SARS – R&D (Part 10)

By Prof Chee Yam Cheng, Editorial Board Member

Editorial note:
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INTRODUCTION

This article is written in December 2003, a year after SARS first reared its head in Southern China, when patients suffering from a flu-like illness were thought not to have a new disease. It was on 10 February 2003 that the government of Guangdong province and Guangzhou city announced the epidemic situation of this disease in Guangdong. The disease was not named as yet and Dr Carlo Urbani (from the WHO) had not yet come to Hanoi, Vietnam to investigate the outbreak there, which occurred in early March. Up to 9 February, there had been 305 cases with five deaths in Guangdong province, of which 225 cases with two deaths were from Guangzhou.

When WHO finally declared the global health alert on 12 March, it also issued the clinical case definition for the disease, now named SARS – Severe Acute Respiratory Syndrome. The clinical criteria then used was fever of more than 38ºC, lower respiratory tract illness (cough, difficulty in breathing, shortness of breath), radiographic evidence of lung infiltrates consistent with pneumonia/respiratory distress syndrome or autopsy findings consistent with these, and no alternative diagnosis could fully explain the illness. All four criteria had to be fulfilled to define a patient with SARS. A suspect SARS patient did not fulfill all the four criteria, especially the lung pathology. And important in the clinical history was a history of contact with a SARS patient or travel to a SARS-affected country.

Today, the SARS case definition has changed because the laboratories are able to do certain tests for the coronavirus, which has been identified as the causative agent for this new disease, the first in the 21st century. I will therefore elaborate on the R & D of SARS – the research and diagnostic achievements to date.

SARS CASE DEFINITION

This is taken along three aspects – clinical, laboratory and epidemiological. A patient with SARS is defined as a case that meets the laboratory criteria of SARS and is not required to meet all the components of the clinical criteria (stated above) and epidemiological criteria. A patient suspected of SARS is a case that meets the epidemiological criteria, but not all the components of clinical and laboratory criteria.

What then are the laboratory criteria? A patient is diagnosed as suffering from SARS when he has symptoms and signs that are clinically suggestive of SARS and has positive laboratory findings for SARS coronavirus based on one or more of the following diagnostic criteria.

1. PCR positive for SARS-CoV. PCR positive using a validated method from:
(i) at least two different clinical specimens (e.g. nasopharyngeal and stool); or
(ii) the same clinical specimen collected on two or more occasions during the course of the illness (e.g. sequential nasopharyngeal aspirates); or
(iii) two different assays or repeat PCR using a new RNA extract from the original clinical sample on each occasion of testing.

2. Seroconversion by ELISA (Enzyme Linked Immunosorbent Assay) or IFA (Indirect Fluorescent Antibody Assay):
(i) negative antibody test on acute serum followed by positive antibody test on convalescent phase serum tested in parallel; or
(ii) fourfold or greater rise in antibody titre between acute and convalescent phase sera tested in parallel.

3. Virus isolation:
Isolation in cell culture of SARS-CoV from any specimen and PCR confirmation using a validated approach.

(Reference: WHO document 14 August 2003 "Alert, Verification and Public Health Management of SARS in the Post Outbreak Period".)

Finally, the epidemiological criteria relate to persons with an epidemiological link to a case of SARS. This judgement of contact history is based on the risk assessment made by the specific epidemiologist investigating the case using two criteria:

1. Travel (including transit in an airport) within ten days of onset of symptoms to an area with current or previously documented or suspected community transmission of SARS (see Table 1).
2. Close contact within ten days of onset of symptoms with a person known or suspected to have SARS.

So although WHO declared Singapore free of local SARS transmission on 31 May 2003, in the above table, US CDC takes a later date for Singapore.

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TABLE 1. TRAVEL CRITERIA FOR SUSPECT OR PROBABLE CASES OF SARS.

