World Health Organization

SUMMARY OF THE DISCUSSION AND RECOMMENDATIONS OF THE SARS LABORATORY WORKSHOP, 22 OCTOBER 2003

An informal SARS Laboratory Workshop was held at WHO, Geneva, on Wednesday, 22 October 2003, to discuss aspects of the laboratory diagnosis of SARS coronavirus (CoV) infection pertaining to the standardization of test protocols and reagents, the development of a panel of positive control sera for diagnostic serology, the development of a set of strategic plans or algorithms to provide guidance to laboratories about testing of specimens from patients with atypical pneumonia in non-epidemic periods, and biosafety and biocontainment issues in laboratories working with live SARS CoV. The workshop brought together 27 members of a new enlarged laboratory network from 15 countries and regions and a further 7 observers.

The major discussion points and recommendations were as follows:

(i) Diagnostic tests, their standardization, and quality assurance

All major, active diagnostic laboratories described the tests they currently employ and the tests they have used for comparison and validation. Currently, a variety of different SARS tests are available both for commercial and for in-house use. Almost all laboratories that were represented in the meeting have developed their own in-house tests, for nucleic acid detection (e.g. RT-PCR), as well as for serology (e.g. IF, ELISA, Western blots, neutralisation tests etc. with a range of native and recombinant antigens). The "overall" sensitivity of the RT-PCR was shown to be around 70% within the first days after disease onset, however, highly dependent on the type of specimen collected and increasing with time collected after onset. Most experience had been gained with the Artus and Roche commercial tests and various in-house tests, and the overall view was that they were generally comparable given similar specimens (by type of specimen, collection method and time post onset). There was less uniformity in serological tests, but in general IF tests were similar, and some ELISAs were equivalent. The major problems with serological tests were with possible cross-reactions between coronaviruses, and especially between SARS CoV and human coronavirus 229E, and with recombinant expressed antigens such as the nucleo-protein gene in some expression systems, especially expressed in the *E.coli* versus the baculovirus system.

The meeting agreed on the urgent need for quality control, standardization of test protocols and reagents, the need for verification of initial cases/clusters in non-epidemic periods, and the establishment of international reference and verification laboratories.
The meeting also recognized the need for repositories of human and animal SARS CoV strains, and for sera monoclonal antibodies and human convalescent serum samples.

The recommendations were that:

1. **There should be a process of standardization of laboratory tests and protocols.**

2. **There should be a process of quality assurance of laboratory tests and protocols**

3. **There should be established a network of international reference and verification laboratories for SARS.**

4. **There should be a process of verification of laboratory confirmed cases of SARS CoV infections during the post-outbreak period, preferably by an external laboratory which is also an international reference laboratory network. In addition, during an established outbreak of SARS the first case(s) in any new cluster fulfilling the WHO SARS Alert criteria and sporadic cases consistent with the clinical case definition of SARS should be tested to confirm the diagnosis. WHO advises that a statistical sample of clinically compatible cases is tested during a sustained outbreak to ensure that there is no diagnostic confusion with other infectious conditions that mimic SARS clinically.**

5. **There should be repositories established of SARS CoV strains from human and animal origin.**

(ii) Development of a panel of positive control sera

The participants discussed the need for a panel of positive control sera for use in SARS serological diagnosis. It was acknowledged that the availability of well-characterized sera was limited and indeed was decreasing. It was suggested that individual countries be asked to assist, and that it might be possible to invite donation of sera from known cases of SARS. In addition, it would be particularly useful for such a control panel to have antisera to the other human coronaviruses, 229E and OC43. The National Institute for Biological Standards and Control (UK) offered to aliquot, freeze dry and store the sera.

The recommendation was that a panel of positive control sera is established for SARS CoV and related human coronaviruses for use in sero-diagnosis. The participants felt there was considerable urgency for this panel, given the perceived problems of cross-reactions and the imminent influenza season. Participants from the People's Republic of China and Canada suggested that their governments be approached to request assistance in assembling the sera required from voluntary donors.

