

## The role of national and international veterinary laboratories

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### Summary

In the field of diagnostic test validation, World Organisation for Animal Health (OIE) Reference Laboratories (RLs) have a pivotal role and provide the international community with impartial advice and support in diagnostic test selection, development and validation which can be applied to the specialist diseases for which they are designated. National RLs provide an invaluable function in supporting the introduction, ongoing validation and application of validated diagnostic tests to international standards. Experienced staff with extensive knowledge of test validation systems and access to specialist facilities for conducting work are available to monitor changes or advancements in technology. They consider their relevance and value to evolving diagnostic test requirements. Reference Laboratories often have a broad mandate of activity linking research or development programmes and surveillance activities to benefit the continual assessment and, if necessary, improvement of diagnostic tools. Reference Laboratories maintain or have access to unique biological archives (known positive and negative sample populations) and produce international reference standards, both of which are vital in establishing the necessary and detailed validation of any diagnostic test. Reference Laboratories act either singularly or in collaborative partnerships with other RLs or science institutes, but also, when required, and with impartiality, with the commercial sector, to ensure new tests are validated according to OIE standards. They promote and apply formal programmes of quality assurance (including proficiency testing programmes) for newly validated tests, ensuring ongoing monitoring and compliance with standards, or, as required, indicating any limitations or uncertainties. Reference Laboratories publish information on test validation in the scientific

literature and on relevant websites, as well as disseminating information at workshops and international conferences. Furthermore, they can offer training in the processes and systems underpinning test validation.

### **Keywords**

Bio-archives – Consultancy – Impartiality – Reference Laboratory – Standard compliance – Test accuracy.

### **Introduction**

The *Concise Oxford English 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’ (1). Within the specialised context of this paper, a Reference Laboratory (RL) is specifically designated and acts at either national or international level or both. Reference Laboratories, irrespective of scope, are considered by the veterinary and scientific community at large to produce authoritative information, opinions and advice. National laboratories are appointed by the Competent Veterinary Authority in the country concerned, sometimes in consultation with other bodies (e.g. with the Competent Authority for human health if the disease in question is zoonotic). International laboratories are formally endorsed through the World Organisation for Animal Health (OIE) and their Members. (The Food and Agriculture Organization of the United Nations [FAO] also can independently appoint international RLs.) In support of the delivery of RL mandates, the laboratory will perform a variety of procedures and provide a range of services. Functions central to diagnostic test validation science are embodied in the terms of reference of OIE international RLs (2), which state that their responsibilities are to:

- use, promote and disseminate diagnostic methods validated according to OIE standards
- recommend the prescribed and alternative tests or vaccines as OIE standards
- develop reference material in accordance with OIE requirements, and implement and promote the application of OIE standards
- store and distribute to national laboratories biological reference products and any other reagents used in the diagnosis and control of the designated pathogens or diseases
- develop, standardise and validate according to OIE standards new procedures for diagnosis and control of the designated pathogens or diseases

- provide diagnostic testing facilities, and, where appropriate, scientific and technical advice on disease control measures to OIE Members
- carry out and/or coordinate scientific and technical studies in collaboration with other laboratories, centres or organisations
- collect, process, analyse, publish and disseminate epidemiological data relevant to the designated pathogens or diseases
- provide scientific and technical training for personnel from OIE Members
- maintain a system of quality assurance, biosafety and biosecurity relevant for the pathogen and the disease concerned
- organise and participate in scientific meetings on behalf of the OIE
- establish and maintain a network with other OIE RLs designated for the same pathogen or disease and organise regular inter-laboratory proficiency testing (PT) to ensure comparability of results
- organise inter-laboratory PT with laboratories other than OIE RLs for the same pathogens and diseases to ensure equivalence of results
- place expert consultants at the disposal of the OIE.

Further, national RLs can provide valuable support to diagnostic test validation in their area of competence. Specifically, under their mandates these activities include:

- collaborating with other national and international RLs, and participation in training courses and in inter-laboratory comparative tests organised by these laboratories
- coordinating the activities of official laboratories designated at a country level with a view to harmonising and improving the methods of laboratory analysis, testing and diagnosis and their use, ensuring well-validated methods are applied
- where appropriate, organising inter-laboratory comparative testing or proficiency tests between official laboratories, ensuring an appropriate follow-up of such tests and informing the Competent Authorities of the results of such tests and follow-up; poor performance in such tests may identify tests that have not been appropriately validated

- where relevant, validating the reagents and lots of reagents, establishing and maintaining up-to-date lists of available reference substances and reagents, and of manufacturers and suppliers of such substances and reagents, and maintaining reference collections of pests of plants and/or reference strains of pathogenic agents
- actively assisting the Competent Authority in the diagnosis of outbreaks of diseases, by carrying out confirmatory diagnoses, characterisation and epizootic or taxonomic studies on pathogen isolates, all using appropriately validated methods.

