For information

SARS Expert Committee

Laboratory Investigation by Department of Health

Purpose

This paper aims to give a brief summary on the role and efforts of the Pathology Service of the Department of Health (DH) in laboratory investigation in connection with the Severe Acute Respiratory Syndrome (SARS) outbreak in Hong Kong.

DH Pathology Service

2. The DH Pathology Service provides laboratory services to Government clinics and health centres, as well as public hospitals and institutions for both public health functions and patient care. It comprises different laboratories specializing in bacteriology, virology, cytology, histopathology, immunology, neonatal screening, as well as haematology and chemical pathology. Laboratory support for disease surveillance, outbreak investigation, and responding to biological incident is mainly provided by the bacteriology and virology laboratories, mainly the Government Virus Unit (GVU) in the lead, involving also the Public Health and Microbiology Laboratories.

3. DH Pathology Service maintains close liaison with the World Health Organization (WHO) and other international institutions regularly on the surveillance of infectious diseases, and the GVU has been designated as a WHO National Influenza Centre since 1962. Laboratory surveillance has been expanded since the isolation of influenza A (H5N1) virus in 1997. Except for the Prince of Wales Hospital which conducts laboratory investigation in its own Pathology Department, respiratory specimens obtained from patients in public and private hospitals as well as sentinel clinics are cultured for isolation of respiratory viruses including influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus (RSV), herpes viruses and enteroviruses. All viruses isolated are characterized in details. For influenza viruses, those showing differences with the current circulating strains are sent to the WHO Collaborating Centre in the US and UK.

At Initial Stage of the Outbreak of Atypical Pneumonia

4. In early February 2003, following media reports on atypical pneumonia (AP) in the Guangdong Province caused by unknown agent, enhanced surveillance of AP was started in Hong Kong and it was agreed that severe community acquired pneumonia (SCAP) cases in the Hospital Authority (HA) hospitals would be referred to DH for epidemiological and laboratory investigations. In this regard, the GVU had been regularly receiving lists of SCAP cases from the HA since 12 February, but some patients' specimens were not sent to the GVU, making laboratory investigations not possible for these cases. The GVU therefore took the initiative to contact the microbiology department of the major hospitals and afterwards managed to obtain specimens from the severely ill patients for laboratory investigations. Some of the cases handled are highlighted in the ensuing paragraphs.

5. Upon notification on 13 February of two suspected AP cases involving a father and his son returning from Fujian, the GVU promptly conducted extensive laboratory investigations and later confirmed influenza A (H5N1) infection on the two patients on 19 and 20 February respectively. The WHO was immediately alerted on the same days when the H5N1 infection was confirmed and the prompt action and reporting had enabled the WHO to take early alert, informing the international communities and health agencies via a press release issued on 19 February and putting the WHO Global Influenza Surveillance Network on alert.

6. On 24 February, DH was alerted that a SCAP patient from the Mainland had been admitted to Kwong Wah Hospital. The patient died on 4 March. The GVU and the University of Hong Kong (HKU) conducted extensive investigations on the patient. However, the results were all negative, ruling out influenza infection, except that serology study showed a fourfold rise in adenovirus antibody titre.

7. In early March, DH was informed of a case of influenza B with severe pneumonia who was transferred from Hanoi to Hong Kong for treatment and who had previously infected a number of health care workers in the hospital which he had stayed in Vietnam. The patient was admitted to the Princess Margaret Hospital on 6 March and finally succumbed on 13 March. Extensive investigations were conducted by the GVU and HKU on clinical specimens, including polymerase chain reaction (PCR), cell culture and serology for influenza, parainfluenza, RSV, adenovirus, enterovirus, hantavirus, metapneumovirus, mycoplasma, rickettsia, chlamydia and legionella. However, no causative agent was detected.

International Collaboration

8. On March 15, WHO issued a global warning on the emergence of SARS and provided information on the disease including recommended SARS case definitions. At the same time, the WHO set up a network of scientists from 11 laboratories in 9 countries/territories to secure the participation of laboratories with scientific expertise in the detection of wide range of viruses and concentrate intellectual resources, with a view to expediting identification of the causative agent and developing a robust and reliable diagnostic test. GVU is among the three laboratories from Hong Kong participating in the network; the other two are from the HKU and the Chinese University of Hong Kong (CUHK).

9. GVU participated actively in the WHO laboratories network and the international collaboration involved daily teleconference; posting and exchange of laboratory findings including EM pictures, primer sequences through a secure WHO website; testing protocols etc. Samples from SARS patients were exchanged enabling simultaneous analysis and sharing of results in real-time. Several etiologic agents have been proposed during the course of investigation for causative agent. Locally, the CUHK had the initial finding on paramyxo-like virus or human metapneumovirus on 18 March. Later on 21 March, the HKU, using special cell line, successfully isolated an agent from the specimens from SARS patients. Several paired sera from SCAP cases as well as archived sera from other cases previously obtained by GVU were tested against the newly identified agent in immunofluorescent test. The results of the tests confirmed the agent reacted in high titres with the SARS sera and on 22 March, electron microscopy done by the GVU showed an enveloped virus of 70 -130nm resembling coronavirus. The same finding was also obtained at the Centres for Disease Prevention and Control (CDC), US.