Area	First date of illness onset for inclusion as reported case	Last date of illness onset for inclusion as reported case*
China (mainland)	1 November 2002	13 July 2003
Hong Kong	1 February 2003	11 July 2003
Hanoi, Vietnam	1 February 2003	25 May 2003
Singapore	1 February 2003	14 June 2003
Toronto, Canada	1 April 2003	18 July 2003
Taiwan	1 May 2003	25 July 2003
Beijing, China	1 November 2002	21 July 2003

(Source: CDC, US, July 18, 2003.)

* The last date of illness onset is ten days (one incubation period) after removal of a CDC travel alert. The case patient's travel should have occurred on or before the last date the travel alert was in place.

ANIMAL RESERVOIRS

A SARS-like virus had been found in a broad range of animals, ranging from snakes and birds to mammals. Fourteen United Nations and Chinese experts visited farms and markets in Guangdong, the epicentre of the virus in South China, in search of a possible animal carrier of the virus. They were surprised to see so many different species were capable of infection. The French expert Mr François Monton said: "What is surprising is we got positive results from mammals, birds and reptiles. This is very strange because usually we don't find viruses affecting so many animals." Mr Hume Field, an expert from Australia's Animal Research Institute said: "There may be many animals that are capable of being infected but they might not be capable of transmitting the virus to people." WHO is training thousands of medical workers in China to prevent infectious diseases from spreading in hospitals. "Whether or not SARS returns, China must have a strong surveillance network already in place," said Mr Henk Bekedam, the WHO's representative. *(Straits Times, 22 August 2003, pg. A2, col. 5-7.)*

KOCH'S POSTULATES FULFILLED

Dutch researchers proved that the new coronavirus is the source of SARS by completing tests that met all accepted scientific standards. *(Straits Times, 16 May 2003, pg A4 col 1-3.)* Virologists at Amsterdam's Erasmus Medical Centre, led by team leader Albert Osterhaus, said: "It is important in terms of combat strategies against the disease that you can unequivocally define what the primary cause is. It will speed up diagnostics. It will speed up antivirals development and it will speed up vaccine development because now we know what we have to focus on." The tests they carried out met standards set by Koch's postulates. It involved cross-checking to ensure that the disease can be clearly traced to a given virus and not to other pathogens that may lurk in samples taken from patients.

Other groups working on SARS met the first three criteria of isolating the virus from diseased hosts, cultivating it in host cells and proving that the agent passes though a laboratory filter that traps bacteria. Professor Osterhaus announced they had successfully carried out the other three Koch tests. These were: inducing the disease in the same or a comparable host; re-isolating the pathogen from the sick animals; and detecting a specific response to the virus from the body's immune system. These experiments were carried out on macaque monkeys.

This laid to rest the theory that an atypical paramyxovirus or chlamydia species found in samples from SARS patients in China and Hong Kong, caused SARS.

DIAGNOSTIC KITS

Once it was clear that the coronavirus was the causative agent of SARS, scientists began in earnest to sequence its complete genetic code. Roche Diagnostics announced that it could roll out diagnostic test kits within six to eight weeks of the genome sequence. Current diagnostic measures depend on antibodies in cell and tissue cultures but are severely limited by the fact that they are only able to detect the presence of SARS 14 to 21 days after infection. By that time, most patients would have reached a critical stage of illness.

Roche was using the molecular testing approach to detect SARS two to three days after infection by identifying the genetic material of coronavirus. The test result could be ready in one hour. This would be one step ahead of the diagnostic kit which Artus, a German biotechnology company, was developing. The Artus kit required two hours to diagnose SARS. Both kits employ the polymerase chain reaction (PCR) technique. The Roche version employs also the light cycle, an instrument that allows the diagnostic results to be processed in a significantly shorter time. This same process is used by Roche to diagnose patients with HIV, hepatitis A virus and tuberculosis.

