

Severe acute respiratory syndrome coronavirus

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *severe acute respiratory syndrome coronavirus*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Severe acute respiratory syndrome coronavirus (SARS-CoV) infection

1.1.1 Definitive Criteria (1)

- *Virus isolation in cell culture of SARS-CoV from any specimen, with PCR confirmation using a validated method.*
- *Positive on a validated NAD test specific for SARS Co-V*
 - on at least 2 different clinical specimens (e.g., nasopharyngeal swab and stool)
OR
 - the 2 or more samples from the same site collected on 2 or more days during the course of the illness
OR
 - 2 different assays or repeat NAD tests using a new RNA extract from the original clinical sample on each occasion of testing.
- *Seroconversion or a four-fold or greater rise in antibody by neutralization, enzyme-linked immunosorbent assay (ELISA*) or immunofluorescence assay (IFA)**

*Virus antibody detected by EIA or IFA should be confirmed by neutralization to exclude serological cross-reactions with other circulating coronaviruses.

1.1.2 Special Considerations

- SARS *coronavirus* is a biosafety level (BSL) 3 organism and the handling of viable virus, such as isolation or neutralization assays, should only be attempted in a PC3 or PC4 laboratory (2, 3). Untreated clinical specimens that may contain SARS-CoV should be handled in the laboratory with enhanced BSL- 2 precautions (2, 3). Specimens that contain inactivated virus may be manipulated using routine BSL- 2 precautions (2, 3).
- In the absence of current SARS-CoV transmission worldwide (since 2004), testing should be selective (4). Positive test results should be confirmed at a WHO SARS Reference and Verification Network Laboratory (5).
- Diagnostic approaches should be discussed with the relevant PHLN laboratory before sample collection is performed.
- WHO Reference and Verification Laboratories in Australia (5):
 - Victorian Infectious Disease Laboratory, 792 Elizabeth Street, Melbourne, 3000, Victoria, Australia. Tel: +61 (3) 9342 9600; Fax: +61 (3) 9342 9666
 - Animal Health: CSIRO Australian Animal Health Laboratory, Private Bag 24, East Geelong, Victoria, 3219, Australia Tel: +61 (3) 5227 5000; Fax +61 (3) 5227 5555.

2 Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) caused a viral pneumonia that had an approximately 10% mortality rate, during a pandemic in 2002 and 2003 (6). It originated in Southern China and closely related strains have been found in horseshoe bats, palm civets and other mammals (6). It was thought to have crossed the species barrier to humans due to the practice of using wild game as a source of food (6). In 2004, a virus of a separate lineage to the pandemic strain caused a localised outbreak, and illustrated the potential for reemergence (6). The majority of cases of SARS were from person to person transmission (6). Contact with respiratory droplets or fomites were the most significant routes of transmission (6). The virus has been found in tears, urine and feces and these may act as sources of infection (6).

Coronaviruses are RNA viruses in the family *Coronaviridae* (7). Some *coronaviruses* (eg. HKU1, OC43, NL63, 229E) routinely circulate amongst the human population and cause mainly mild respiratory tract infections (7). They have a lipoprotein envelope that surrounds the nucleocapsid (N) protein and genomic RNA (7). The envelope contains membrane (M) proteins, envelope (E) proteins and spike (S) proteins (7). S proteins form projections giving the virus the characteristic corona appearance (7). In SARS-CoV, the S protein binds to angiotensin-converting enzyme 2 (ACE2), the main receptor for the virus on host cells (6). ACE2 is found on lung, heart, kidney, vascular endothelium, intestine, liver and testicular tissue (6).

The incubation period for SARS is usually 2-14 days (6). The clinical presentation typically consists of the systemic and respiratory symptoms of a viral pneumonia (6). Rhinorrhoea and sore throat are less common. Deterioration usually occurs 1 week following the onset of symptoms and was often

associated with diarrhea (6). Other characteristics include liver dysfunction, dizziness, seizures, myositis and petechiae (6). Urinalysis may be abnormal and is associated with high urinary viral loads (6). The peak time for transmission is 5 or more days after the onset of symptoms (6). The peak viral load in nasopharyngeal secretions is 10 days after symptoms (6). Higher nasopharyngeal and serum viral loads are associated with more severe pneumonia and a higher mortality (6). Pregnancy and comorbidities are risk factors for mortality (6).

The differential diagnosis of SARS includes other causes of viral pneumonia, such as *influenza viruses*, *respiratory syncytial virus*, *parainfluenzaviruses*, *rhinoviruses*, *metapneumovirus*, *enteroviruses* and *other coronaviruses* (1). *Middle East Respiratory Syndrome coronavirus* (MERS-CoV) was described in 2012, and is also associated with severe viral pneumonia (8). Non-viral causes of atypical pneumonia include *Legionella pneumophila*, *Legionella longbeachae*, *Mycoplasma pneumonia*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Coxiella burnetii*.