After Coronavirus Found

10. Soon after the initial identification of coronavirus, a sequence of the virus genes was made available to the GVU by the US CDC. This information enabled the GVU to design primers used in PCR to detect the virus in clinical specimens. The Unit ordered a few primers and promptly carried out comparison of their sensitivity. The evaluation was completed on 26 March and DH started testing patients' specimens for coronavirus on 27 March. The availability of the PCR test was just in time to supplement the investigation of the cluster of cases in Amoy Gardens, enabling effective laboratory investigations on the cases. Upon continuous efforts in improving the test, the GVU successfully designed another pair

of primers to replace the initial pair on 30 March. At the same time, the GVU started growing virus in suitable cell line and determining the growth characteristic of the virus. Large amount of infected cells were necessary to test for antibody to the SARS virus. The work was tedious and required to be done in high risk laboratory. For safety consideration, only experienced staff were assigned to the assignment. By early April, the GVU have established sensitive cell line and gathered enough experience to start virus culture and antibody testing.

Challenges

11. With the SARS outbreak continuing and the introduction of the PCR, culture and immunofluorescent antibody tests, the GVU had to cope with enormous workload, including testing for hospital patients, contacts and surveillance cases. There was an urgent need to produce the testing results in very short time and the GVU managed to produce results with 24-hour turnaround time. At the same time, the Unit needed to do experiments to evaluate different tests and study virus shedding pattern. All these promoted teamwork and propelled the laboratory to move forward rapidly. Different testing methods and testing strategies were developed and tested quickly and implemented within a very short period of time. Given the new virus and the existence of little knowledge but many unknowns, the Unit needed to constantly monitor new developments in virus characterization. Moreover, GVU was requested to evaluate a few test methods developed by overseas institutions as well as the primers used in the WHO training workshop in China from April onwards. At the same time, for preventive measures, the Unit also started working on survival time of the new virus under different physical and chemical conditions. Priority had to be set with limited resources.

12. The Amoy Gardens cluster which surfaced in late March brought a new scope to laboratory investigation. By early April, in addition to the clinical samples, GVU had to embark on large scale environmental testing with thousands of requests and samples from the Food and Environmental Hygiene Department, Agriculture, Fisheries and Conservation Department, Environmental Protection Department and Drainage Services Department. This presented a new challenge to the laboratory investigation work since environmental testing was technically more demanding and required highly skilled staff.

13. For SARS investigation, DH Pathology Service has so far performed over 20,000 PCR, virus culture and immunofluorescence antibody test on clinical specimens and environmental samples (see Appendix). From end of March, 10 Medial Laboratory Technician II and 1 Scientific Officer (Medical) were deployed from other specialties to cope with the huge increase in workload, in addition to the

42 staff of the GVU. It was difficult to deploy more senior staff due to the complex nature and specific skill requirements of the laboratory work involved. The laboratories had to operate seven days a week and worked late into the evening in order to cope with the workload and the staff of the virology laboratory was put on roster to work on extended hours. The manpower was stretched very thin.

14. On top of managing the laboratory work and developing testing methods, the Unit were constantly requested to give advice on various virus-related and infection control issues raised by different departments as well as the public. Inter-laboratory interaction in Hong Kong as well as outside also took up much of our time. Packaging and sending requested specimens for research and diagnostic development to local and overseas laboratories stretched further the much needed manpower. As the specimens are carefully labeled, properly stored and completed with all the relevant information, the WHO requested the GVU to set up specimen bank with aliquots of catalogued specimen for use in the diagnostic development. As this involved sorting, labeling, aliquoting, storing and keeping records of thousands of specimens, this initiative could only be carried out slowly with whatever remaining resources.

15. One of the difficult problems facing the laboratory work was data management. From early April, results were e-mailed to Pathology Department of different hospitals. At the same time, the test results had to be analysed and transmitted to DH rapidly and the HA information technology unit also required daily updates of testing records. To cope with these demands, GVU had redeployed two staff from its already tight staff pool to take up data management tasks on nearly full-time basis.

Department of Health
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Appendix

Laboratory tests performed for the investigation of SARS outbreak

(March-May 2003)

	March	April	May
Clinical specimens:			
RT-PCR	582	5948	3006
Culture	938	5200	2290
IF antibody tests		1779	4453
Sequencing		344	32
RFLP		44	321

	April	May
Environmental samples:		
RT-PCR	916	1319
Culture	916	75
Sequencing	108	20