Reviews and reports

Laboratory needs for emerging infectious diseases

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The medical laboratory plays a critical role in recognizing, emerging and re-emerging infectious diseases by establishing a specific etiology for disease syndromes seen by clinicians, and by reporting new or unusual pathogens that such laboratories might encounter. The laboratory may also serve as a key surveillance point for information gathering and dissemination, as is the case with information about antimicrobial resistance. For the laboratory to have maximum impact, it must be able to perform competently a series of essential steps. These include the collection of appropriate clinical specimens, appropriate handling during transport, appropriate labelling and access for the microbiologist to relevant clinical observations, to better allow the laboratory to ensure that testing is done for all possible causes of the symptoms seen. The laboratory will also benefit from current knowledge of the existing epidemiological situation. The chain of events is completed when the laboratory provides results to the attending medical staff, to allow clinical management of the individual case, and information to the epidemiologist, for trend analysis and monitoring, or alert should a new pathogen be recognized.

Appropriate specimen collection involves ensuring that the attending medical staff is knowledgeable about the samples to be obtained—whole blood, serum, cerebrospinal fluid or other specimens. It also requires that the proper containers be available, especially if the specimens are to be transported outside the hospital or clinic. This often means having plastic tubes (nunc tubes or equivalent) that can be securely transported without risk of breakage in transit. Although such information seems obvious, clinical specimens often arrive at reference laboratories without proper information as to the source of the specimen and where results should be sent. Without this important information, the laboratory cannot contribute effectively. Finally, if clinical specimens are to be shipped by air freight or courier service, care must be taken to ensure that the material is properly packaged in a shipping container that meets International Air Transport Association (IATA) regulations.

Formal guidelines are available from IATA and should be consulted prior to arranging international transport of infectious specimens. Of course, the laboratory to which the specimens are being sent should be notified in advance that the shipment is being made, so that it may assist with receiving activities (customs clearance, transport from airport to laboratory, etc.).

Once arrived at the laboratory, care must be taken to ensure that each specimen is ap-

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propriately logged into a tracking system so that it can be followed and later archived, if appropriate (Table 1). The critical needs of the laboratory, however, centre around three basic issues: having the proper equipment and consumable supplies to conduct the tests needed; having the reagents necessary (antigens, antibodies, positive and negative controls, and so on) to test for the pathogens of interest; and having staff adequately trained in the conduct of the tests required, some of which are complex. Each of these needs may be significant limiting factors to the successful recognition of emerging infectious diseases. For example, diagnostic reagents for even common viral diseases such as dengue may not be commercially available. Thus, the virus laboratory must rely upon its own locally produced reagents or on reagents supplied by other cooperating groups. These may lack appropriate quality control in production and can vary considerably in quality from batch to batch.

Table 1 **Requirements for appropriate laboratory analysis**

- Equipment requirement met
- Appropriate safety precautions in place
- Required reagents available
- Properly trained staff
- A quality control programme in operation
- Specimen log maintained
- Specimens archived, as required
- Communications ability

Diagnostic reagents

The availability of high quality diagnostic reagents may be the greatest limiting factor to effectively addressing new and re-emerging infectious diseases, especially in the case of viral diseases. For example, the WHO network of collaborating centres for arboviruses and haemorrhagic fevers was recently surveyed to determine what diagnostic tests were routinely conducted and if the collaborating centres had on hand diagnostic reagents for several common arbovirus and haemorrhagic fever diseases. This network involves 36 laboratories in 27 countries that were selected for their technical expertise and special capabilities, and they often serve as national reference centres for viral diseases. These laboratories are called upon to assist in the identification of new or unusual viruses and serve as referral facilities to many national laboratories. We found that virtually all these reference laboratories had the necessary equipment to perform routine virus serology and cell culture–based isolation, and many also had access to laboratory animals for isolation attempts and antisera production. Unfortunately, significant problems were found with respect to availability of diagnostic reagents. Only about two thirds had reagents to diagnose dengue, only half could diagnose yellow fever and only about a quarter had reagents for haemorrhagic fever viruses such as Lassa or Ebola. These laboratories had good reagent coverage for diseases known to occur in their own area, but lacked reagents for other common viral illnesses occurring elsewhere, so that for example it might prove difficult to confirm the diagnosis of an infected traveller from another region.

