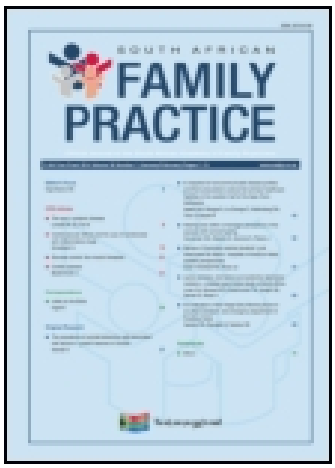

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Developments in the laboratory diagnosis of SARS—coronavirus infections

AA Hoosen MBChB, M Med (Microbiol), FC Path (Microbiol Path)[a] & G Lecatsas PhD (Wits), DSc (UP)[b]

[a] Department of Microbiological Pathology, University of Limpopo (Medunsa Campus), Pretoria

[b] Department of Virology, University of Limpopo (Medunsa Campus), Pretoria

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Developments in the laboratory diagnosis of SARS – coronavirus infections

Hoosen AA, MBChB, M Med (Microbiol), FC Path (Microbiol. Path)
Department of Microbiological Pathology, University of Limpopo (Medunsa Campus), Pretoria

Lecatsas G, PhD (Wits), DSc (UP)
Department of Virology, University of Limpopo (Medunsa Campus), Pretoria

Correspondence to: Prof AA Hoosen, Dept. of Microbiological Pathology, University of Limpopo (Medunsa campus) Email: hoosen@ul.ac.za

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Summary

A novel coronavirus, the SARS – coronavirus (SARS-CoV) is the infectious agent that has been implicated in the recent epidemic outbreak of severe acute respiratory syndrome (SARS). This paper briefly reviews the features of the infectious agent (SARS-CoV), the outbreak and the development of an impressive array of laboratory tests in a short period of time since detection of the infectious agent. These laboratory tests are useful tools for identifying and confirming infections with the SARS-CoV. Early disease is best detected by polymerase chain reaction (PCR) based tests whilst detection of specific antibodies is the preferred diagnostic approach after 10 days of the onset symptoms.

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SARS coronavirus

Severe acute respiratory syndrome (SARS), which developed into a pandemic, originated in the Far East between March and June, 2003. Cases in China and Hong Kong accounted for approximately 85% of all cases world-wide.[1] Cases also occurred in Canada and a number of outbreaks occurred in hospitals in both Hong Kong and Canada. The causative agent was soon isolated and identified as a coronavirus by means of cell culture and characterised morphologically by electron microscopy. Once these characteristics were identified it was possible to search for the reservoir of the virus which now appears to be the civet cat which serves as food in certain areas of the Far East particularly China.

Coronavirus is a genus belonging to the family Coronaviridae, the other genus being Torovirus. Coronaviruses infect a large group of species including cattle, rabbits, dogs, cats, mice, turkeys, chickens and humans. Consequently, the search for the virus would centre on food sources derived from these animal species. Important animal coronaviruses are infectious bronchitis virus (IBV) of chickens, murine hepatitis viruses (MHV) and transmissible porcine gastroenteritis. The first human coronavirus isolated was B814, a coronavirus causing cold symptoms and the second 229E. Both these viruses (B814 & 229E) were found by electron microscopy, to be identical in morphology to IBV. These viruses were originally isolated in organ cultures of human embryonic trachea and subsequently grown in tissue culture in fibroblasts.[2]

Coronaviruses are now classified as belonging to three antigenic groups based on antigenic and genetic homologies. These are group I (229E plus several animal strains) group II (OC43, MHV and several animal