The management of SARS cases consists of supportive therapy and the prevention of transmission through prompt recognition of cases, the implementation of infection control measures and the tracing of contacts (6).

For patients in health care facilities, it is recommended that precautions remain in place for 10 days following the resolution of the illness (9). Australian guidelines advise convalescent patients remain at home for at least 7 days and then have clinical assessment (10). The US CDC recommends outpatients remain in the home as much as possible for 10 days following the illness (11).

Respiratory transmission is thought to be primarily through droplet spread, however, since there may be the potential for airborne transmission, airborne precautions (fit tested N95 masks) in addition to contact precautions (gown, gloves, protective eyewear) are recommended for routine care (6, 10). Ideally, hospitalised patients should be accommodated in single, negative pressure (respiratory isolation) rooms (10). Powered air-purifying respirators (PAPR) are recommended to provide additional protection for procedures that generate aerosols, such as the use of nebulized therapy, diagnostic sputum induction, bronchoscopy, airway suctioning and tracheostomies, positive pressure ventilation via face mask (BiPAP, CPAP), and high-frequency oscillatory ventilation (10).

The SARS-CoV is stable in the environment and can remain viable in the 2-3 days on dry surfaces (6). It was found to be viable in stool specimens for 4 days and respiratory secretions for greater than 7 days (6). It is inactivated by disinfectants, such as bleach (sodium hypochlorite) and 70% ethanol, and these are recommended for cleaning surfaces (6, 10).

3 Tests

Algorithms for the testing of suspect cases of SARS-CoV infection are based on clear epidemiological risk factors and clinical features (fever $\geq 38^{\circ}\text{C}$ or history of fever and respiratory symptoms): see references (1) and (4). Tests for other causes of viral and atypical pneumonia should also be performed (see Section 2.0). Selective testing for SARS-CoV and the verification of results by reference laboratories is necessary to avoid alarm based on false positive results.

3.1 Specimen collection and transportation

Recommended specimens are upper respiratory (eg. nasopharyngeal swabs / oropharyngeal swabs), lower respiratory (eg sputum), blood (serum and plasma), and stool (12). Tissue from biopsies or post-mortem examinations can also be used (12). However, the aerosol-generating collection procedures (eg nasopharyngeal aspirates and bronchoalveolar lavages) should be avoided if possible due to the risk to staff (12). If necessary, they should be performed in a negative-pressure room with respiratory precautions in place. See reference (12) for more details.

To minimise inhibition of RT-PCR, swabs should be dacron or rayon (not calcium alginate) and have plastic (not wooden) shafts (13). Swabs and other specimens for viral isolation can be placed in viral transport medium (13). Specimen containers should be sterile, leak-proof, properly sealed and placed in bags with clean (non-contaminated) external surfaces: double bag, if necessary (2). Use a “no touch technique” when packing samples (2). Specimens should not be transported in pneumatic tube systems, due to the potential for leakage and widespread contamination (2). Most specimens should be shipped on ice (13). It is recommended that peripheral laboratories make aliquots of original specimens that can be sent unopened to reference laboratories to verify positive results (5). Sequential testing is also recommended (13).

3.2 Laboratory Precautions

The initial laboratory processing of untreated samples should be performed using enhanced BSL2 precautions (2, 3). These are using BSL2 facilities with BSL3 precautions, including respiratory protection such as N95/P2 masks, impermeable disposable gowns and full eye protection (2, 3). Routine BSL2 precautions can be performed on specimens that have been treated to inactivate the virus, such as nucleic acid extracts for PCR (2, 3). Any procedure involving potentially viable virus requires full BSL3 precautions and should not be attempted outside a PC3 or PC4 laboratory (2, 3). For more details see references (2) and (3).

3.3 Nucleic acid testing (NAT)

3.3.1 Suitable specimens

SARS-CoV has been found in respiratory secretions, cerebrospinal fluid, urine, blood (plasma and serum), stool and tissue (6). These are suitable specimens for nucleic acid tests. See reference (12) for recommended specimens to be collected from suspected SARS cases.

3.3.2 Nucleic Acid Tests

Suitable NAT are RT-PCR assays or other methods that have been validated for the detection of SARS-CoV. Most primers target the *pol* 1b open reading frame or the nucleocapsid gene (6, 14). Assays that target regions conserved between SARS-CoV and other coronaviruses can be used as a routine test (7). Specific assays for SARS-CoV identification can then be performed on positive samples, if warranted on clinical and epidemiological grounds (4).