In PCR, a target sequence of 100 – 600 base pairs uniquely specific to the coronavirus would be replicated with primers marking each end of the target sequence. The sequence flanked by the two primers is then amplified. *(Medical Tribune, May 2003, pg. 13.)*

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In Singapore, it was reported on 3 April 2003 *(Straits Times, pg. 4)* that "Singapore may have SARS test soon." Diagnostic tests that can confirm infection in patients were being validated but would only be ready in a fortnight. Singapore General Hospital's (SGH) virologist Dr Ling AE was reported to have said that the diagnostic tests which were already being used on some suspected SARS patients here, were based on kits of DNA unique to the coronavirus. The tests were being validated on known SARS patients and then used to confirm SARS infection in patients already in hospital. Dr Ling said they had succeeded in growing the virus. Also tests on antibodies produced by patients locally, as well as molecular tests on the virus itself, showed that the offending virus was the same as samples drawn from victims in Hanoi and Bangkok.

Mention must be made of the contributions of our Infectious Disease doctor who en route back from New York in March 2003 was detained and quarantined in Frankfurt, Germany, because he had SARS. From him came multiple samples of blood and other body fluids, which contributed to the kit as a diagnostic test for antibodies to the SARS virus.

WHO CONFERENCE ON SARS RESEARCH

This took place in Singapore on 19 June 2003 jointly organised by the Ministry of Health, A*STAR and National University of Singapore (NUS). Five plenary lectures were delivered on the molecular biology of the coronavirus, priorities in SARS research, aetiology of SARS, vaccine development and SARS therapeutics. Dr Michael Lai from the Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, told us that the SARS virus represents a novel group of coronavirus that is distinguishable from known human and animal coronaviruses. Evolutionally, it is situated at an equal distance from Groups II and III coronaviruses. As a family, coronaviruses usually cause respiratory and enteric infections. Of the four to five structural viral proteins, the S (Spike) protein is responsible for receptor binding and induction of neutralising antibody; it is a candidate protein for vaccine and a prominent determinant of viral tissue tropism and pathogeneses.

The experience from animal coronavirus suggests that coronaviruses tend to develop persistent infections, with a long-term carrier state. Viruses may continue to evolve as a result of recombination and mutation. The viruses may cause disease as a result of both direct cytocidal effects and immune-mediated mechanisms, the latter especially evident with feline and murine coronaviruses. Vaccines vary in efficacy. However, for feline coronaviruses, the vaccines may actually potentiate the disease.

Earlier on 29 May 2003, a report titled: "SARS: From civet to man or other way round" began by saying: "News coming out this week makes the transmission line increasingly clear. The coronavirus jumped from civets to humans and the rest became history as SARS raged around the world." *(Straits Times, pg. 16, col. 2-5.)* The virus was uncovered in five out of six civets – nocturnal animals related to the mongoose, which have characteristically striped faces, long tails and cat-like bodies – but not in five other species in the same live animal market in Shenzhen, Guangdong, Southern China. The Head of Microbiology at the University of Hong Kong, which collaborated with the Shenzhen Centre for Disease Control in this study, believes that genetic information shows that the coronavirus "has been jumping from the civet to human." Comparing the SARS coronavirus genome with that of the civet coronavirus reveals two findings. First, all 17 SARS viral genome sequences in the public domain show that based on the specific mutations they share, the virus falls into two broad mutation groups. One strain is linked to the Metropole Hotel in Hong Kong from where the global outbreak started, and the other to the Mainland Strain, which remains distinct from the former and accounts for most of the cases in China. These two strains suggest two independent jumps from animal to man, but not necessarily, both from civets.

Secondly, the virus in civets showed four strains identical to the two human strains except that they all had a sequence of 29 base pairs that both human strains do not have. It is more likely for a virus to lose genetic material in jumping across species, than for it to add genetic material in making that jump. This implies that a jump from civets (where the virus has the 29 base pairs) to humans (where it does not) is more likely than the other way around – that loss came specifically from a gene that makes the S (Spike) protein forming part of the virus shell that permits the virus to enter human cells. This missing piece seems to have made the virus so infective that human-to-human transmission became possible. Although 29 out of a total of 29,000 base pairs in the SARS genome may be miniscule, they could make all the difference between an innocuous virus and a deadly one.