Figure 1: SARS-coronavirus

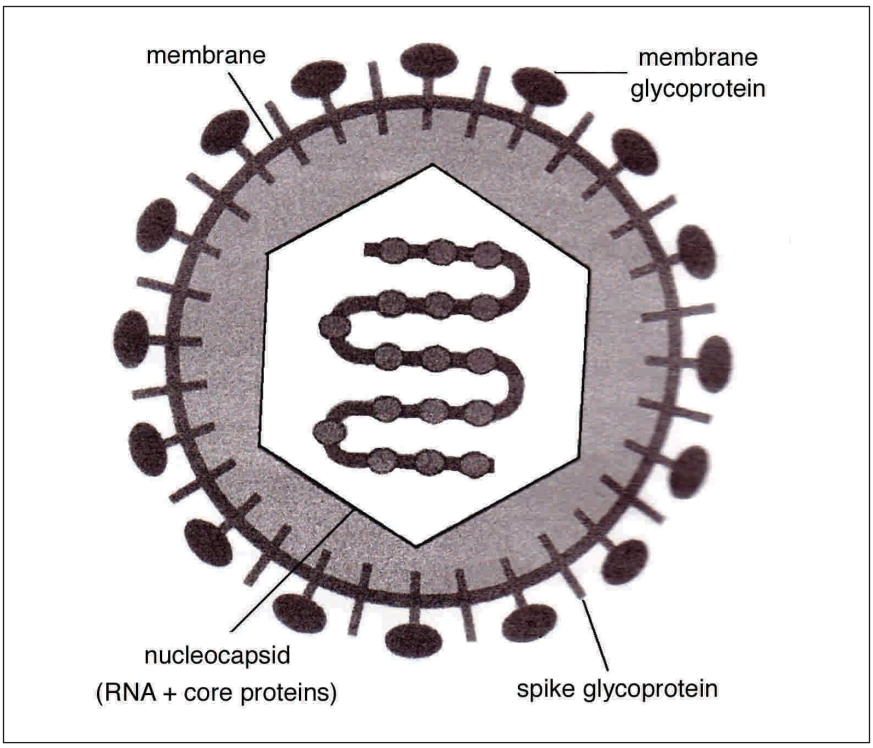

Figure 2: SARS case definition scheme (WHO) [3]

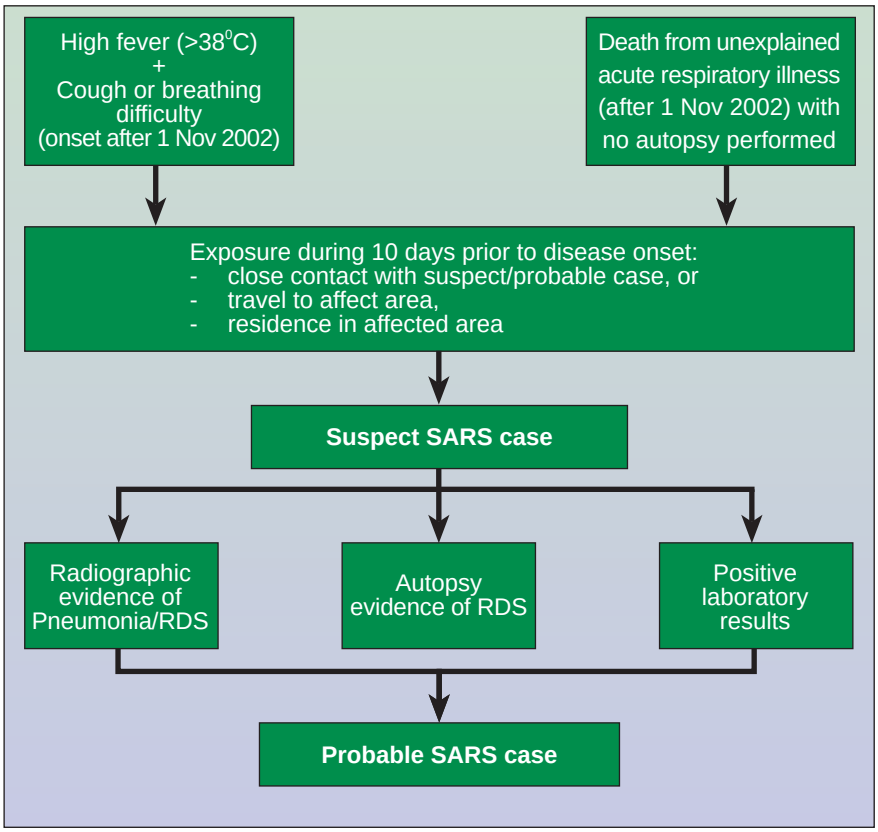

Table I: Laboratory methods for the confirmation of suspected cases (WHO) [4]