(iii) Algorithms for the laboratory diagnosis of SARS in the post-outbreak period

The participants discussed the problems inherent in the positive predictive value of testing when population prevalence of a disease is extremely low. The risk of false positive results from SARS CoV testing will be very high in the post-outbreak period given that there is no evidence currently that the virus is circulating in human populations. In addition, other common respiratory diseases causing atypical pneumonia or respiratory distress syndrome (RDS), such as influenza may stimulate inappropriate testing for SARS CoV. This situation should be minimized by clinical and testing algorithms reflecting the local epidemiology of atypical pneumonia or RDS, and by national and local risk assessments of the likelihood of the re-emergence or introduction of SARS. The algorithms should be flexible enough to meet contingencies but also fit into the current epidemiological framework described in the document *"Alert, verification and public health management of SARS in the post-outbreak period"* which is posted on the WHO website (http://www.who.int/csr/sars/postoutbreak/en/).

The recommendation was that algorithms be developed that would provide guidance to clinicians and laboratories about the testing of specimens from atypical pneumonia or RDS cases for SARS CoV. The algorithms should be congruent with those published on the WHO website, and flexible enough to meet specific clinical needs.

It should be noted that while these are based on laboratory requirements, specific requests from infectious diseases physicians should be considered on a case by case basis and if necessary discussed further with the requesting physician. The triage process should include ensuring that testing for SARS-CoV is only undertaken when there is compelling clinical and epidemiological evidence that SARS may be the cause of an outbreak 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.

WHO recommends testing in the following situations:

Low risk areas

- **In the event of a SARS Alert i.e. a cluster in an acute care facility fulfilling the clinical case definition of SARS and with onset of illness in the same 10-day period and where no other cause can fully explain the illness OR**
- **Sporadic case(s) or cluster(s) fulfilling the clinical case definition of SARS epidemiologically linked to a laboratory in which SARS CoV is being studied or in which clinical specimens potentially infected with SARS CoV are being processed or stored.**

Nodal areas

- **Testing during a SARS Alert as above OR**
- **Sporadic case(s) or cluster(s) fulfilling the clinical case definition of SARS epidemiologically linked to a laboratory in which SARS CoV is being studied or in which clinical specimens potentially infected with SARS CoV are being processed or stored.**

In addition, based on the local risk assessment some nodal areas may implement enhanced surveillance for SARS, including the testing of cases of atypical pneumonia and/or RDS not fully explained by another cause, bearing in mind that in the post-outbreak period the pre-test probability of true positive results will be very low.

Zone of potential re-emergence

- **Testing during a SARS Alert as above OR**
- **Sporadic case(s) or cluster(s) fulfilling the clinical case definition of SARS epidemiologically linked to a laboratory in which SARS CoV is being studied or in which clinical specimens potentially infected with SARS CoV are being processed or stored.**

- **Routine testing of cases of atypical pneumonia and/or RDS not fully explained by another cause as part of enhanced surveillance for SARS, bearing in mind that in the post-outbreak period the pre-test probability of true positive results will be very low.**

It is recognized that tests for SARS CoV might be requested for other specific cases such as cases of atypical pneumonia or RDS from travellers from the zone of potential re-emergence, but it is hoped that these should be kept to a minimum.

In areas where access to high dependency care is unavailable or limited, consideration should also be given to SARS CoV testing if there is compelling clinical and epidemiological evidence that an outbreak of acute severe respiratory disease may be SARS after exclusion of more common diagnoses. This requires a sound understanding of the local epidemiology of respiratory disease.

(iv) Biosafety in the laboratory, and inventory of SARS CoV cultures

The importance of laboratory biosafety was clearly demonstrated with the occurrence of a laboratory-acquired case of SARS CoV infection in Singapore last month. The participants discussed a number of biosafety issues, including the biocontainment level for culturing SARS CoV and working with live SARS CoV, the biocontainment level under which SARS CoV cultures and clinical specimens were stored, and the need to have national inventories of SARS CoV and some form of national certification of labs working with SARS CoV.