To overcome this problem, WHO has tried to emphasize regional self-sufficiency in reagent production. To do this, WHO has worked closely with key laboratories in most WHO Regions to facilitate reagent production, often assisting with production and distribution costs. For example, in Brazil WHO arranged a collaborative agree-

ment between the Instituto Evandro Chagas in Belém to produce dengue and yellow fever suckling mouse brain antigens for use in IgM capture immunoassays. These bulk antigens were then given to Dr Robert Shope, now of the University of Texas at Galveston, who inactivated them and packaged them in small volumes. He also conducted quality control tests to ensure their reactivity and safety. These reagents are now available to assist laboratories in need and can be requested directly from Dr Shope at: Department of Pathology, 301 University Blvd, Galveston TX 77555–0609, USA: Fax: +1 409 747 2429; e-mail: robert.shope@utmb.edu. A similar effort has been advanced by the Centers for Disease Control (CDC) in Atlanta, Georgia, working closely with Dr F. Pinheiro at the Pan American Health Organization (PAHO), focusing on dengue reagents alone; PAHO has also assisted in the development of other national reference laboratories for dengue as well.

Another way to overcome the reagents problem is to make greater use of currently available biotechnology to produce expressed antigens and labelled monoclonal antibodies. For example, CDC Special Pathogens Branch has contracted to produce a large volume of expressed antigen for the Sin Nombre virus, cause of the recently recognized hantavirus pulmonary syndrome. This antigen is designed for use in enzyme immunoassays, is completely safe and non-infectious and is easily transported. Interested laboratories may request this antigen directly from the Special Pathogens Branch, NCID, CDC, 1600 Clifton Road NE, Atlanta GA 30333, USA; Fax: +1 404 639 1118. Another example is the broadly cross reactive antiflavivirus monoclonal antibody developed and distributed by CDC Fort Collins. This antibody is directly labelled to serve as a conjugate in IgM capture immunoassays and appears to function equally well in both yellow fever and dengue assays, and probably in assays for many other flaviviruses as well. This monoclonal antibody is available from Dr Shope when the suckling mouse brain antigens mentioned above are requested, or directly from the CDC laboratory in Fort Collins, Colorado (request from Division of Vector-borne Infectious Diseases, NCID, P.O. Box 2087, Fort Collins CO 80522; Fax: +1 970 221 6476).

Positive control sera

To ensure the quality of serological tests, it is important to include positive and negative control sera. Often, however, these are difficult to obtain, especially for low incidence diseases. Nonetheless, WHO has attempted to collect these key sera, and at present Dr Shope has on hand small quantities of positive control sera for dengue, yellow fever, tick-borne encephalitis, Puumala and Hantaan hantaviruses and perhaps others. These too have been dispensed in small volumes and are available if requested. Clearly, these are valuable reagents, and WHO would prefer laboratories to use them only in a reference capacity to validate their own locally acquired positive control sera. WHO would also welcome donations of positive control sera. If necessary, WHO can assist in the costs of obtaining these important sera. Dr Shope should be contacted directly to arrange shipment and payment for positive control sera.

Virus laboratory containment

One of the most important aspects of a global strategy to monitor emerging diseases, especially those caused by viruses, is ensuring that the proper level of biosafety con-

tainment is available to allow safe handling of pathogenic viruses. Most viruses are classified at one of four distinct biosafety levels, depending upon the seriousness of the disease they produce, their transmissibility and the availability of effective treatment or vaccination. Most viruses are classified as biosafety level 1 or 2. Level 1 organisms are thought not to cause human disease, while level 2 organisms are indigenous and of moderate risk, causing illness of varying but usually not life-threatening severity. Basic microbiological precautions are enforced, including no eating or smoking in the laboratory. Biosafety level 2 laboratory conditions include limited access, no children under 16 years of age, biohazard warning signs posted, all staff wear laboratory coats, wastes are autoclaved and a safety cabinet is used for all procedures that might generate aerosols. Biosafety level 3 laboratories have limited access through a double-doored airlock or changing room, directional air flow, high-efficiency particulate air (HEPA) filtered exhaust air; workers wear protective clothing. Biosafety level 4 conditions have locked door access, special treatment of sewage, all items leaving the laboratory are sterilized first, directional air flow, back-up HEPA filtered exhaust air, individual room supply and exhaust air, emergency power supply and back-up exhaust fans. Most work at biosafety level 4 is conducted with the investigator either inside a protective suit that is supplied with independent air, or in sealed safety cabinets ("glove boxes"). The physical plant required to support a biosafety level 4 laboratory is considerable, and maintenance expenses are high. Consequently, only a few exist, and all serve as major referral laboratories as needed.

Up to this point this report has focused on some general considerations of the needs of the laboratory in responding to new and emerging infectious diseases. Below is provided a few examples of how the laboratory assists with monitoring emerging diseases.