### **The need for Reference Laboratories**

An RL is a centre of excellence and technical expertise for a particular disease. Generally, an RL will employ experts knowledgeable about the disease and fit-for-purpose diagnostic methods. In some cases, RLs also have experts that are knowledgeable about the preparation and evaluation of effective vaccines for the disease may also be available. The role of an RL is to provide an independent, impartial, but definitive, opinion, within the scope of current scientific knowledge, on matters concerning the diagnosis and control of the disease in question. Rarely will the RL have controlling authority over other laboratories with which it regularly engages. Where an RL has such authority, this is usually as a result of its role as a central or national RL (2). As test methods change and there is greater use of emerging technologies (including information technology that can produce standardised digital outputs), there are more opportunities for collaboration between regional laboratories, which leads to improved knowledge of disease diagnosis and control. The role of the RL is principally to provide unbiased advice that is founded on the best scientific knowledge available and, where definitive advice is not possible, to ensure that any uncertainties are understood.

In order to be able to meet the ongoing challenges in test validation and harmonisation, in often rapidly advancing fields (e.g. vaccine design and new diagnostic methods), it is important that the RL remains active and aware of developments in its relevant field. There is a continuous demand for rapid, reliable diagnostics and interventions, so awareness of developments in new diagnostic methods and requirements for new vaccines or standards is important. This horizon scanning and preparedness to respond requires RLs to have active science programmes in their specialist disease that are outward facing and utilise scientific literature, digital technology, acquisition of new knowledge through disease events and, importantly, collaboration with other laboratories, science institutes and the commercial sector.

Finally, RLs are in a privileged position, being in receipt of biologicals from around the world. This carries responsibilities and RLs must avoid conflicts of interest. For example, if RLs are in a

position to commercially exploit discoveries, including new or variants of pathogens, they have a responsibility to make data available to the international community early on, especially when it is fundamental to providing critical insights into test modifications. RLs must also manage the, sometimes, competing interests of individual countries and the wider international community. If an RL helps a country to detect disease it will wish to report this to the OIE, but the country may not, resulting in delayed or restricted publication of results.

## Organisation of Reference Laboratories

### Personnel requirements

Personnel in RLs should have experience of working with the disease in the field and should be familiar with its clinical appearance, pathological features, biological properties, epidemiology, transmission and control. Importantly, they should understand all the appropriate diagnostic laboratory methods for the disease and should be able to provide a critical interpretation of the laboratory test results, including an assessment of the accuracy and predictive value of individual tests. They should have access to relevant clinical material and be able to apply their expert knowledge to the correct selection of laboratory tests for defined purposes, in accordance with OIE principles (see Gardner *et al.*, this issue [3]). Staff should understand test performance criteria, test validation, fitness-for-purpose demonstration and technical troubleshooting of test failures or anomalous test results. To ensure that they have the multi-disciplinary scope of knowledge required, RLs increasingly employ a diverse team of specialists (including technicians, epidemiologists, pathologists, veterinarians, researchers, data scientists and statisticians).

### Equipment and facilities: importance of maintaining World Organisation for Animal Health standards

All RLs, regardless of their particular remit, need to be adequately and sustainably resourced and equipped to effectively respond with an appropriate and sustainable testing strategy. Robust laboratory infrastructure is critical in view of the increasing numbers of disease incidents which continue to challenge RL activities globally. Biocontainment controls must reflect the OIE standards pertaining to biosafety and biosecurity (4) to minimise laboratory safety lapses, which may lead to pathogen escape and other unwanted consequences for RLs. Novel tests developed and recommended by RLs must also consider the resources and regulatory limitations of national RLs.

Regional RLs and field veterinarians play a critical role both in recognising suspect clinical cases, which is the first step in disease investigation, and in ensuring that diagnostic assays remain fit for purpose.

Research facilities which enable animal experimentation in the target livestock species, in line with animal welfare standards, are available at RLs with the appropriate containment level for biosafety and biosecurity (5). Carefully designed *in vivo* studies primarily serve to investigate various aspects of the relationship between pathogen and host. Studies include pathogenesis studies and trials of candidate vaccines, which may involve collaborations with the biotechnology industry. The resultant clinical samples can be further exploited for test validation, thereby embracing the 3Rs principles (replacement, reduction and refinement of animal testing) (6), and are valuable alternatives to live animal testing where appropriate field samples are unavailable.

### **Financial support and commercial activity**

Most RL activities are funded internally or by their national authorities. One way to alleviate any funding difficulties is for the RL to charge for some of their services, for example, the provision of reference materials or training. The OIE has recognised the right of an RL to make appropriate charges if a particular service would otherwise be impossible; however, such an action tends to detract from the overall principle of open access to information and materials, which is the principle that underpins the very concept of RLs. In the area of test validation, the RL may be placed in a challenging position. The maintenance of bio-archives on behalf of the national or international community is a substantive and ongoing cost to an RL. Third parties do not have ready access to such materials, and it can often be logistically challenging and costly for RLs to supply them (e.g. shipment costs for infectious substances). For this reason, detailed test validation is best conducted by RLs themselves, but in a manner that allows timely release of information and data, ideally through peer-reviewed publication. The RL needs to maintain impartiality and so commercial endorsement of diagnostic kits or products should ideally be avoided. However, it is sometimes essential that the RL works with industry to facilitate test validation.