In the New England Journal of Medicine, 10 July 2003, on pages 187-8, three doctors from the Chinese University of Hong Kong reported on the genome sequence variations of their patients with SARS. They confirmed that at least two strains of SARS coronavirus had emerged, and that by mid March 2003 (when Singapore was hit), these two strains of the SARS coronavirus had already been found in patients in Hong Kong. This observation meant that there was more than one source of infection present at the beginning of the SARS epidemic in Hong Kong. Therefore, they concluded that even if there had been no outbreak in the Metropole Hotel, SARS could have probably broken out eventually in Hong Kong. This was based on using the glycoprotein sequences as a molecular epidemiologic tool.

In the plenary lecture on SARS vaccine development presented by the Director of the WHO Initiative for Vaccine Research, Geneva, Switzerland, she stated that WHO would give support to activities concerned with with (a) repositories of well characterised biological specimens, (b) a database of viral genomic nucleotide sequences, (c) studies on SARS immunology and pathogenesis, (d) standardisation of laboratory

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assays to evaluate immune responses, (e) standardisation of animal models, (f) product development, (g) facilitation of clinical trials in developing countries, and (h) regulatory issues related to the licensing of SARS vaccines. I suppose these principles are applicable to any new infectious disease that afflicts mankind. Particular to SARS is the phenomenon of the "super-spreader", which Singapore was unfortunate to have had at least one from the beginning of the epidemic. Why and how does this happen? Can we identify such a person early in the course of illness?

SARS LABORATORIES

As SARS has proved itself to be a deadly disease (just like smallpox was in yester-years), specimens of this coronavirus are kept in various research laboratories around the world coordinated by the WHO. Handling of such specimens is therefore serious business. Bioterriorists would love such a virus for their intended operations. Although WHO has issued guidelines on biosafety for laboratories, two lapses have occurred – one in Singapore and the other in Taiwan. Both sparked off fears of a SARS outbreak globally.

WHO strongly recommends Biosafety Level 3 (BSL3) as the appropriate containment level for working with live SARS-coronavirus. Laboratories currently conducting research on this virus represent the greatest threat for renewed SARS-CoV transmission through accidental exposure associated with breaches in laboratory biosafety. So, WHO has strongly recommended that national governments maintain a registry of such laboratories. Any laboratory accidents, such as accidental spillage of material suspected of containing SARS-CoV should be reported to the appropriate authority and all people potentially exposed to SARS-CoV resulting from such accidents should be closely monitored for 10 days for evidence of infections.

BSL 2 FACILITIES

According to the WHO Laboratory Biosafety Manual, the following procedures could be performed by personnel trained in the use of appropriate BSL 2 work practices:

1. Routine diagnostic testing of serum and blood samples (including haematology and clinical chemistry).
2. Manipulations involving neutralised or inactivated (lysed, fixed or otherwise treated) viral particles and/or incomplete, non-infectious portions of the viral genome.
3. Final packaging of specimens for transport to diagnostic laboratories for additional tests. Specimens should already be in a sealed, decontaminated primary container.

However, certain BSL 3 work practices may be performed in BSL 2 facilities. Examples of activities requiring BSL 3 working practices for work with SARS-CoV in BSL 2 facilities include:

1. Aliquoting and/or diluting specimens.
2. Inoculation of bacterial or mycological culture media.
3. Performance of diagnostic tests that do not involve the propagation of viral agents in vitro.
4. Nucleic acid extraction procedures involving untreated specimens.
5. Preparation and chemical or heat fixing of smears for microscopic analysis.

BSL 3 PRACTICES

These include:

1. Any procedure that may generate aerosols or droplets should be performed in a biological safety cabinet (e.g. sonication, vortexing);
2. Laboratory workers should wear protective equipment, including disposal gloves, solid front or wrap around gowns, scrub suits or coveralls with sleeves that fully cover the forearms, head covering and, where appropriate, shoe covers or dedicated shoes, eye protection and a surgical mask, or full face shield, because of the risk of creating aerosols or droplets exposure when performing specific manipulations.
3. Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be unloaded in a biological safety cabinet.
4. Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against enveloped viruses should be sufficient if used according to the manufacturer's recommendations. Generally, 5% bleach solutions are appropriate for dealing with biohazardous spillage.
5. Biological waste contaminated with suspect or confirmed SARS specimens or with SARS-CoV should be properly treated before disposal.

