



WHO SARS International Reference and Verification Laboratory Network: Policy and Procedures in the Inter-Epidemic Period

1. INTRODUCTION

WHO announced that the last chain of human transmission of SARS coronavirus (SARS-CoV) was broken on 5 July 2003, and thus the epidemic was over. For cases of SARS to reappear, the virus has to re-emerge from one of three sources: from an animal source, from a laboratory accident, or from undetected transmission cycles in human populations. However, regardless of the source of re-emergence, its rapid detection and specific diagnosis will be crucial in preventing any re-emergence of new cases which could initiate a new epidemic. Since 5 July there have been a number of laboratory confirmed SARS-CoV infections resulting from laboratory accidents (Singapore and Taiwan, China) and from exposure to animal sources or environmental contamination (China). Although none of these cases has been fatal nor resulted in secondary transmission, the resurgence of SARS leading to an epidemic remains a distinct possibility. In the post-epidemic period, all countries must remain vigilant for the recurrence of SARS and maintain their capacity to detect and respond to the re-emergence of SARS should it occur.

With the approach of the northern winter and the possibility of increased influenza activity, a meeting was held of an expert laboratory group on 22 October, 2003, to address the issues of detection and diagnosis, and the management of false positive results which are likely to occur when a large number of tests are conducted in a situation of very low disease prevalence.

The major recommendations made by the expert group were:

1. To establish a quality assurance programme and standardization of laboratory tests and protocols;
2. To establish a WHO SARS Reference and Verification Laboratory Network to undertake verification of suspected cases of SARS-CoV infection, with terms of reference requiring such laboratories to be active members of the quality assurance programme;
3. To require all sporadic (non-epidemic) cases of suspected SARS-CoV infection to be verified by a WHO Reference and Verification Laboratory external to the country in which the case occurred;
4. To acquire and assist in the development of a panel of positive SARS-CoV control sera;

5. To develop algorithms for assessing the need to test patients for SARS-CoV infection based on an epidemiologic and geographic risk assessment;
6. To endorse the guidelines for biosafety in laboratories handling diagnostic specimens potentially containing SARS-CoV or culturing SARS-CoV, which should be done in biocontainment level 3 facilities where tests involve virus propagation, or in biocontainment level 2 facilities with level 3 work practices where tests involve manipulations of live virus without propagation;
7. To strongly recommend that countries maintain an inventory of laboratories working with SARS-CoV, and an inventory of cultures of SARS-CoV in those laboratories.

These recommendations are shown in full at
<http://www.who.int/csr/sars/guidelines/en/SARSLabmeeting.pdf>

This document will only address recommendations 1 -3. The other recommendations will be addressed elsewhere.

2. STRUCTURE AND FUNCTION OF THE LABORATORY NETWORK

The WHO SARS Reference and Verification Laboratory Network was initiated in late 2003. It was based largely on the laboratory network established by WHO in March 2003 during the SARS epidemic, with some additional laboratories to provide greater geographic representation. The laboratories are listed in Annex 1. Each laboratory was invited officially to participate in the network, and in so doing, agreed to accept the terms of reference which included the requirement to take part in regular quality assurance programmes. This latter issue is important as a number of different tests are used or are available, or may be developed in the future, for both virus detection and serology, some of unproven reliability, and it is essential that there is scientific and public trust in the outcome of all diagnostic tests used for the diagnosis of SARS-CoV infection.

2.1 Indications for testing

WHO recommends that clinicians, epidemiologists and laboratory experts consult on persons under investigation for SARS in the post-epidemic period. In low risk settings where false positive results for SARS-CoV are more likely, the triage process should ensure that testing for SARS-CoV is only undertaken when there is clinical and/or epidemiological evidence that SARS may be the cause in an individual or cluster of patients to avoid the inappropriate use of scarce resources and the risk of overwhelming the health system by unnecessary activation of hospital-based and public health response teams.

2.2 Clinical specimens required for laboratory diagnosis

The reliability of laboratory diagnostic tests for SARS-CoV infection depend crucially on the type of clinical specimens collected, the time of collection and the mode of collection. In addition, it is essential that the specimens are collected by well trained

personnel. Thus it is important that, at a minimum, respiratory samples are taken during the first week of illness, together with plasma or serum specimens for virus isolation and for acute phase serology. Respiratory samples should ideally include nasopharyngeal aspirates, provided full infection control procedures are in place to protect staff and other patients. Respiratory and stool specimens should be routinely collected for virus isolation or detection by RT-PCR during the first and second weeks, recognizing that these specimens may be the most likely to yield virus. Serum specimens should also be collected for serology in the second and third weeks to detect a rising titre by testing acute and convalescent sera in parallel. Evidence from animal coronaviruses suggest that persistent infection with SARS-CoV could possibly occur in humans, and indeed prolonged shedding of virus may occur in stools, but the role of this virus as a potential source of reinfection is not known.

