases of the pandemic, which began in Wuhan, China in December 2019, only persons who returned from Wuhan, then the province of Hubei, China with ARI, were considered for testing. With the progression of the epidemic, testing of those with ARI after return from travel to countries with COVID-19 activity was indicated.

Following the evolution of COVID-19 to a pandemic and local transmission in most jurisdictions in Canada, testing approaches were broadened with an initial focus on case identification for contact tracing and testing to support the containment strategy. Testing focuses on: persons with ARI who are travelers returning from areas with local COVID-19 activity; hospitalized persons; contacts of outbreak cases; institutionalized persons; healthcare workers; remote, isolated and/or indigenous communities; and vulnerable populations. Once the case numbers increase with more extensive community transmission and pressures on testing resources, the goal of testing may need to be prioritized to support the mitigation strategy including:

- testing persons at risk for serious disease,
- those likely to transmit virus within a healthcare facility or vulnerable community setting,
- those from whom COVID-19 disease would have an impact on delivering healthcare or critical infrastructure,
- those for whom exposure would put them at risk of testing positive.

Additional groups may be considered for testing, depending on the stage of the pandemic, local policy, and availability of reagents.

COVID-19 testing should be completed for patients who meet testing criteria regardless of whether another pathogen is identified. Early data suggests that up to 30% of patients with COVID-19 can have co-infection with other respiratory viruses.

Further information on laboratory testing for COVID-19 is available from the Canadian Public Health Laboratory Network (CPHLN) COVID-19 Best Practices document.

When to Suspect the Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Limited data suggests that MERS-CoV can present as a co-infection with other viral pathogens. As such, in addition to specimens that are negative for conventional pathogens, those that do have other identified pathogens **but are consistent with suspect cases of MERS-CoV based on the PHAC case definition, or alternatively provincial testing guidelines,** should be tested. The details regarding testing and some control materials for method development are available from the National Microbiology Laboratory (NML). To date only a few PPHLs have developed the capacity to test for this pathogen in-house; all other PPHLs should forward the suspect specimens to the NML for further testing.

When to Suspect a Novel Influenza Virus (Including H7N9)

Influenza viruses that are positive on the initial influenza identification test but cannot be subtyped using RT-PCR should be further characterized. Laboratories that have the capacity to further characterize the specimens by novel subtyping PCRs or sequencing methods (*e.g.* sequence the HA, N, M, or other genes) to determine the subtype of the virus should do so. Those that lack this capacity should rely on the NML for further characterization. However, given that subtyping assays are usually less sensitive than the identification assays, weak positives may not be able to be typed. Based on local experience, each laboratory should evaluate these on a case-by-case basis in concert with their local clinicians and public health colleagues.

Influenza positive specimens outside the influenza season or obtained from patients with a history of exposure to animals (*e.g.* pigs or chickens) and should be routinely submitted to the PPHL and/or NML for characterization

NOTE: *While initial analysis of in-house assays used by many labs suggest they should be effective in identifying H7N9, it is difficult to determine the effect on the sensitivity of testing. This is particularly true of the performance of commercial assays whose primer sequences are not known. It is important for laboratories to have vendors supply information about the ability of their assays to detect novel influenza viruses. Laboratories using Level of Detection Tests should monitor viral sequences and their match to the primers and probes in their assays.*

If a Front Line Laboratory Suspects a Novel/Emerging Respiratory Pathogen:

The initial tests (as outlined above) would be similar but supplemental testing will be required at the PPHL. The Laboratory should communicate with the clinician to ensure that the following specimens are collected:

- A second NPS/endotracheal aspirate or BAL to be used for confirmation by the NML.
  - A viral throat swab (in viral transport media) – A number of avian influenza A viruses including the H7N9 have been detected in throat swabs. Multiple specimen types should be collected when novel influenza viruses are considered, and when possible, include both upper and lower respiratory tract specimens.
  - Acute and convalescent sera collection may be appropriate, depending on the specific virus suspected, and advice from NML and PPHLs. Serology is not recommended for patients suspected of influenza A(H7N9) or MERS-CoV infection. SARS-CoV-2 serology assays have been developed by several commercial providers and are being evaluated by NML and some PPHLs. These include ELISA-based and imunochromatographic point of care tests. Their role in clinical testing and public health is yet to be clarified as insufficient data is available on sensitivity, specificity, and positive and negative predictive values. Testing guidelines will be developed once assay performance characteristics have been elucidated and assays are validated for clinical testing.

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If a PPHL Suspects a Novel Respiratory Pathogen:

- The PPHL should notify the patient's healthcare provider, local public health unit, and MOH immediately when a suspect specimen is identified.
- All specimens with suspected novel respiratory pathogens (as outlined below) must be forwarded to the NML for confirmatory testing. If a novel respiratory pathogen causes an epidemic or pandemic, with local transmission, only early specimens will be sent to NML for confirmatory testing. In addition, testing may be implemented at hospital or community laboratories, as has occurred during the SARS-CoV-2 pandemic.
- Specimens suspected to contain a novel respiratory virus should be handled using CL2 with enhanced personal protective equipment if manipulated outside a biosafety cabinet.