The following activities should be performed in containment laboratories (BSL 3) by personnel trained in the use of appropriate BSL 3 work practices:

1. Performance of diagnostic tests that involve propagation of viral agents in vitro and in vivo.
2. Work involving the replication of SARS-CoV in cell culture and / or storage of cell culture isolates.
3. Recovery of viral agents from cultures of SARS-CoV specimens.
4. Manipulations involving growth or concentration of SARS-CoV.

WHO SARS LABORATORY WORKSHOP

This was held in Geneva on 22 October 2003. Participating laboratories included the Virology Section, Department of Pathology, Singapore General Hospital, laboratories from the People's Republic of China and Hong Kong SAR. The workshop brought together 27 members of a new enlarged laboratory network from 15 countries and the region, and a further seven observers. In its report under the heading "Biosafety in the laboratory and inventory of SARS-CoV cultures" is the statement: "The importance of laboratory biosafety was

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clearly demonstrated with the occurrence of a laboratory acquired case of SARS-CoV infection in Singapore last month." Four recommendations were issued:

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 viruses should be under-taken 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 diagnostics of SARS-CoV, that national governments institute a process by which laboratories wishing to work with SARS-CoV be licensed to do so.

And on 18 December 2003, the WHO re-emphasised this in its website, titled: "WHO post-outbreak biosafety guidelines for handling SARS-CoV specimens and cultures."

SINGAPORE LABORATORIES

On 10 September 2003, the news was bad. The *Straits Times* first page headline was: "It's SARS, but an isolated case." A 27-year-old laboratory researcher and post-doctoral student at the NUS Department of Microbiology tested positive for SARS. He had been hospitalised on 3 September. His research involved the West Nile virus. On 23 August, he had spent 30 minutes at the Environmental Health Institutes (EHI) laboratory in Science Park II. On 26 August, he was back at the NUS laboratory and developed fever at about midnight. He saw a family doctor on the 27th, the Emergency Department at SGH on the 29th, a Chinese sinseh on 1 September, and on the 3rd, returned again to SGH where he was warded. On 8 September, the SARS test results came back positive and he was transferred over to the CDC at Tan Tock Seng Hospital (TTSH). The next day, extra tests confirmed he had SARS and the Health Ministry issued quarantine orders to 25 people who had been in contact with him. Eight were family members, two were at the sinseh's clinic, eight were at the ED of SGH, three were hospital visitors, and four were discharged patients of SGH. *(Straits Times, 10 September 2003, pg. H1.)*

As events turned out, it was indeed just an isolated case and WHO's confidence in our authorities' ability to keep the situation under control was justified. The spokesman for the Western Pacific, Mr Peter Cordingley told the *Straits Times*: "Like the Singapore authorities, we believe this is an isolated case. There is no established human-to-human transmission of the virus. We are confident the Singapore authorities can keep the situation under control. The WHO has no plans at this stage to even consider issuing a travel advisory against Singapore". *(Straits Times, 10 September 2003, front page.)*

The Dean of the Faculty of Medicine said there was "zero chance" of infection at the Department of Microbiology because researchers there worked only on dead viruses, which were not infectious. The National Environment Agency's Director General in whose BSL 3 laboratory (where live virus is kept), the researcher worked, said: "There seems to be some coincidental link, but I would be really surprised if it is from the laboratory." He gave three reasons. One, the researcher was working with the West Nile viruses, not the SARS virus. Two, the researcher had visited the laboratory on 23 August, six days after anyone had done SARS work at the institute. It was unlikely that the SARS virus could survive for more than two days, let alone six, and infect the researcher. Third, the laboratory was well designed and followed strict procedures as laid down by the WHO and the US CDC. The next day, the *Straits Times* further reported that the BSL 3 procedures at the NEA laboratory meant those working on the virus had no contact with it and NEA was confident that the strict safety measures it had, made lab infections unlikely. *(Straits Times, 11 September 2003, pg. H 2, col. 3-7.)*