Clinical samples should be separated into three aliquots at the time of collection, or in a secure laboratory which is clean and in which there is no ongoing work on SARS-CoV strains. One aliquot should be used by the local diagnostic laboratory, the second aliquot should only be used, unopened, by the national reference laboratory, and the third aliquot should be retained for use by the WHO SARS Reference and Verification Laboratory should verification be necessary.

2.3 External verification

External verification is necessary in the event of positive clinical specimens reported by either the local diagnostic laboratory or the national reference laboratory. Selection of an external member of the WHO SARS Reference and Verification Laboratory Network to undertake verification is a choice of the national health authorities. WHO will be happy to advise if required. Appropriate arrangements for shipment should only be made after the selected Network member laboratory has indicated that it is able to accept the shipment. All packaging for shipping specimens must conform to international regulations. These are referred to in the document, *WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and cultures* which is found on the WHO website at http://www.who.int/csr/sars/biosafety2003_12_18/en/

It is important to recognize that the most effective diagnostic tests now available are those which provide information on the viral genome, especially RT-PCR or realtime PCR of genomic fragments or cultured virus. The importance of the RT-PCR test is that it can be used to make a relatively early diagnosis, and quality assurance of a number of laboratories has demonstrated that most laboratories are able to perform these tests with a low incidence of false positives. A positive RT-PCR test should be repeated by the national reference laboratory using the second, unopened aliquot, as described above, and also repeated by the local diagnostic laboratory using a second, independently collected sample. Subsequent isolation of the virus under appropriate biocontainment facilities is particularly valuable for phylogenetic and epidemiological investigations.

Serological testing is improving, although quality assurance has recently indicated a significant level of missed positive specimens and of false positive results. Where acute and convalescent phase paired specimens are available and show four-fold or greater rise in titre when tests are carried out in parallel, but no PCR product has been available or virus isolated, viral neutralization assays should be performed by the national reference laboratory and by a WHO SARS Reference and Verification Laboratory for

final confirmation and to ensure that the rising titre is not due to a second human coronavirus. Western blots may be useful if a second related virus is suspected.

Additional and more specific diagnostic tests may become available in due course with third generation technologies, and may provide greater specificity, safety and speed.

3. THE WHO STRATEGY FOR DIAGNOSING SARS, AND THE ROLE OF THE SARS REFERENCE AND VERIFICATION LABORATORY

The establishment of the international laboratory network to provide timely, accurate and effective isolation, identification, and verification of infection with SARS-CoV is crucial for strengthening surveillance and control of SARS. The network will also contribute to improving our understanding of the virus and the disease it causes.

The proposed process is a three-tier network of laboratories (i.e. local laboratories, national laboratories, and international reference laboratories), each with defined responsibilities.

1. **A local laboratory** should provide services relating to the initial diagnosis of SARS-CoV infections using standard procedures. It would usually be able to undertake RT-PCR and serological tests, but unless it has suitable biocontainment facilities, it would not undertake virus isolation and/or culture. Positive diagnostic results indicative of SARS-CoV infection in non-epidemic periods would then be sent to the designated national reference laboratory for confirmation and further investigation, and the national public health authority notified. Infection control measures should be initiated. Should there be compelling clinical and epidemiological evidence in addition to these preliminary laboratory findings, then the national public health authority should inform the WHO Regional Office and WHO headquarters in Geneva.

2. **A national reference laboratory** should undertake confirmation of the results of persons under investigation, and conduct further investigations including virus isolation. At this stage, a positive result might be announced *internationally* as a probable case, depending in part on epidemiological and clinical findings, and unopened aliquots of all specimens collected from the patient sent for verification to an external member of the WHO SARS Reference and Verification Laboratory Network. The choice of the Verification Laboratory will be made by the national reference laboratory or by the national public health authority.

3. **Members of the WHO SARS Reference and Verification Laboratory Network** would be responsible for providing verification of all probable cases of SARS-CoV infection, and would do so whenever requested by any national health authority or a national reference laboratory. Verification or confirmation of a suspect case would be necessary for elevating a suspect case to be a confirmed case. The WHO SARS Reference and Verification Laboratory Network will also undertake diagnostic functions for those countries which do not have a national reference laboratory with the necessary biocontainment facilities. WHO will facilitate testing at one of the Network laboratories for Member States without their own SARS-CoV testing facilities.

