National and international veterinary reference laboratories for infectious diseases

S. Edwards & D. Alexander

Virology Department, Central Veterinary Laboratory (Weybridge), Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Summary

Reference laboratories play an increasingly important role in the harmonisation of laboratory diagnostic tests and the standardisation of veterinary vaccines. This is particularly important in building confidence between international trading partners. The authors review aspects of the organisation, designation and support of reference laboratories for infectious diseases of animals and discuss the principal activities which such laboratories would normally perform. These activities include advice and consultancy, publications and communication, training, research, disease surveillance, maintenance of culture collections, evaluation of reference methods, preparation of reference materials and organisation of inter-laboratory comparisons.

Keywords

Introduction

The Concise Oxford Dictionary defines reference as ‘the act of referring a matter for decision, settlement or consideration to some authority; or the scope given to this authority’. Within the specialised context of this paper, a reference laboratory is one that is considered by the veterinary scientific community at large to produce authoritative information, opinions or advice. In support of this activity, the laboratory will perform laboratory procedures, including the analysis of samples submitted by other laboratories, the archiving of biological materials, and the preparation and validation of reference materials for use by other laboratories. The reference laboratory may also serve as a focus or facilitator in organising – or even ‘policing’ – comparative testing for different national or regional laboratories.

The need for reference laboratories

The fact that variability is an overriding feature of all attempts to measure biological systems is well recognised by those who work with such systems. The sources of this variability are many, complex and interactive with one another, so that the biologist rarely deals with absolutes but rather with relative measurements, and must often resort to descriptive phrases in the absence of defined quantitative criteria. The practical consequence of this for veterinary laboratories is considerable difficulty in maintaining consistency of results, both within a laboratory and even more so between laboratories. Although difficult to explain, it is well recognised that on occasion, two or more laboratories apparently following identical procedures can produce different results when testing duplicate aliquots of the same material. Only the most detailed investigation of every aspect of the methodology used (the exact specification of reagents used, the calibration of the equipment and the skills of the personnel) can shed light on the reasons for such discrepancies. Such a detailed investigation is not feasible for each and every procedure used in a typical laboratory, thus the opportunity to call upon the services of a reference laboratory to help resolve such matters is of considerable value. Discrepancies between laboratories become even more apparent when different methodologies are used to measure the same phenomenon (for example, antibody titre in a serum sample). The reference laboratory may be asked for comment on the relative merits of different tests, whether those tests can be considered as equivalent, and indeed the level of response in each case that may be considered to represent a ‘positive’ result.
The rationale for using a reference laboratory is that it represents a centre of excellence for the particular disease, i.e., that it generally has access to experts knowledgeable about the disease and the control of that disease, together with others who have specialist skills in the diagnostic methods appropriate for the disease, and in some cases the preparation and evaluation of effective vaccines for the disease. Thus the reference laboratory is relied upon to provide a definitive opinion (within the scope of current scientific knowledge) on matters concerning the diagnosis and control of the disease in question. The reference laboratory does not usually have a controlling authority over other laboratories with which it has dealings. If the reference laboratory has such an authority, this is usually as a result of its role as a central or national laboratory (10); the role of the reference laboratory is principally to provide unbiased advice that is founded on the best scientific knowledge available.

Organisation of reference laboratories

Personnel requirements

A reference laboratory designation usually applies to a specific disease or group of diseases. The designation implies that the laboratory is staffed by key personnel with an extensive knowledge of the disease(s) concerned. They should be familiar with the disease in the field, its epidemiology, transmission, control, clinical appearance and pathological features. They should understand all the appropriate diagnostic laboratory methods for the disease and should be able to provide a critical interpretation of laboratory test results, including the assessment of accuracy and predictive value of individual tests. The specified disease expert in a reference laboratory would normally be expected to command a position of authority in the scientific community as a result of publications, presentations and current research or investigative activities on the disease (6).