"WHO and US experts to fly in to help check labs" – This was the response by the Ministry of Health (MOH). *(Straits Times, 12 September 2003, pg. 4, col. 6-7.)* Four of them came to help MOH investigate the practices, facilities and equipment at the EHI at Science Park II and the microbiology laboratory at NUS. This independent panel was to "establish whether the laboratories could have been the source of infection." The panel duly completed its investigations and the EHI laboratory was found to have had breaches in its safety procedures. This led to calls for punitive action on those responsible but there was no punishment. The Minister for the Environment came out publicly with an apology and took the responsibility for the safety lapse. The patient/researcher fully recovered and nobody else got infected.

In the *Straits Times* report on the panel's findings, EHI was found most wanting in safety standards. Four labs were reported upon, and in response to certain statements in the report, the respective chiefs gave their statements as follows. *(Straits Times, 24 September 2003, pg. H2.)*

At EHI: Strict new measures needed at high risk BSL 3 and BSL 2 laboratories. Dr Ooi EE, head of EHI said: "Our plan is first of all, to shut down the BSL 3 facility and decontaminate, but more importantly, go back and focus on dengue while we review our long term plans and look for a facility for BSL 3 work."

At SGH: The Pathology Department needs a dress code for BSL 3 laboratories and regular refresher safety courses. Professor Tan SK, CEO, SGH said: "We'll ensure we implement the recommendations as soon as possible."

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At NUS: Staff and students of the Microbiology Department lack culture of safety. Professor John Wong, Medical Faculty Dean said: "We have our office of safety, health and environment, and we'll work with it to develop further the recommendations."

At DSO: The facility, which also handles organisms with bioterrorism potential, is generally safe. Mr Quek TB, CEO, DSO National Laboratories said: "Most recommendations are already being planned or in the process of being implemented."

TAIWAN LABORATORY

After WHO had its conference in October 2003 on "Biosafety in the Laboratory" following the case in Singapore and issued its guidelines, yet another researcher got infected, this time from a laboratory in Taiwan. He was a 44-year-old military researcher, Lieutenant-Colonel Chan, who works at the highly secretive Institute of Preventive Medicine under the National Defence Medical Centre, a top military medical research body. Five researchers came to Singapore for a meeting with fellow researchers on flight CI 661 (China Airlines) on 7 December and returned to Taiwan on flight 662 on 10 December, Lt Col Chan recovered from SARS, and his Singapore friends at the meeting who were quarantined did not contract SARS. The safety breach? Carelessness, said the Taiwan officials. *(Straits Times, 18 December 2003, pg. A3, col. 1-6.)* A test tube containing a SARS sample had spilled in the laboratory where he was working. He did not wear protective gloves and a gown. "He was in a hurry to get ready for the Singapore trip, so he was rushing to finish his disinfection work and was careless," said Taiwan CDC Chief Su Yi-Jen. Lt Col Chan was in Singapore from 7 to 10 December and was well, but took ill on his return to Taiwan. After two incubation periods, i.e. 20 days later on 31 December 2003, Taiwan was given the all clear again.

WHAT RISKS AND ANY PUNISHMENT?

In *Streats* on 23 December 2003, Mr Robin Gauguly wrote a piece titled: "SARS: Let us be 100% tough. It's time to cover a dangerous clink in our armour – sloppy laboratory researchers who don't follow procedures and endanger all of us and our livelihoods." He felt the government could do more; he asked, when is it a criminal negligence? Doctors are sometimes excused for making mistakes but are sued for being negligent. Is the case of researchers different? Entire societies can be put at risk. The government has put strict guidelines in place for research laboratories. But it is time to go one step further, he suggested. "It is time to enact laws which can be used to severely punish irresponsible researchers. It is time to let them know that there is no room for carelessness." As a comparison, he cited Parliament amending the Infectious Diseases Act on 25 April 2003 so that even first time quarantine breakers could be jailed for six months and fined $10,000. I ask you, he said, who is more dangerous? Someone who may not have SARS at all or a careless researcher who handles live samples of virus with secant respect for safety? Why should the first be punished and the second be excused for "mistakes"? Good questions.