Laboratory methods	WHO recommendations on interpretation of laboratory results
A. Confirmed positive PCR for SARS-CoV	At least two different clinical specimens (e.g. nasopharyngeal and stool) OR the same clinical specimen collected on two or more days during the course of the illness (e.g. two or more nasopharyngeal aspirates) OR two different assays or repeat PCR using the original clinical sample on each occasion of testing
B. Seroconversion by ELISA or IFA	Negative antibody test on acute serum followed by positive antibody test on convalescent serum OR Four-fold or greater rise in antibody titre between acute and convalescent phase sera tested in parallel
C. Virus isolation	Isolation in cell culture of SARS-CoV from any specimen AND PCR confirmation using a validated method

strains) and group III which contains only IBV. Coronaviruses are round, membrane-bearing viruses characterised by club-shaped surface projections which are 20nm in length.

They have a diameter of approximately 100 to 150nm. The virus buds from internal cellular membranes (Golgi apparatus and endoplasmic reticulum) and not from the cell membrane. The viral genome is the largest of known RNA viruses being 27 to 32 kb in size. The surface projections are composed of the S-glycoprotein. Some members of group II coronavirus have an additional surface glycoprotein designated HE. The M (membrane) protein is also embedded in the viral envelope. These viruses can cause both gastrointestinal and respiratory infections. Although both types of infections occur in humans it should be emphasised that cross species infections also occur, and this is a hallmark of the recent SARS outbreak. Both 229E-like and OC43-like virus infections occur in winter and spring. Enteric coronaviruses do not appear to be associated with seasonality. The respiratory route of infection is the main mode of transmission. Respiratory coronaviruses have a short incubation period of about 2 days and the peak of respiratory symptoms occurs by day 4 following infection.

SARS case definitions

Initially the diagnosis of SARS cases was based on clinical and epidemiological information, however, molecular and serological tests for detecting the SARS-CoV have been rapidly developed. SARS cases are classified as suspect or probable, based on clinical, epidemiological and laboratory criteria defined by the World Health Organisation (WHO) [3] (Figure 2). A suspected case of SARS that is positive for SARS-CoV by one or more assays (Table I) is classified as a probable case.[4]

Diagnosis of SARS

According to the World Health Organisation (WHO) SARS case definition[3], a case should be excluded if an alternative diagnosis can fully explain the illness. For example, influenza viruses, parainfluenza viruses, *Chlamydia pneumoniae, Mycoplasma pneumoniae* and *Legionella pneumophila* infections can also cause atypical pneumonia. Positive laboratory test results for these agents serve as exclusion criteria.

Laboratory diagnostic methods for confirmation of suspected cases: PCR for SARS-CoV

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) allows for direct detection of SARS-CoV genetic material in various patient specimens, such as respiratory secretions, blood, stools, or body tissues. Positive PCR results are highly specific and mean that there is genetic material (i.e. RNA) of the SARS-CoV in the specimen that has been tested. This does not necessarily mean that the active virus is present, or that it is present in a sufficient quantity to cause an infection.

Negative PCR results do not

exclude the presence of the SARS-CoV in a patient. Besides the possibility of obtaining false-negative test results, specimens may not have been collected at a time when sufficient virus or its genetic material was present. The sensitivity of PCR tests for SARS depends both on the type of specimen and the time of testing during the course of the illness. Sensitivity can be increased if multiple specimens are tested. The specificity of PCR tests for SARS is excellent if the technical procedures used follow quality control guidelines. False positive results may arise as a result of technical problems (e.g. laboratory contamination), so every positive PCR test should be verified. Nasopharyngeal aspirates (NPA), throat or sputum samples are the most useful clinical specimens in the first 5 days of illness, but as the disease progresses viral RNA can be detected more readily in stool specimens.[5] The viral load is unusually low in the early symptomatic phase of SARS and for respiratory specimens reaches its peak level at approximately 10 days after the onset of the disease[6]. In contrast, viral loads in many viral respiratory tract diseases are usually high during the initial disease process.