Influenza

A global network of over 100 virus laboratories collaborates successfully each year to determine which influenza viruses are circulating and, by contributing recent isolates to three international reference centres, lays the foundation for the selection of the following year's influenza vaccine. Local or national laboratories make routine virus isolates from patients as the influenza season starts. They have limited diagnostic reagents on hand that allow them to determine the basic characteristics of the isolates made, such as influenza A or B, and tentative haemagglutinin and neuraminidase types. Representative isolates are then sent to one of three international reference centres (CDC, USA; London, United Kingdom; or Melbourne, Australia) for detailed genetic and antigenic characterization. The results of these studies are then summarized each February during a special meeting of the laboratory directors and representatives of control authorities. The meeting concludes by deciding which antigens should be included in the next influenza vaccine, and the following day this information is provided to the commercial manufacturers. This network of collaborating laboratories is one of the most successful examples of active monitoring for new and emerging infectious diseases, since virtually every year the influenza strains in circulation change, and accurate laboratory information is required to ensure that the appropriate modifications are made to the vaccine. The critical elements mentioned above in specimen acquisition, transport, testing and delivery of results are key to the success of the programme.

Monitoring antibiotic resistance

The growing threat of antimicrobial resistance is of great concern globally. In order to address this problem, WHO has collaborated in development of a computer program called WHONET that assists hospital microbiologists to monitor nosocomial infection trends within their own hospitals and to share their results with other hospitals in a systematic manner nationally, regionally and globally. The needs of the system are simple: the hospital laboratory must routinely conduct quantitative antibiotic sensitivity testing on clinical isolates made; a microbiologist or other skilled individual must manage the system and review the information gathered; and the laboratory must have a computer available on which to run the program.

At present well over 100 laboratories around the world participate in the WHONET system, and efforts are under way to expand the network significantly. In developing the network, it has become clear that two critical factors greatly influence the success of any laboratory in participating in WHONET: the availability of high quality disks to measure antibiotic sensitivity; and participation in an active quality control and proficiency testing programme, so that technical problems can be recognized and resolved promptly. The problem of disk quality is a challenge yet to be resolved, but will be approached in a similar manner to the reagent problem for viral diseases by attempting to develop regional self-sufficiency. The problem of quality control and proficiency testing is being addressed now through a collaborative initiative with CDC Atlanta, which has agreed to periodically send out test organisms of known sensitivity profiles to collaborating laboratories. This programme has already begun, with good cooperation seen. Laboratories wishing to join the WHONET network should contact Dr E. Tikhomirov, EMC, World Health Organization, Geneva, Switzerland; Fax: +41 22 791 4878.

Special pathology laboratory needs

It is appropriate to mention the key role that the pathology laboratory can play in identifying new and emerging infectious diseases. In many respects, the pathology laboratory may have the fewest needs in terms of specimen transport, since it often requires only formalin-fixed tissues, which are noninfectious and thus more easily transported. As but one example of the benefit of having an expert pathology referral laboratory, we summarize the recent advances made by Dr Sherif Zaki of CDC in identifying Ebola virus in tissues of fatal cases seen in Africa. Clearly surveillance activities in some areas of developing countries are a challenge since it is often difficult or impossible to obtain and transport specimens from remote medical clinics to reference diagnostic facilities. In response to this challenge, Dr Zaki and his colleagues at CDC have developed a procedure to examine formalin-preserved skin snips from fatal cases for the presence of Ebola antigen. The specimens are easily obtained and transported, yet allow a confirmed diagnosis to be made. Although the procedure has only been validated for Ebola, studies are continuing to determine if it has value for other diseases.

Summary

The diagnostic laboratory plays a critical role in addressing the challenge of emerging infectious diseases. To be most successful, it

requires appropriately obtained and handled specimens that have been transported safely and efficiently and have been accompanied by adequate patient clinical and epidemiological information. Reagent availability is a critical limitation to the range of diagnostic tests that the laboratory can perform, and adequate training opportunities must be made available to ensure that staff are prepared to conduct the technically demanding tests that may be required to identify new or unusual pathogens. To ensure the quality of the results generated, quality control and proficiency test programmes are essential. It is likewise critical that the appropriate biosafety containment conditions exist within the laboratory to allow safe testing of pathogenic organisms. Finally, prompt communication of results to the attending medical staff and to epidemiological monitoring programmes are essential. In laboratories specifically interested in new or re-emerging infectious diseases, a systematic programme to archive clinical specimens may prove to be extremely valuable in recognizing new strains or changing incidence patterns.