In addition, the reference laboratory should provide a team of technical support staff who are experienced in performing the relevant diagnostic tests and in providing the underlying technological support and performance monitoring. It is important that personnel policies, salaries and conditions of employment are such as to encourage stability among the scientific personnel, which leads to improved reliability and continuity of scientific services (5).

Equipment and facilities

To be prescriptive about the type of equipment and facilities required by a reference laboratory is impossible and inappropriate, as this will depend upon the nature of the disease, the categorisation of the infectious agent and any local regulations regarding the handling of the agent or pathological materials. One prerequisite is that the reference laboratory should be able to handle the organisms specified in its role to the highest appropriate containment levels (6). For international laboratories in which carrier materials may be received from other countries, the containment levels should reflect those organisms likely to be contaminating the carrier materials unless previously inactivated. Sufficient equipment should be available to perform the range of tests required for the particular disease, and there should be sufficient spare capacity to handle unexpected peaks of work, for instance during an epidemic. All equipment in the reference laboratory must be serviced regularly, and records should be kept to demonstrate that the equipment is performing within expected parameters. The reference laboratory should have facilities for long-term storage of strains and reference materials and, wherever possible, key materials should be stabilised in a form that is independent of temporary interruptions to power supplies (e.g., lyophilised). There should be access to adequate transport facilities for shipment of reference materials to other laboratories within the sphere of influence (national or international) of the reference laboratory, and the reference laboratory should establish a satisfactory system for the receipt of clinical and laboratory samples from the field (3). This may require arrangements for import permits or legal authority to hold and work with particular pathogens.

The reference laboratory should have a well-organised office system which ensures that all records are kept up to date and are archived in an accessible manner. The records should include lists of pathogens and strains held, antisera and other reference materials, details of diagnostic submissions and test results, and quality control records related to the monitoring of equipment and test performance. Facilities for electronic communication with other laboratories or, at the very least, an efficient postal system, are desirable.

Designation

A reference laboratory cannot designate itself. The director of a laboratory seeking reference status should apply to the appropriate authority and provide relevant information in support of the candidacy. National reference laboratories are normally designated or nominated by the national government authorities, and these authorities will determine the scope of the designation. In some cases, national laboratories are officially recognised by international bodies as the reference point for laboratory matters concerning the disease in that country. This is the case in the European Union (EU), where Directives for the control of specific diseases (e.g., classical swine fever [hog cholera], Aujeszky's disease, Newcastle disease) list a national laboratory for that disease in each member state. The national laboratory then has a co-ordinating role for maintaining standards in any regional or other laboratories within the country, as well as a responsibility for communication with the relevant EU-designated international reference laboratory.

For international reference laboratories in particular, the balance between the requirements for personnel and facilities
must be weighed carefully. It is not sufficient to assume that a disease 'expert' will necessarily have adequate facilities available; nor should it be assumed that the presence of the best available facilities at an institute will necessarily attract an expert of sufficient calibre to be accepted as such by the international scientific community.

International organisations which recognise reference laboratories in the area of infectious animal diseases include the Office International des Epizooties (OIE), the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) of the United Nations (7). Additional recognition may be granted by continental or world regional organisations. Each of these organisations has a slightly different focus, and thus their expectations from a reference laboratory will vary somewhat (2). The OIE is particularly concerned with the effects of contagious animal diseases on international trade in animals and animal products, and this role has assumed a greater significance since the adoption by the World Trade Organisation (WTO) of the sanitary and phytosanitary agreement (11). The OIE thus places great importance on the development of written standards for disease control and diagnosis, which in turn lead to a need for defined operational parameters for diagnostic and vaccine-producing laboratories. The FAO has an advisory and supportive role for countries seeking to control animal diseases, and designates reference laboratories to develop diagnostic capability, to standardise reagents, to characterise causative agents and to provide training. The WHO is involved in the publication of international standards, in the designation of international reference preparations and also in the stimulation of collaborative research by the appointment of laboratories as collaborating centres for research on specified diseases.

