The first cases of SARS appeared in southern China in November 2002. In March 2003 the causative agent of this disease was identified as a novel coronavirus. Since its first reported occurrence in humans, the virus has infected more than 8000 people and caused more than 750 deaths. Between February 2003 and June 2003 cases of the disease were predominantly reported in Asia, but were also reported in North America and Europe.

SARS-CoV is spread mainly by close person-to-person contact, for example, through respiratory secretions from coughing or sneezing. The virus can also spread from surfaces or objects contaminated with infectious droplets. The incubation period for SARS is typically 2-7 days, after which patients develop high fever (>38°C) and respiratory symptoms, mainly pneumonia. About 10 to 20 percent of patients have diarrhoea. Other symptoms are headache, an overall feeling of discomfort and body aches.

The overall mortality from SARS is around 10%, with levels as high as 50% in the over-65 age group. Currently there is no specific and effective therapy or method of prevention for SARS.

The initial diagnosis of SARS was based on clinical and epidemiological criteria. During the initial outbreak, molecular and serological tests for detecting infections with the SARS-CoV were developed. Direct or indirect identification of the virus by these diagnostic tests has become an important means of diagnosing the disease, of understanding the pathways of transmission, and of studying SARS epidemiology.

SARS case definitions

SARS cases are classified as suspect or probable based on clinical, epidemiological and laboratory criteria [1] defined by the World Health Organisation (WHO) [Figure 2]. A suspected case of SARS that is positive for SARS coronavirus by one or more assays [Table 1] is classified as a probable case.

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

The polymerase chain reaction (PCR) allows direct detection of SARS-CoV genetic material in various patient specimens, such as blood, stool, respiratory secretions or body tissues. Positive PCR results are very specific and mean that there is genetic material (RNA) of the SARS-CoV in the sample. This does not necessarily mean that the active virus is present, or that it is present in a quantity great enough to infect another person.

Negative PCR results cannot 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. To date the optimal type of sample to use at different times after the onset of symptoms has not yet been determined. 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.

In contrast with many other viral respiratory tract diseases, the viral load is unusually low in the early symptomatic phase of SARS. Depending on the specimen, the viral load of SARS-CoV in SARS patients reaches its peak level at approximately day 10 after the onset of the disease [2, 3]. Nasopharyngeal aspirates (NPA), throat swabs or sputum samples appear to be 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 [4, 5].

Seroconversion determined by ELISA or IFA

Antibodies against SARS-CoV become detectable with high sensitivity around 10 days after the onset of infection, but they can be 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 (including the human coronaviruses HCoV-229E and HCoV-OC43) are not known. Several serological studies with SARS patient sera using immunofluorescence tests (IIFT) and/or ELISA showed sensitivities between 92 and 99% [3, 4, 6, 7]. A comparison of IIFT, ELISA and PCR [Figure 5] showed that PCR predominantly enables fresh infections to be identified. In later stages of the illness (~10 days after onset) antibody determination using IIFT or ELISA is the most reliable method for identifying infections with SARS-CoV.

**Virus isolation**

The presence of the infectious virus can be detected by inoculating suitable cell cultures (e.g. Vero cells) with patient specimens (e.g. respiratory secretions, blood or stool) and propagating the virus in vitro. Cell culture is a very demanding test. Once isolated, the virus must be identified as SARS-CoV using further tests (predominantly nucleic acid-based). Positive results indicate the presence of living SARS-CoV in the sample.

**Differential diagnosis**

According to the WHO SARS case definition, 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* can also cause atypical pneumonia. Positive laboratory test results for these agents may serve as exclusion criteria.

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 possible SARS patients. Furthermore, members of the WHO network laboratories [8] have agreed to test samples of suspected or probable SARS patients from countries which may not have the laboratory capacity (PCR technology and biosafety level 3). Guidelines for the safe handling of SARS specimens are also described on the WHO web site [9].

### Table 1. Laboratory methods for confirmation of suspected cases.

<table>
<thead>
<tr>
<th>Laboratory methods</th>
<th>WHO recommendations on interpretation of laboratory results</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Confirmed positive PCR for SARS-CoV</td>
<td>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</td>
</tr>
<tr>
<td>B. Seroconversion by ELISA or IFA</td>
<td>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</td>
</tr>
<tr>
<td>C. Virus isolation</td>
<td>Isolation in cell culture of SARS-CoV from any specimen AND PCR confirmation using a validated method</td>
</tr>
</tbody>
</table>

**References**

8. World Health Organisation. WHO collaborative multi-centre research project on Severe Acute Respiratory Syndrome (SARS) diagnosis. www.who.int/csr/sars/proj ect/en

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**Figure 3.** PCR amplification of SARS-CoV nucleic acids.

**Figure 4.** Euroimmun IIFT: antibodies against SARS-CoV.

**Figure 5.** Comparison of Euroimmun IIFT, ELISA and PCR results from 34 sera of SARS patients.