3.1 *The need for caution in serological diagnosis*

Much still remains to be determined about SARS-CoV and related viruses, especially the role played by other human (OC43 and 229E) and animal coronaviruses in eliciting serological cross-reactions and in generating antigenic recall (original antigenic sin). Some degree of caution needs to be exercised in the interpretation of serological data in non-epidemic periods, and especially where no viral sequence data are available. It is expected that virus neutralization tests should distinguish antibodies specific to SARS-CoV where cross-reacting antibodies appear to be present in other serological tests. Co-infection with a related human coronavirus may be more difficult to distinguish, although the use of expressed proteins in Western blots may help to sort this out. It is recognized that there could be instances where interpretation is complicated by these and other factors, and it is therefore suggested that representatives of the WHO SARS Reference and Verification Laboratory Network act as an *ex officio* expert panel of review should this prove necessary in difficult cases.

Other roles of the WHO SARS Reference and Verification Laboratory Network include training in SARS diagnosis within a regional framework, and the provision of advice concerning diagnostic methodologies and reagents.

Laboratory safety is a major issue when working with SARS-CoV. All laboratories concerned with SARS diagnosis, confirmation and verification are urged to take all necessary precautions, as described in the document, *WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and cultures* which is found on the WHO website at (http://www.who.int/csr/sars/biosafety2003_12_18/en/)

The strategy implicit in the above procedures has been used successfully in the verification of the two laboratory-acquired cases in Singapore and Taiwan China, and most recently in verifying the two sporadic cases from Guangdong, China. WHO believes the transparency used in diagnosis, confirmation and verification of these cases greatly assisted the generation of public trust in the ability of national health authorities to diagnose and control such cases.

4. INTERPRETATION OF LABORATORY RESULTS

The interpretation of laboratory results should follow the following guidelines. These guidelines take into account any problems that might conceivably arise if related viruses to SARS-CoV occur in animals and can cause human infections, and also if antibodies to SARS-CoV cross-react with other human coronaviruses, OC43 or 229E, or the reverse, that antibodies to OC43 or 229E cross-react with SARS-CoV. Co-infections between SARS-CoV and a human coronavirus may remain difficult to diagnose serologically, although multiplex PCR tests could provide a method to detect genomic fragments of both viruses.

4.1 *RT-PCR-positive specimen and/or virus isolation*

If a specimen is RT-PCR positive for SARS-CoV, or a virus is isolated from a specimen (specimen may be blood, respiratory, stool, urine etc), then a second unopened aliquot of the same specimen should be found to be positive in a second laboratory, AND a second independently-collected specimen must also be found to be positive. It is important to sequence the PCR products to verify SARS-CoV as the infecting virus

rather than a related virus. Care must be taken to ensure that the region selected for amplification is specific for SARS-CoV and not conserved amongst other known coronaviruses.

4.2 Serology

If acute and convalescent phase sera are collected at least 8-10 days apart, a four-fold or greater rise in antibody titre when tested in parallel by IFA, ELISA (using well-characterized antigen), or other serological test, should be considered indicative of a suspect case, but confirmation should be obtained by virus neutralization. This caveat of virus neutralization is strongly recommended because of the possibility of cross-reacting antibodies with at least one other human coronavirus, OC43. Until the basis of cross-reactivity and the conditions under which cross-reactivity occurs are better understood, the use of virus neutralization should be mandatory in verification.

5. Reporting of laboratory results

For the purposes of the international reporting of SARS, Member States are requested to officially report to WHO probable and laboratory-confirmed cases only. However, given the global attention given to SARS rumours, informing WHO of clusters of acute respiratory disease and/or high risk individuals under investigation for SARS will facilitate the accurate dissemination of information to other Member States, the media and the public.

All cases which are confirmed as positive by the national reference laboratory (probable cases) should be then sent to at least one external member of the WHO SARS Reference and Verification Laboratory Network for verification before being announced internationally as a laboratory-confirmed case. It must be remembered that *case confirmation* requires the inclusion of clinical and epidemiological data.

Members of the WHO SARS International Reference and Verification Laboratory Network, to which specimens are sent for verification, should report the results of verification tests first to the national public health authorities which sent the specimens, and then, with their permission, to the WHO Regional Office and WHO Headquarters in Geneva. The results should remain confidential until released by the requesting national authority.

Please note that this document will be subject to change and updating as new diagnostic techniques or other relevant information becomes available.

ANNEX 1: WHO SARS REFERENCE AND VERIFICATION LABORATORY NETWORK

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