In the case of the OIE, applicants for reference laboratory status need to provide information in the following categories:

- name and curriculum vitae of expert
- names of the laboratory and the director of the laboratory
- experience in diagnostic testing for the disease
- additional expertise in the disease (agent characterisation techniques, molecular techniques, monoclonal antibody techniques, vaccine development or evaluation)
- experience in standardisation of diagnostic tests
- reagent production and shipping capability
- current and completed research projects on the disease and work on methods development, including a list of relevant publications
- experience in training and consultation on the disease.

Financial support
In general, the international organisations do not enjoy sufficient resources to sponsor adequately the reference laboratory activities that they would like to support. The actual level of support varies from nothing to a limited contribution towards the direct costs of materials or shipment. For this reason, the activities of many international reference laboratories are limited to those which they can support from their own internal resources, or from funding provided by their national authorities (6, 7). There is little doubt that this imposes a severe restriction on the amount of reference laboratory activity in the veterinary world. Where a supranational authority has legal and financial powers, as is the case for the EU, then specific funding for a reference laboratory may be provided for the more important diseases, particularly those in OIE 'List A' (9). The size of the budgets needed to operate such a reference facility adequately (for example, European currency unit [ECU] 150,000 [ECU1 = US$1.11] for classical swine fever and ECU100,000 for Newcastle disease in 1997) demonstrates the problems faced by other international reference laboratories which have no specific funding for the task. In addition, by providing an operating budget, the funding authority is able to specify exactly which tasks the reference laboratory is required to perform.

One way to alleviate the funding difficulties is for reference laboratories to charge for their services, in particular for reference materials supplied and also for the costs of providing training. The OIE has recognised the right of OIE reference laboratories to make appropriate charges: however, such an action tends to detract from the overall principle of open access to information and materials which underpins the very concept of reference centres. Nevertheless, not to charge for reagents may be impracticable: for example, the cost to the EU Reference Laboratory for Avian Influenza of supplying the national laboratories with antisera to identify influenza viruses to the subtype level has been calculated to be in excess of half of the entire budget of the laboratory for one year.

Evaluation and audit
Upon designation as a reference centre, a laboratory is required to ensure that work and control methods in place are of the highest possible standard. At present it is not possible to require that a reference laboratory has any formal accreditation, as many veterinary laboratories lack such certification, even though their work may be universally recognised as world class. There is also as yet no clear system for accreditation at the international level. However, in some countries where moves to introduce accreditation schemes in all veterinary laboratories monitored by the national reference laboratory are in progress, the question of international recognition of quality of the national reference laboratory has been raised. As the move towards quality assurance gathers force, some form of international accreditation will be inevitable for national and international reference laboratories (3).
A reference laboratory should have a well-documented quality system, and this should include procedures to ensure that bench techniques, diagnostic tests, reagent production and clerical records are all adequately controlled. A clear audit trail should exist to follow any one submission through the analytical stages to production of the final report, together with archiving of the data and, if appropriate, of the biological material itself. The body which is responsible for designation of the reference laboratory should have the right to query the control procedures, to audit the processes and, if not satisfied, to withdraw the designation. Where more than one reference laboratory exists for a particular disease, cooperation between these laboratories should be established to provide external quality checks and inter-laboratory comparisons, thereby ensuring greater consistency in the wider world of veterinary laboratory activities.

Reference laboratory activities

Advice and consultancy

As opportunities for world trade in animals and animal products increase, there is a growing need for regulatory authorities to have access to reliable, dispassionate, in-depth knowledge about diseases, so that appropriate measures can be taken to reduce the risks of transmission, to improve animal health and welfare and to provide consumer protection. Increasing use is being made of risk analysis methodology, for which reliable data inputs are essential. Reference laboratories, and the experts who work in those laboratories, are a most important source of informed knowledge about diseases. Although the information revolution in the late 20th Century has opened up much wider accessibility to published material, considerable added value is obtained by using the knowledge and experience of specialised experts to put such information in context, and also often to extend the data with unpublished material gained through knowledge networks.