  Note: *Virus culture should not be conducted on respiratory specimens in a CL 2 laboratory when a novel, or emerging pathogen is suspected as they are RG 3 pathogens, Virus culture, if required, may be considered in a CL 2 setting if the specimen has been tested for these pathogens and is negative by RT-PCR.*

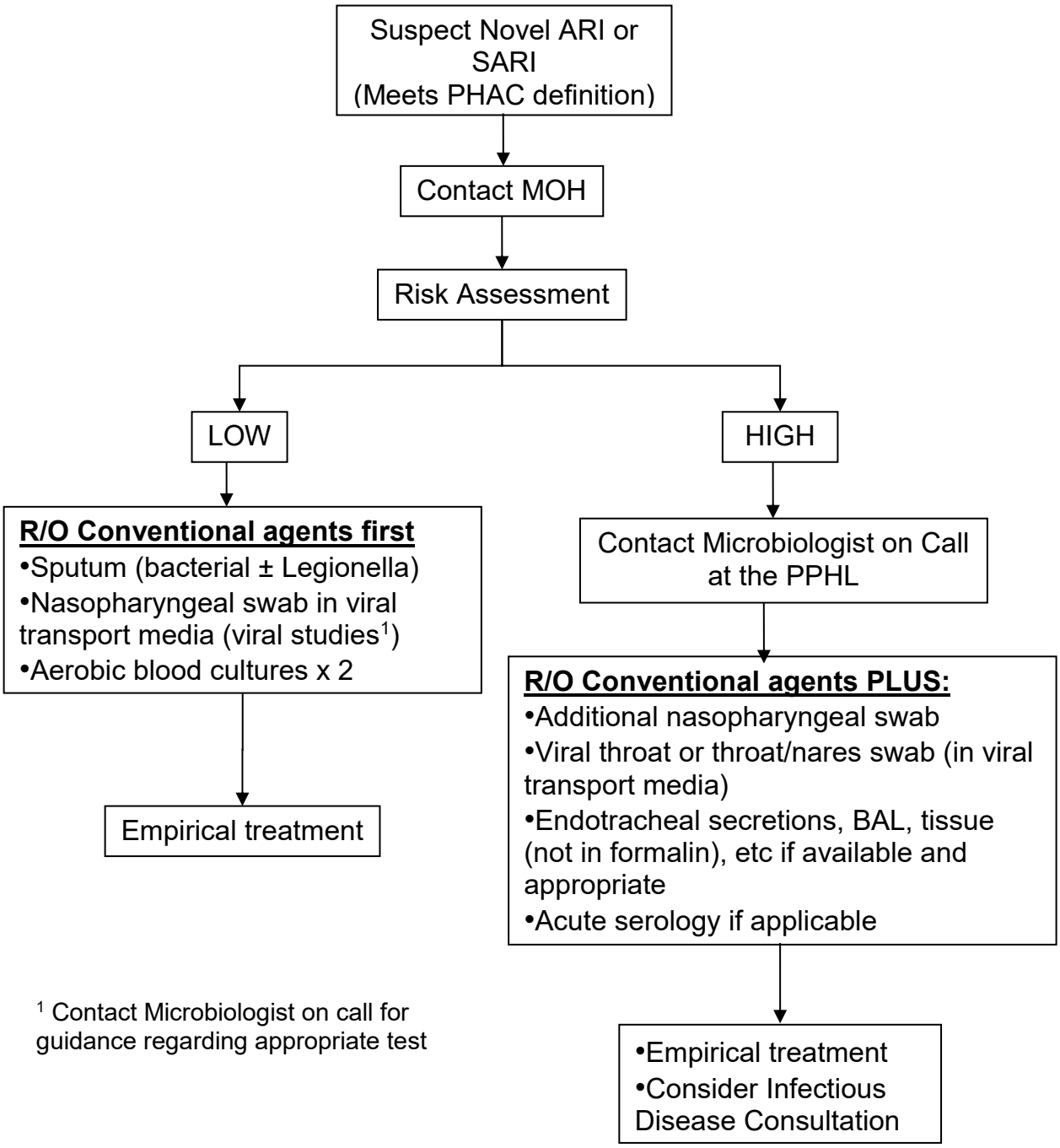
Specimen Transport

Specimens should be transported to the laboratory as soon as possible, preferably within 72 hours on ice packs. If a longer delay is anticipated, specimens should be frozen at -70°C, and transported on dry ice. However, Specimens should not be frozen at -20°C, as this may affect the recovery of the virus if culture is required. If -70°C / dry ice is not available specimens should remain at 4°C and shipped as soon as possible. Specimens should be transported as diagnostic specimens per the usual practice for seasonal influenza specimens, and no enhanced precautions are necessary. See the PHAC SARS-CoV-2 Biosafety Advisory for more information.

Please ensure that the specimen tube is labeled and requisition is completed correctly and fully, with matching patient names and unique identifiers, and relevant clinical information.

Figure 1: Laboratory Protocol

•**Think** about the possibility of an emerging respiratory infection, *e.g.* novel influenza virus
•**Tell** the local medical officer of health or local public health official; notify your local laboratory and provincial public health laboratory (PPHL) that you are suspecting a novel pathogen.
•**Test** for pathogen only after appropriate consultation and based on clinical symptoms



[1] Contact Microbiologist on call for guidance regarding appropriate test