3.3.3 Sensitivity and Specificity

The sensitivities of various nucleic acid tests is summarised in table 4 of reference (6). A RT-PCR that targeted the *Orf 1b* had a sensitivity of >80%, when nasopharyngeal aspirates were used as specimens. Clinical sensitivity may be improved by testing multiple samples from each patient (7).

A comparison of 6 different RT-PCR methods for SARS-CoV detection demonstrated clinical specificities that ranged from 94 to 100% (15). Non-specific amplification of DNA from circulating *coronaviruses* and contamination are potential sources of false-positive results. Selective testing based on clinical and epidemiological features, the sequencing of products, the collection of multiple samples or multiple extractions, and the verification of results at reference laboratories serve to minimise the risk of false positive results (5). Where feasible, the products of RT-PCR assays should be sequenced to confirm the identity of amplified nucleic acid.

3.3.4 Internal Controls

Positive and negative controls for nucleic acids test should include: one negative control for the extraction procedure, a water negative control for the run, one positive control for extraction and the run and a patient sample spiked with a weak positive control to check for inhibitors (16).

3.3.5 Quality Assurance

The RT-PCRs for SARS-CoV at WHO Reference and Verification Laboratories were validated with external quality control specimens during the outbreak. Other PHLN laboratories with SARS-CoV NAT capacity have participated in local and international QAP.

3.3.6 Special Considerations

Negative nucleic acid test results do not exclude infection. While a positive result is indicative of the presence of SARS-CoV nucleic acid, the virus may not be viable or present in sufficient quantity to be cause infection in other people (17).

3.4 Serology

3.4.1 Suitable specimens

Acute and convalescent serum. See reference (12) for the recommended timing of collections.

3.4.2 Serological Tests

Validated serological tests include IFAs and ELISAs and these are compared in reference (6). Virus neutralisation should be performed on positive results to exclude cross-reactions with other *coronaviruses* (1).

3.4.3 Sensitivity and Specificity

Results for sensitivity and specificity of various serological assays are compared in reference (6). Sensitivity is increased in from the second week of illness onwards (6). A study of IFA using fixed SARS-CoV antigen showed a sensitivity and specificity of 100% from day 21 to 37, from the onset of fever (18). However, 2% of the uninfected controls had a non-specific reaction with uninfected cultured

cells. Patients who have infection with SARS-CoV may also cross-react on serology for HCoV-229E and HCoV-043 (19, 20). Due to the potential for cross-reactivity between SARS-CoV and other coronaviruses, confirmatory viral neutralization is recommended (1).

3.4.4 Suitable Internal Controls

Positive (high, low) and negative controls should be included to validate each test run.

3.4.5 Quality Assurance

The IFA for SARS-CoV at WHO Reference and Verification Laboratories was validated with external quality control specimens during the outbreak.

3.4.6 Special Considerations

No detection of antibody after 21 days from onset of illness indicates that SARS-CoV infection was not present (17).

Viral neutralisation should only be performed in a PC3 or PC4 laboratory by trained staff and with appropriate precautions (1).

3.5 Viral isolation

3.5.1 Suitable specimens

Virus has been found in respiratory secretions, cerebrospinal fluid, urine, stool and tissue and these are suitable specimens for culture. See 3.1.

3.5.2 Cell culture

Compared to most other *coronaviruses*, SARS-CoV, grows well in cell culture (14). However, cell culture techniques are not usually used for diagnostic purposes, due to the infectious risk and reduced sensitivity compared to PCR from clinical specimens (14). SARS-CoV grows in various cell lines including Vero, Vero-E6, Caco-2, FRhK-4 and R-Mix Cells (Diagnostic Hybrids Inc) (6). SARS-CoV grows poorly some cell lines routinely used for respiratory virus cell culture: Rhesus monkey kidney, MDCK, Hep-2, MRC-5, AGMK, RDE and LLC-Mk2 (14). SARS-CoV can be detected with electron microscopy of viral isolates (7). Specific identification can be performed with antibody neutralization or nucleic acid amplification and amplicon sequencing.

3.5.3 Special Considerations

SARS-CoV is a biosafety level 3 organism. Any culture of the virus should only be attempted in a PC3 or PC4 laboratory, by trained staff and with appropriate precautions (2). A negative cell culture result does not exclude the presence of the infection (17).

4.0 Agreed Typing & Subtyping Methods

Family *Coronaviridae*, Genus *Coronavirus*, Group 2b, Species SARS-CoV.

SARS-CoV can be subtyped according to nucleotide sequence analyses using multilocus sequence typing, helicase and RNA polymerase, and full-length sequences (7, 21, 22).

4.1 Laboratory Nomenclature for National Database Dictionary

SNOMED CT concept

SARS-coronavirus (organism) Code 415360003

5 References

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