The reference laboratory expert should, in addition to being familiar with the disease and the historical literature on the subject, maintain a currency of knowledge through reading scientific literature, through attending international conferences, and through dialogue and collaborations with other experts in the field. The expert should have access to up-to-date disease-incidence data, be aware of the advantages and disadvantages of different diagnostic techniques, and have an understanding of the role and value of vaccines in control of the disease. The expert should be available to provide factual advice and consultancy to all enquirers.

World trade disputes

As world trade becomes less restrictive due to the ideals of the General Agreement on Tariffs and Trade as implemented by the WTO, disputes between countries (or commercial companies) and national laboratories over disease status and the interpretation of diagnostic results which affect imports and exports are likely to become more frequent. It is very easy for 'sophisticated' developed countries to dismiss unjustifiably the findings of workers in developing countries as erroneous due to inability or incompetence, and an increasing number of international reference laboratories are likely to be referred to as places of arbitration or definitive testing.

Research and development

The reference laboratory is required to maintain current awareness of scientific developments, both in relation to the specific disease for which it holds the designation, and in the area of biotechnological innovations which might have benefits for diagnosis or control of the particular disease. In many cases, the reference laboratory itself will be conducting research projects leading to the development of new or improved diagnostic and analytical methods. The application of genetic sequencing technology to the characterisation of infectious agents provides a good example which has led, in many cases, to a much clearer understanding of disease epidemiology and of the biological variation and evolution of specific organisms.

Publications

A reference laboratory will normally be located within an institution which is conducting scientific research, disease surveillance or diagnostic testing, and in many cases all three. These activities are therefore expected to result in scientific publications related to either new understanding of disease processes or new techniques in diagnosis or control. Presentations at national and international conferences should be encouraged. The subsequent publication of such material in scientific journals is equally important (5). Diagnostic and surveillance activities generate much valuable data on trends in disease incidence and distribution, but regretfully much of this information never reaches the public domain. The reference laboratory should play a key role in making such information available and in providing analysis and interpretation of observed trends. An example of such interpretative analysis, in a more generalised context, is provided by the annual Veterinary Investigation Diagnosis Analysis (VIDA) which is based on a record of every submission made to veterinary investigation laboratories in Great Britain (4).

The ability of the reference laboratory to communicate with national and regional laboratories, with national veterinary authorities and with international organisations is essential (3). In some cases, the role may extend to that of co-ordinator or information clearing house to assist different organisations in working towards common goals, and to avoid unnecessary duplication of effort. The opportunities provided by electronic media and the Internet will give a new impetus to the obligation of reference laboratories to promulgate disease information.

Although the dissemination of information by publication is desirable, the role of the reference laboratory in achieving this
objective may not be simple. For international reference laboratories, there may be conflicts of interest between nationally and internationally perceived disease risks resulting in delayed or restricted publication of results. In addition, the ability to exploit the commercial potential of discoveries, including the isolation of organisms, may result in conflict between the reference laboratories and those submitting material. There should be a clear agreement between reference laboratories and those submitting material regarding the publication of results obtained by the reference laboratory from that material, and there is a case for extending this agreement to the supply of submitted organisms and materials to third parties for research purposes.

Training

With regard to the harmonisation of laboratory procedures, it is very important that diagnostic laboratories have the opportunity to send staff to reference laboratories for training in specific techniques, and also for refresher courses or to attempt to resolve problems encountered in the home laboratory. On occasion, a reference laboratory may arrange to run a specific training course or workshop for multiple participants, and this will be especially relevant where a new disease emerges (e.g., bovine spongiform encephalopathy) or a new technology is introduced to diagnostic work (e.g., polymerase chain reaction techniques). In addition, the reference laboratory would normally be expected to host ad hoc visits from individual scientists wishing to work alongside experienced colleagues to gain competence in a particular area.