ROCHE DIAGNOSTICS

On 16 July 2003, Roche Diagnostics announced that it had developed a sensitive and accurate SARS research kit. The work began in March. In May, it said it was collaborating with the Genome Institute of Singapore (GIS) to produce such a kit. Special pieces of genetic material called primers developed by GIS are a critical ingredient in the new test. These primers are short stretches of artificially created genetic material which match a corresponding stretch on the SARS virus. GIS Deputy Director Dr Ren Ee Chee explained that GIS's work in uncovering mutations in the virus helped it to develop primers based on "stable" parts of the SARS genetic code, so that the tests could correctly detect the disease even in mutated samples.

Roche said the test is close to 100% accurate, and it can detect SARS before patients show symptoms and results can be provided in an hour. Following this, regulatory approval for the test was sought from the FDA of the US and the CE of the EU. This could take up to 18 months. *(Straits Times, 18 July 2003, pg. 4, col. 1-7.)* Roche took all of eight weeks to develop the test – its shortest time ever for coming up with such a product. The quick turnaround was accomplished because virology institutes and government agencies around the world collaborated with it. The test is based on PCR technology. PCR acts like a kind of genetic photocopier, allowing scientists to detect even minute samples of genetic material be it from blood, spit or stool samples.

SARS VACCINE

We have no vaccine yet, but on 19 December, the *Straits Times* reported that the race is on with a flurry of research activity. Among the front runners is France's premier Pasteur Institute, which has linked with Europe's largest drug maker, GlaxoSmithKline, to develop one. *(Straits Times, 19 December 2003, pg. A2, col. 3-7.)* Hong Kong has its version and China has three kinds of vaccine ready for tests on patients. A genetically engineered vaccine had shown promising results in monkeys, and the team from Pittsburgh University, Pennsylvania, hopes to start clinical trials before the end of 2004. The Canadians too have a vaccine ready for human testing. In Singapore, NUS said its effort had been hindered by safety lapses that led to a local scientist becoming infected with SARS in September.

So if there are vaccines ready for testing, but there is no more SARS, how would we know if these vaccines are effective? SARS has infected 8098 people worldwide and killed 774 to date.

SARS GENES?

Researchers in Taiwan said a genetic susceptibility may explain why SARS affected South East Asia so badly. They found certain

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HLA types common in the people of Southern Chinese descent that made patients in Taiwan much more likely to develop life-threatening symptoms of SARS. Their research was published in BMC Medical Genetics. Researchers at Mackay Memorial Hospital in Taipei examined the HLA genes in 37 probable SARS, 28 with fever but no SARS, and 101 HCW not infected but exposed to SARS. 190 normal, healthy unrelated Taiwanese were used as controls. They found that patients with severe SARS were likely to possess HLA-B 4601. This gene is not seen in indigenous Taiwanese (1.5% of the population) and none developed SARS. Further this gene is seldom seen in European populations. *(Straits Times, 3 October 2003, pg. H11. col. 1-4.)*

SARS TREATMENT

There is no cure presently. Hospitals have tried everything from controversial drugs to Traditional Chinese Medicine (TCM). *(Straits Times, 31 May 2003, pg. A6.)* Hong Kong, like Taiwan, used Ribavirin on an empiric basis. The Director of the Infection Prevention Department at the National Taiwan University Hospital, said Ribavirin is effective if used within 10 days of infection. Professor Yuen of the Department of Microbiology, University of Hong Kong, noted that 90% of the first 138 SARS patients in HK showed improvement when given Ribavirin and high doses of steroids. A combination of TCM and Western Medicine was endorsed by the HK Hospital Authority for treatment of SARS patients after a two-week trial on 21 patients. WHO recommends that TCM be used only as a supplement. What are these treatments?

