SARS: seeking a real-time solution

– Tracey Wright and Raewyn Poole –

The outbreak of Severe Acute Respiratory Syndrome (SARS) clearly illustrates that the emergence or re-emergence of infectious diseases is a constant threat. In late February, the world became aware of the existence of SARS in Vietnam after an alert from WHO scientist Dr Carlo Urbani (who later died from the disease). However, SARS originated in southern China in November 2002. Globally, the total number of confirmed cases has now reached 8221 (as at 27 May 2003). While patients with SARS have been identified in more than 30 countries, the majority of cases have occurred in China (7648), including Hong Kong (1728) and Taiwan (596). To date, control of the SARS outbreak has focused on preventing its transmission, and there has been an emphasis on the development of diagnostic tests to accurately identify the virus in patients with suspected SARS infections. However, a number of compounds are currently under investigation as potential treatments for the disease.

SARS is a respiratory illness that has recently been reported in Asia, North America and Europe. In general, SARS begins with a fever of $> 38^{\circ}\text{C}$. Other symptoms may include headache, an overall feeling of discomfort, and body aches, and some patients also experience mild respiratory symptoms. After 2 to 7 days, patients may develop a dry cough and have trouble breathing. No treatment beyond good intensive and supportive care has been consistently shown to improve prognosis. SARS appears to be transmitted primarily through close person-to-person contact; most cases have involved people who cared for or lived with someone with SARS, or had direct contact with infectious material (e.g. respiratory secretions) from a person with the disease. It also is possible that SARS can be spread more broadly through the air or by other ways that are currently not known.

Race to develop diagnostics

Shortly after the WHO announced in April that its global collaborative research network had identified a new type of coronavirus as the probable causative agent for SARS, research teams in Canada, the US, Hong Kong, Singapore and China separately reported that they had sequenced SARS-associated viral genomes. With the genome sequenced, the race was on to develop a real-time PCR-based diagnostic test.

With a number of companies working on a SARS diagnostic, the CDC has called for accuracy over speed in the race to develop tests for the quickly spreading disease. The WHO has also sounded a note of caution. In a statement released on 25 April, the WHO noted that *"it is extremely important . . . to understand the limitations of currently available tests. Their use as a basis for treatment decisions may give a false sense of security that can allow persons carrying the SARS virus . . . to escape detection"*. In particular, the WHO mentioned the PCR test developed by artus GmbH, which the company claims could provide a result within 2 hours. The WHO says that the test is probably specific but lacks sensitivity; i.e. a negative result should not be considered conclusive without extensive supporting clinical evidence.

Abbott and artus align

In mid-May, Abbott Laboratories commenced a worldwide marketing and distribution agreement with artus GmbH for what is believed to be the first commercial PCR-based test to detect a form of the coronavirus linked to SARS. Hamburg-based biotechnology company artus developed the virus detection assay together with the Bernhard-Nocht

Institute of Hamburg, Germany. In mid-April, artus launched the SARS test in Asia and Europe. The company has also provided the test to laboratories under the direction of the WHO and other major clinical sites conducting studies on the SARS virus. Initial results of different sample types (sputum, swabs, stool and tissue) from these WHO laboratories are promising.

The team of virologists at Bernhard-Nocht Institute was part of WHO's research network that identified the virus causing SARS. Based on the discovered genetic sequence, the Bernhard-Nocht Institute developed a real-time PCR diagnostic test that produces results within 2 hours. From that test, artus developed a ready-to-use system (RealArt HPA-Coronavirus RT PCR Reagents). The PCR assay directly detects parts of the new coronavirus in provoked sputum samples.

Under the terms of their agreement, artus will manufacture the test and Abbott will market and distribute it, initially for use on the Roche LightCycler thermal cycler system in North America, the UK, Germany, and Austria. The test will be available worldwide on the Applied Biosystems ABI Prism 7000 Sequence Detection System in the near future. Abbott will also help artus submit the test to the FDA and will market and distribute it through its molecular diagnostics alliance with Celera Diagnostics.

Roche aiming for July

Roche Diagnostics plans to launch a diagnostic test for the coronavirus that causes SARS by the end of July. In May, Roche Diagnostics and the Genome Institute of Singapore signed a deal to jointly develop a SARS detection kit based on Roche's PCR technology. Work has already begun at the Genome Institute of Singapore to develop a primer set for real-time PCR detection on the Roche LightCycler system. The Genome Institute of Singapore is one of the institutes that sequenced the SARS genome.

Focusing on SARS

At the start of May, Focus Technologies announced it had developed a first-generation real-time PCR test to detect the presence of the coronavirus associated with SARS. The company said its reference laboratory in Cypress, CA, has begun performing SARS-associated coronavirus testing to help clinicians diagnose disease in suspected patients. The test identifies genomic RNA from the coronavirus associated with SARS via reverse transcription of specific viral genomic RNA sequences followed by PCR amplification. The assay is based on previous work by the WHO and the Bernhard-Nocht Institute to identify PCR primers for the virus.

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Prodesse to develop multiplex test

Prodesse, Inc. is developing a molecular test using its multiplexing technology for the detection of the coronavirus causing SARS. The company is considering adding SARS to its Hexaplex assay, thereby allowing clinicians and epidemiologists to screen patients with SARS-like symptoms not only for SARS, but also for the far more common viruses that cause similar, severe respiratory diseases. This would give healthcare professionals far more information than the recently announced tests that detect only the SARS virus. The patented multiplexing technology used in Prodesse's products is able to detect and differentiate many different pathogens within only a few hours.