Training, whether on an individual or group basis, must be properly planned, appropriate resources allocated and funding secured. Training must also be tailored to the needs of those attending. The trainees should also recognise that this activity is a major commitment by the reference laboratory and should approach training sessions with an appropriate sense of dedication.

As well as offering formal and individual practical training of this nature, reference laboratories are contributing increasingly to the production of interactive multi-media systems, which enable a much wider target audience to be reached and also permit individuals to progress at their own rates and to focus on the areas of specialism which are relevant to the local situation. A good example of such a system is the 'Advanced Veterinary Information System' (AVIS) created by an international consortium which has produced a series of modules on rinderpest. Modules on Newcastle disease, foot and mouth disease and others are currently in production with the assistance of experts from the relevant reference laboratories.

Surveillance

Animal disease surveillance is important for identification of:

- outbreaks of epizootic 'List A' diseases or other diseases exotic to the country or region
- zoonotic disease risks
- new and emerging animal diseases
- changing trends in endemic diseases.

A reference laboratory should be actively involved in disease surveillance within its own country, and international reference laboratories should also ensure they have access to data from other countries in their region or, if possible, from around the world (even if these reference laboratories do not carry out the surveillance themselves). International bodies, such as the FAO, the OIE and the WHO, have an important role in collecting and publishing such data. Whenever possible, surveillance should be structured and targeted and thus an essential prerequisite is to define the purpose for which surveillance is being performed. There also remains a certain value in collating and analysing data acquired in the course of regular diagnostic testing. However, this should be interpreted in the context of the type of samples being tested and the reasons for submission to the laboratory.

Characterisation of infectious agents and maintenance of culture collections

Reference laboratories will usually be involved in primary diagnostic activities, at least for the local area. However, they have a particularly important role in providing confirmatory testing in support of local diagnostic laboratories. This may include confirming a positive test result by repeating the test under defined conditions or, more likely, the use of more elaborate confirmatory tests which require specialised equipment and/or experience. The reference laboratory would then be expected to extend the basic diagnostic information by studying the characteristics of the infectious agent involved in a particular outbreak, and to relate these to the characteristics of other outbreaks in order to derive epidemiologically relevant information which may assist in future control programmes.

Infectious disease agents characteristically show biological variability, both temporally and geographically. It is important for disease control programmes that this variation is monitored and reported, as it may lead to changing requirements for diagnostic tests or for vaccine constituents. An essential part of this activity is for the reference laboratory to maintain an archive, with documentation, of representative organisms isolated and characterised. The precise criteria for archiving any particular strain will obviously depend on the organism, the extent of inherent variability of that organism and any unique features of the isolate in terms of biological characteristics, or site or time of isolation. As an example, the World Reference Laboratory for Newcastle disease at Weybridge, United Kingdom, received nearly 2,500 viruses from 55 countries over a nine-year period. The identity of all the submissions was confirmed, and further characterisation was then performed on selected isolates according to criteria such as those mentioned above.
Evaluation and definition of reference methods

An ideal laboratory method is one that always gives the correct result, regardless of the nature of the sample or the location at which the test is performed. In practice, all laboratory methods have limitations and the purpose of defining reference methods is to provide a 'gold standard' against which other tests can be compared. This can assist greatly in the determination of the standard parameters (diagnostic sensitivity, diagnostic specificity, predictive value) of a new or modified test (8).

For some diseases, the reference method itself is recognised to have significant deficiencies, and reference laboratories and research institutions will be working to develop improved methods which might, in time, replace the current reference method. In such a case, the reference method will provide a poor indicator of the value of the new method, which should therefore be fully validated from first principles using an independent technique to identify populations of known positive and known negative samples (8).