Serological tests

Antibodies against SARS-CoV become detectable with high sensitivity at about 10 days after onset of infection, and are undetectable prior to this by current testing methods. Positive antibody test results indicate that there has been an infection with SARS-CoV. Seroconversion from negative to positive or a four-fold rise in antibody titre in the serum of a convalescent patient compared with that patient's serum during acute illness denotes a recent infection. A negative serological result 21 days after onset of symptoms indicates absence of SARS-CoV infection. Cross-reactions with antibodies to other agents like other human coronaviruses are said to be rare[7], however, one ought to be cognisant of this. This needs to be seriously considered in the post SARS outbreak situation.

Several serological studies with SARS patient sera have been reported and these show varying sensitivities and specificities.[5,6,8] The reference serological method is the neutralisation test and this was compared to enzyme linked immunosorbent assay (ELISA), immunofluorescence assays (IFA) and the immunochromatic test (ICT). Antibody determination using IFA or ELISA was the most reliable method for identifying infections with SARS-CoV. The ICT had a poor sensitivity.

In the study reported by Wu *et al,* 799 sera specimens from 537 probable cases of SARS were tested for antibodies to SARS CoV by the neutralisation test, IFA, ELISA, and ICT.[8] The sensitivity, specificity, positive predictive values and negative predictive values were 98.2%, 98.7%, 98.7% and 98.4% for the ELISA; 99.1%, 87.8%, 88.1% and 99.1% for the IFA; 33.6%, 98.2%, 95.7% and 56.1% for the ICT respectively.

Virus isolation

Patient specimens such as respiratory secretions, blood, or stool can be inoculated in suitable cell lines for growth of the infectious agent. Cell culture requires considerable expertise, is time consuming and quite demanding. Vero cells have been used for culture. After isolation, the virus has to be confirmed and this is usually done with nucleic acid based tests. Positive results indicate presence of viable SARS-CoV, whilst negative cell culture results do not exclude SARS. These viruses were originally isolated in organ cultures of human embryonic trachea and subsequently grown in tissue culture in fibroblasts. Although most coronaviruses are highly species specific, under certain experimental conditions some human strains may infect different species though, for example, intra-cerebral inoculation of African green monkeys. Serial passaging in heterologous cell lines can extend the host range. This leads to the virus being able to employ a larger variety of receptors on the cell surface. Coronaviruses show a marked degree of tissue tropism. Closely related viruses may show different tropism, some tending towards respiratory infections and others to gastrointestinal infections. These tropisms are influenced by both host cell surface characteristics and by viral S-glycoprotein variation. Although coronaviruses replicate in the cytoplasm the role of the nucleus in this respect is unknown. Coronaviruses usually cause lytic infections although persistent infections are also known to occur depending on the particular virus strain and host cell.[9]

Conclusion

The development of an impressive array of laboratory tests in a short period of time since detection of the infections agent is really impressive. These laboratory tests are useful tools for identifying and confirming infections with the SARS-CoV. Early disease is best detected by polymerase chain reaction (PCR) based tests whilst detection of specific antibodies is the preferred diagnostic approach after 10 days of the onset of symptoms. Every laboratory confirmation of SARS should be undertaken in a national or regional reference laboratory and reported to the WHO. The WHO encourages each country to designate a laboratory at national level for the investigation and shipment of specimens from the investigation and shipment of specimens from possible SARS patients. In South Africa, the National Institute of Communicable Diseases (NICD) of the National Health Laboratory Services (NHLS) would be the designated facility. Guidelines for the safe handling of SARS specimens are also described on the WHO web site.[10]

See CPD Questionnaire, page 45

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