Laboratory tests for SARS: Powerful or peripheral?

Ron A.M. Fouchier, Ab D.M.E. Osterhaus

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ß See related article page 47

It has been 6 months since the World Health Organization (WHO) officially declared the global outbreak of severe acute respiratory syndrome (SARS) to be under control. Since then, public health officials involved in the SARS outbreak, be it clinicians, epidemiologists, laboratory scientists, outbreak management teams or politicians, have turned to extensive introspective analyses. Important lessons can be learned from these evaluations in the event that SARS reappears or outbreaks of new and emerging infections occur.

In this issue (page 47), Patrick Tang and colleagues, participating in the Ontario Laboratory Working Group for the Rapid Diagnosis of Emerging Infections, report on their experience with the laboratory investigation of SARS during the outbreak in Toronto.[1] They point out the difficulties encountered within the diagnostic laboratories and the continual changes in the options that were available to them. During the first weeks of the outbreak no diagnostic tests were available, but later, when the first generation of molecular assays and serologic tests became available, additional problems arose, such as the absence of a "gold standard" for the tests, the lack of uniformity of the tests (i.e., how they were conducted and which primer sets were used), and the lack of information on which specimens to collect from patients and how to collect them. Despite these problems, the authors have conducted detailed retrospective analyses of the laboratory data collected during the outbreak in Toronto and have put them in context with the available clinical and epidemiological data. After all, when it comes to an outbreak such as this one, it is better to draw careful conclusions from imperfect data than to draw no conclusions at all.

The main conclusion of their study is that the evaluation of the clinical presentation and the elucidation of a contact history must remain the cornerstone of SARS diagnosis, which is in keeping with recent WHO recommendations and with medical practice in general.[2] Because the tests available to detect the SARS-associated coronavirus (SARS-CoV) are not sufficiently sensitive to identify early infection — when transmission of the virus may already be occurring — cases need to be identified using alternative (clinical and epidemiological) strategies.

To this end, the case definition of SARS has recently been updated, in part to help distinguish the illness caused by SARS-CoV from atypical pneumonia caused by other pathogens.[2] Molecular diagnostic tests, such as reverse-transcriptase polymerase chain reaction (RT-PCR), amplify minute quantities of viral RNA and thus may help to detect SARS-CoV early in the course of illness. Molecular tests may also be useful in clinical and epidemiological investigations of clusters of infected patients. However, at present, neither negative results nor single positive results of molecular tests can be considered conclusive for the detection of SARS-CoV. In Tang and colleagues' study, even after repeat testing with RT-PCR, only 54% of the patients for whom diagnostic test results were available had a positive result. In addition, as pointed out by the authors, the presence of other pathogens, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, does not exclude the possibility of coinfection with SARS-CoV. The role that these and other pathogens, such as human metapneumovirus, may play in SARS still needs to be determined.

In the Toronto outbreak, the peak positivity rate of RT-PCR occurred 9 to 11 days after the onset of symptoms, which is similar to the late peak of virus titres observed in clinical specimens collected from patients in the Hong Kong outbreak.[3] Stool samples and samples collected from the lower respiratory tract appeared to be the best choice for sampling because they yielded the highest detection rates. The authors recommend that sputum and stool samples be the preferred type of specimen obtained for RT-PCR because their collection does not require invasive procedures, which may increase the risk of nosocomial infection.[4]

As with all serologic tests available to identify infectious diseases, those used to detect SARS-CoV antibodies are primarily useful retrospectively because the antibodies reach detectable levels later in infection. The first generation of serologic tests appear to give consistent results for samples from patients in Toronto and elsewhere, and they now provide a powerful tool to identify or confirm SARS cases retrospectively. In the series reported by Tang and colleagues, convalescent serum samples were positive in 96% of the patients from whom paired samples were col-

lected. Thus, the current serologic tests may be a good "gold standard" for new or improved diagnostic tests to detect SARS-CoV early in the course of illness in a largely seronegative population. Poon and colleagues[5] recently developed a second-generation RT-PCR assay capable of detecting SARS-CoV in up to 88% of respiratory tract samples obtained within the first 3 days after illness onset in confirmed SARS cases in the Hong Kong outbreak. In addition, they showed that the viral load is unusually low early in the course of SARS, as compared with the viral load in other respiratory illnesses. This provides a plausible explanation for the poor detection of SARS-CoV in early samples using the first-generation RT-PCR assays.

The push is now on to design even more sensitive tests. Most of the current RT-PCR tests for SARS-CoV are designed to detect the replicase gene of the virus, because this was the first gene identified. Perhaps alternative target gene sequences such as the nucleocapsid gene may yield more sensitive tests. The nucleocapsid protein coats the viral RNA and is more abundant than the replicase protein in CoV-infected cells and virus particles. This is because coronaviruses rely in part on a transcriptional gradient, in which nucleocapsid messenger RNA (mRNA) is more abundant than mRNA encoding the replicase protein. Since some coronaviruses can also package this nucleocapsid mRNA in virions,[6] it is worth investigating whether RT-PCR assays based on the nucleocapsid gene are more sensitive than those based on the replicase gene.

Second-generation tests using alternative PCR technologies are being tested in a large number of laboratories — institutional, regional and national — in order to prepare for the possible return of SARS. However, it is unlikely that such diagnostic tests will significantly affect the current strategies for outbreak management.[2] As emphasized by Tang and colleagues, it is important that there be an integrated system of institutional, regional and national diagnostic laboratories in order to respond effectively and rapidly to outbreaks caused by new and emerging infectious diseases and that the communication and cooperation between physicians, epidemiologists and laboratory scientists be optimized. The creation of a Canadian Agency for Public Health, as recommended by the National Advisory Committee on SARS and Public Health,[7] would respond effectively to this challenge and bring Canada in line with other highly developed Western countries. Diagnostic laboratories are very important in the early recognition of emerging pathogens and in the development and improvement of diagnostic tests. When incorporated in the outbreak management network from bedside clinicians to politicians, the diagnostic laboratories indeed can play a crucial role in outbreak control, as was shown during the recent global SARS outbreak.

The authors are with the Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands.

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Correspondence to: *Dr. Ron A.M. Fouchier, Department of Virology, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands; fax 311 040-8948; r.fouchier@erasmusmc.nl*