Preparation of standard reference materials

The production and use of standard reference materials have an important role to play in the international and national diagnosis of disease. These materials may take two forms: in-house positive and negative sera and antigens, and international standard preparations. The former are usually supplied by the reference laboratory and used routinely in diagnostic tests. Such 'working' or secondary standards must be distinguished from primary international reference materials (12). Frequently it is more appropriate that the primary reference preparations are produced and stored in laboratories dedicated to biological standards. The problems associated with standard reference preparations are often related to a defined system of storage and a regimen for testing the reagents. It may be most appropriate for institutes dedicated to biological standards to store and organise routine testing which could be performed at a recognised reference laboratory.

Organisation of inter-laboratory comparisons

Designating agencies usually consider the organisation of comparative tests of methods used in the regional or national laboratories as an important function of the reference laboratory. There is little doubt that such testing allows confidence both for the laboratories performing the testing and those requiring their services. However, such tests are extremely expensive, in both reagents and the time needed to prepare reagents, to perform the tests and to analyse results. Comparative tests aiming to examine every facet of a particular test within a statistical degree of confidence may become extremely complicated and proportionately expensive, and it is often practicable to settle for a lower level of comparison. When an inter-laboratory test comparison is conducted for the express purpose of determining the capability of a laboratory to conduct specific diagnostic tests, the process is referred to as proficiency testing, which may become an integral part of laboratory accreditation programmes (see above).

Conclusions

Reference laboratories are not a luxury; at national level, they are important to provide co-ordination and confidence in veterinary laboratory procedures, while in the international arena there is little doubt that their input is essential to ensure adequate disease safeguards that will allow freedom of trade in animals and animal products throughout the world. At present, there is considerable variation in the standards of and services provided by different reference laboratories for different diseases that, nevertheless, have repercussions for international trade. The greatest hindrance to creating homogeneity between existing reference laboratories and establishing further laboratories is funding. If freer world trade is to be achieved, adequate funds must be obtained by designating agencies to allow the establishment and monitoring of adequately equipped (in terms of both personnel and equipment), fully functional centres of excellence covering at a minimum all of the OIE 'List A' diseases.
Laboratoires vétérinaires nationaux et internationaux de référence pour les maladies infectieuses

S. Edwards & D. Alexander

Résumé
Les laboratoires de référence jouent un rôle de plus en plus important dans l'harmonisation des tests de diagnostic et la normalisation des vaccins à usage vétérinaire, contribuant ainsi dans une large mesure à renforcer la confiance entre les acteurs du commerce international. Les auteurs examinent différents aspects de l'organisation et de la désignation des laboratoires de référence pour les maladies infectieuses ainsi que de l'aide qui leur est fournie, et énumèrent les principales activités que ces laboratoires doivent normalement accomplir. Ces tâches comprennent des services de conseil et d'expertise, les publications et la communication, la formation, la recherche, la surveillance épidémiologique, l'entretien des souches de collection, l'évaluation de méthodes de référence, la préparation de réactifs de référence et l'organisation de comparaisons interlaboratoires.

Mots-clés
Harmonisation - Laboratoires - Laboratoires de référence - Maladies infectieuses - Milieux de culture - Normalisation - Santé animale - Services vétérinaires - Techniques de diagnostic.

Laboratorios veterinarios nacionales e internacionales de referencia para las enfermedades infecciosas

S. Edwards & D. Alexander

Resumen
Los laboratorios de referencia intervienen de manera cada vez más decisiva en la armonización de pruebas de diagnóstico de laboratorio y la estandarización de vacunas veterinarias, elementos ambos de especial relevancia para crear un clima de confianza entre países que mantienen relaciones comerciales. Los autores repasan algunos aspectos relativos a la organización, la designación y el apoyo a los laboratorios de referencia para las enfermedades infecciosas de los animales, y resumen las principales actividades que en condiciones normales deberían llevar a cabo tales establecimientos, a saber: servicios de asesoramiento y consultoría, publicaciones, divulgación, formación, investigación, vigilancia de enfermedades, mantenimiento de cultivos, evaluación de métodos de referencia, preparación de materiales de referencia y organización de estudios comparativos interlaboratorios.

Palabras clave
References