In Hong Kong, the disease is managed in three phases. In the first, when viral multiplication is rapid, patients are given antibiotics and/or antivirals like Ribavirin or Kaletra. Phase 1 lasts about one week. In Phase 2, when the body's immune system attacks the virus, steroids are used to prevent the immune system from damaging the lungs. Antivirals are discontinued after 14 days. Steroids are given from the 8th to 21st day. In phase 3, when lungs are severely damaged, assisted ventilation is followed by rehabilitation, if the patient recovers. In addition, TCM may be added to the regime. Herbs include liquorice root, honeysuckle, white mulberry leaves and purple perilla leaves.

A team of Hong Kong researchers reported success in SARS treatment among 31 patients defined as probable SARS. *(Lancet, 2003;361:1615-17.)* Their protocol consisted of a combination of antibacterials: levofloxacin (500 mg once daily) or clarithromycin (500 mg) plus coamoxiclav (375 mg three times daily for young or pregnant patients/patients with tuberculous), ribavirin (400 mg every eight hours for at least three days, then 1200 mg twice daily), and methyl prednisolone (1 mg/kg every eight hours for five days, then 1 mg/kg every 12 hours for five days) changed to prednisolone (0.5 mg/kg twice daily for five days, then 0.5 mg/kg daily for three days, then 0.25 mg/kg for three days before stopping). Patients were given pulsed methyl prednisolone (500 mg twice daily) if their clinical condition or CXR worsened or if lymphopenia persisted.

In China, TCM is used in every phase of treatment. Ionicera powder and forsythia are for shortening fever periods and lowering fevers. In rehabilitation, TCM supplements such as American ginseng and gingko are used. Other TCM ingredients used include Chinese ephedrine, shigao tang and bezoar.

In Taiwan, antibiotics and antivirals are used in Phase 1. In Phase 2, when Ribavirin is no longer affective, intravenous immunoglobulin is used, preferably after 10 days of antiviral therapy. In Phase 3, it is assisted ventilation and TCM to maintain the immune system.

Antisense technology promises new "smart" drugs for cancer and SARS. *(Straits Times, 27 May 2003, pg. H8, col. 1-2.)* This technique aims to kill the genetic messenger carrying diseases. Cancer patients are taking an experimental drug, Genasense in three pivotal trials. The same technique is being used at AVI Biopharma in Portland, Oregon for SARS. AVI said its drug Nevigene which targets West Nile Virus, had been tweaked to take on SARS. Antisense drugs jam vital genetic signals by tackling targeted RNA, which carries DNA's instructions to the body. Antisense scientists create mirror images of the mRNA that is spreading illness. When injected into the body, the mirror image binds with the RNA and prevents it from delivering its message to protein building machinery.

From the New England Journal of Medicine, 18 December 2003 issue, 349:2431-41, on "Current Concepts: The Severe Acute Respiratory Syndrome", under management, comes the bland statement that there is no effective therapy that has been well documented.

And from the Journal of the American Medical Association, 24-31 December 2003 issue, 290:3222-8, is a report of a preliminary study on the use of interferon Alfacon-1 plus corticosteroids in SARS. 22 patients from the North York General Hospital, Toronto were given this treatment between 11 April and 30 May with apparent improvement.

CONCLUSION

Research is important in so many facets of SARS. Hopefully, the diagnostic kits now available would make diagnosis easier and earlier. Even in the asymptomatic patient, the test could be done. There should be less or no confusion with dengue fever. With earlier diagnosis, treatment trials can hopefully get underway faster. With no global SARS outbreak, the laboratories are where the virus resides (besides in wild animal reservoirs). Mishaps in the laboratory can rapidly expose societies and nations to another round of deadly SARS and this need not be in the winter of the Northern Hemisphere. Contrary to earlier predictions, winter is upon us but there is no SARS outbreak – only sporadic cases (two to date). Let us keep hoping for the best. ■