The recommendations were:

1. **To endorse the WHO biosafety guidelines for handling of SARS specimens which states that SARS CoV should be cultured under biocontainment level 3, and that diagnostic activities which do not involve culturing the virus should be undertaken at a minimum of biocontainment level 2 using level 3 work practices.**

2. **That cultures of SARS CoV should be stored at a minimum of biocontainment level 3, and that clinical specimens known to contain SARS CoV be preferably stored at a similar level, but if not possible, that they and clinical specimens suspected of containing SARS CoV be stored at a minimum of biocontainment level 2 within a secure (locked) environment.**

3. **That national governments maintain an inventory of laboratories working with and/or storing live cultures of SARS CoV, and that the inventory should include clinical specimens known to contain SARS CoV.**

4. **That while not wishing to restrict the research and diagnosis of SARS CoV, that national governments institute a process by which laboratories wishing to work with SARS CoV be licensed to do so.**

Participating Laboratories:

Victorian Infectious Disease Laboratory, Carlton, Australia

CSIRO Australian Animal Health Laboratory, Geelong, Australia

Virology, Immunodiagnostics, Perinatal and Vaccine preventable Diseases Laboratory, Ontario Ministry of

Health and Long Term Care, Toronto, Canada

National Microbiology Laboratory, Health Canada, Winnipeg, Canada

National Institute for Communicable Diseases Prevention and Control, China CDC, Beijing, People's Republic of China

Department of Clinical Laboratory, Peking Union Medical College Hospital, Beijing, People's Republic of China

National Institute for Viral Disease and Control and Prevention, China CDC, Beijing , People's Republic of China

Department of Microbiology, Prince of Wales Hospital, Chinese University, China, Hong Kong Special Administrative Region

Government Virology Unit, Public Health Laboratory Centre, China, Hong Kong Special Administrative Region

Department of Microbiology, Queen Mary Hospital, University of Hong Kong, China, Hong Kong Special Administrative Region

Unité de Génétique, Moléculaire de Virus Respiratoires, National Influenza Centre, WHO Collaborating Centre for Reference on Influenza and other Respiratory Viruses, Institut Pasteur, Paris, France

Department of Virology, Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany

Robert Koch Institute, Berlin, Germany

Institute for Medical Virology & Travelmedicine, J.W. Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany

WHO Collaborating Centre for Reference and Research on Influenza and Department of Virology, National Institute of Infectious Diseases, Tokyo, Japan

Institute of Health and Community Medicine, University Malaysia, Sarawak, Malaysia

Department of Medical Microbiology, National Influenza Centre and WHO Collaborating Center for Arbovirus Reference and Research, Kuala Lumpur, Malaysia

National Influenza Centre, Institute of Virology and WHO Collaborating Centre for Arboviruses and Haemorrhagic Fevers, Reference and Research, Erasmus University, Rotterdam, The Netherlands

Virology Section, Department of Pathology Singapore General Hospital, Singapore

Special Pathogens Unit, National Institute for Communicable Diseases, University of Witwatersrand, Sandringham, Republic of South Africa

Early Warning and Preparedness Unit, Swiss Federal Office of Public Health, Division of Epidemiology and Infectious Diseases, Bern, Switzerland

Division of Virology, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Department of Virology, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam

Enteric and Respiratory Virus Laboratory, Health Protection Agency, London, United Kingdom

Communicable Disease Surveillance Centre, Health Protection Agency, London, United Kingdom

Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

Division of Virology, National Institute for Biological Standards and Control, South Mimms, United Kingdom

Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, United States of America

The Jerome L. and Dawn Greene Infectious Disease Laboratory, Columbia University, New York, United States of America

Food Animal Health Research Program, The Ohio State University, Wooster, United States of America

Centers for Disease Control and Prevention, Atlanta, United States of America