Ciphergen and CombiMatrix develop microarrays

Researchers in Beijing, Canada and Singapore are searching for novel protein biomarkers associated with SARS using ProteinChip technology from Ciphergen Biosystems, Inc. According to the company, "*a number of potential biomarkers have already been discovered in SARS patient samples*", using its Expression Difference Mapping protocols on throat swabs, serum and other samples. These biomarkers will now be evaluated for use in developing rapid, protein-based diagnostic tests for SARS.

CombiMatrix has made its new SARS microarrays available to key government and academic researchers. The company produced its first microarrays within 48 hours of publication of the coronavirus genome sequence believed to be responsible for SARS. CombiMatrix' Lab-on-a-Chip platform technology was designed to enable rapid content redesign and overnight synthesis of custom microarrays. The company plans to release 3 types of microarrays: for detection and screening; for genome-wide detection of viral mutations; and for studying expression of viral and/or host genes.

Pharmaceutical industry joins efforts

In April, HHS Secretary Tommy Thompson requested that pharmaceutical companies provide any compounds they had that may be effective against SARS. The US National Institutes of Health (NIH) is testing approximately 2000 antiviral compounds in an aggressive screening programme to identify potential treatments for SARS. In addition, a number of companies have recently announced that they are developing novel agents to treat SARS infections [see *table*]. However, the majority of these agents are still undergoing preclinical evaluation and, even with the US FDA and other regulatory bodies expediting the development and approval process, are unlikely to be commercially available in the near future. Thus, the best hope in the immediate future may lie in antiviral agents that are already approved, and have been extensively tested, for other viral diseases.

Several compounds that are already in clinical studies

for other indications have been identified as potential treatments for SARS. One such compound is Hemispherx Biopharma's mismatched double-stranded RNA ['Ampligen'] and interferon- α -n3 ['Alferon'], which the company is testing against SARS in a collaboration with the Genome Institute of Singapore. 'Ampligen' demonstrated potent activity against human coronavirus strain OC43 in African green monkey kidney cells when a panel of 70 compounds was tested by the Institute for Antiviral Research, in research sponsored by the NIH.

Pfizer has provided a number of experimental compounds to the US government for testing against coronavirus, including the rhinovirus protease inhibitor AG 7088 [rupintrivir]. An article published online in *Science* (May 13, 2003) suggested that inhibition of the viral protease may be the key to developing treatments for SARS, and that compounds such as AG 7088 may therefore form the basis for drugs that are effective against the disease.

Vaccination another possibility

Secretary Thompson met with major vaccine manufacturers GlaxoSmithKline, Wyeth, Merck and Aventis-Pasteur in April to urge them to test their vaccines against coronaviruses. There are also several companies developing novel vaccines against the SARS virus. GenVec has formed a Collaborative Research and Development Agreement (CRADA) with the Vaccine Research Center at the National Institute of Allergy and Infectious Disease of the National Institutes of Health (VRC/NIAID/NIH) to develop a vaccine for SARS using the company's proprietary adenoviral vector technology. The CRADA will be complementary to the funded contract with the VRC/NIAID/NIH to construct and produce an adenovirus-based SARS vaccine. The agreement will govern a preclinical collaboration to evaluate and develop adenoviral vectors expressing modified SARS genes; the VRC/NIAID/NIH will test the preclinical vaccine candidates in preclinical models.

Another vaccine is in preclinical development with Generex Biotechnology Corporation and Antigen Express. This vaccine will utilise Generex's proprietary depot drug delivery and formulation technologies and Antigen Express will contribute its proprietary Ii protein suppression and Ii-Key peptide vaccine-enhancing technologies.

WHO calls international meeting

The rapid pace of discovery and development in the SARS arena has been made possible by the open exchange of information between researchers spread around the world. To consolidate the work done so far, WHO plans to hold an international scientific meeting to review the epidemiological, clinical management and laboratory findings on SARS, and to discuss global control strategies. The meeting will be held in Kuala Lumpur, Malaysia, 17–18 June 2003.

See also *Inpharma* 1383,3, 19 April 2003; see *Inpharma* 1383 p3; 800888926

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800888976

Table. Some drugs and vaccines under evaluation for use against SARS

Product	Company	Remarks
AG 7088	Pfizer	rhinovirus protease inhibitor; one of many compounds provided to the US government for testing
Angiotensin II receptor antagonists	GenoMed	may reduce mortality rate in patients with SARS; GenoMed seeking volunteers for testing
Anticoronavirus antibodies	Medarex/ Massachusetts Biologic Laboratories	fully human monoclonal antibody
Coronavirus antisense compound	AVI Biopharma	provided to NIAID for testing; provided to WHO-affiliated laboratories for cell culture and primate testing on a limited basis
Coronavirus inhibitors	ViroPharma; USAMRIID	one of several compounds from ViroPharma's chemical library being tested
Coronavirus vaccine	Antigen Express/ Generex	agreement in principle for vaccine development
Coronavirus vaccine	GenVec	adenoviral vector vaccine
Hepatitis C polymerase inhibitors	BioCryst	provided to NIAID for testing
Interferon- α -n3	Hemispherx Biopharma	testing in collaboration with Genome Institute of Singapore
Mismatched double-stranded RNA	Hemispherx Biopharma	testing in collaboration with Genome Institute of Singapore
Ranpirnase	Alfacell Corporation	provided to US federal SARS testing programme

» **Editorial comment:** Hemispherx Biopharma's mismatched double-stranded RNA ['Ampligen'] is awaiting approval for use in the treatment of chronic fatigue syndrome in the EU and Canada, and is in phase III trials for this indication in the US. It is also being evaluated in studies involving patients with HIV, hepatitis B, malignant melanoma and renal cancer. Ranpirnase ['Onconase', Alfacell Corporation] is in phase III of development for mesothelioma and in phase II studies for prostate and renal cancer in the US.