### **Evaluation and audit**

National and international RLs play a key role in the standardisation and control of analytical methods. Standards implemented and maintained by RLs have become increasingly required, as they often provide a benchmark against which the performance of laboratories undertaking official testing is compared. Consequently, for RLs to remain in a position to carry out their

responsibilities there is a need for quality performance standards to be maintained. Reference Laboratories meet the fundamental need for rapid, accurate and unambiguous detection of pathogens, using test methodologies that are accredited to ISO/IEC 17025:2017. It is a basic premise of disease control that success in control is entirely reliant on the ability of the Competent Authority to accurately diagnose new cases faster than the disease spreads. For most diseases, rapid unequivocal laboratory diagnosis is therefore a critical tool. Reference Laboratories should use tests that conform to internationally recognised accreditation standards, as this provides assurance of best practice and provides evidence of the RL's competence. Competent Authorities and the public are more likely to trust accredited laboratories to deliver accurate results, which is particularly important, as key strategies and decisions that impact on livelihoods rely on the accuracy of the test results. Accreditation provides further confidence in those test results and is recognised internationally, which allows for a common understanding of baseline performance expectations and enables laboratories to communicate with one another more easily. Reference Laboratories aim to provide both conventional methodology and new technological solutions in order to maintain their position at the forefront of disease diagnosis, response and management. The key advantage of evaluating novel technologies in tandem with accredited tests is that it allows for the relative performance of these test to be compared. This validation of tests is undertaken by RLs according to the principles and standards in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* (7). Review of test validation dossiers, particularly by independent reviewers, is a valuable and integral part of test accreditation.

The implementation of new tests in an official testing laboratory may be facilitated by RLs. Reference Laboratories have a remit to undertake reviews of laboratory procedures, often through laboratory quality assurance exercises, including provision of quality-assured introduction of new validated tests and PT panels. These activities provide continued assurance of the maintenance of standards across a laboratory network. Repeated failure during external quality assurance (EQA) programmes presents cause for concern and can lead to temporary or permanent withdrawal of the test under review.

## **Reference laboratory activities**

### **Advice and consultancy**

Reference Laboratories function within networks, whether national, regional, or coordinated internationally by the OIE and FAO, and their activities include capacity building and implementing laboratory twinning initiatives. Technology transfer and staff training are key when aspiring to test harmonisation, whether regionally or globally. Informed advice founded on

disease understanding is invaluable, as is justification for novel testing strategies, which may feature newly validated diagnostic approaches. The role of the reference expert is pivotal, both in improving standards and in assuring the viability of the RL they represent. They act as a key point of contact for other parties (other RLs, both international and national, commercial companies in diagnostics and vaccines, and international agencies such as the OIE) in the development and validation of new methods (8). Publication will transparently demonstrate the quality and extent of test validation, but validation should continue as an ongoing process. Occasionally, some diagnostic methods are published and appear viable, but in the absence of robust validation (7) these may represent little more than a written method, with no evidence to support their reliability and utility. Reference Laboratory experts must communicate their knowledge through constructive dialogue and demonstrate impartiality on all pertinent themes. Consultancy may be provided via collaborative projects, or even informally, the importance of which cannot be underestimated. There is similar scope for consultancy with industry, where vaccination and test validation may be highly beneficial.

### **Research and development**

Research and development, both scientific and technical, play a pivotal role in retaining the status and global reputation of an RL. To maintain scientific expertise in the provision and validation of diagnostic tests, the RL needs to ensure it has up-to-date knowledge of the disease and pathogen in question. Understanding the circulating strains, transmission, pathology, epidemiology and host ecology is essential to appropriately provide advice regarding the optimal assay and test matrices. Reference Laboratories should employ and encourage the use of standardised *in vivo* and *in vitro* characterisation approaches towards the classification of new pathogens, or variation and changes within existing pathogens, and publish or share scientific data to avoid unnecessary duplication of effort (particularly where animal models are required for pathogen typing or classification). The increasingly available high-throughput molecular screening approaches and more routine use of whole-genome sequencing have exponentially improved the detection of pathogens and the subsequent understanding of their evolution and epidemiology.

Technical expertise underpins the delivery of scientific research and reference activities. Where possible, RLs should have practical knowledge of novel or emerging technologies as applied to their disease or related area so as to determine their suitability for practical application and give greater confidence in globally reported data. The development of new technology for testing is underpinned by inter-laboratory trials and collaborative studies. An example of RL input through international collaboration was the provision of an optimised, well-validated, real-time

polymerase chain reaction (PCR) based on contemporary bioinformatics for the generic detection of all animal influenza viruses (including avian influenza virus [AIV] and swine influenza virus) and human influenza A virus strains (9). These tests meet the global need for effective screening for influenza A viruses, irrespective of origin or host. Assessment and validation of new technologies, particularly diagnostic assays, allows RLs to confidently set quality assurance standards (e.g. cut-off values or test pass/fail criteria) and establish any limitations (e.g. sample matrix, sensitivity or specificity) before the assays are widely used in diagnostic laboratories. The validation and sharing of dedicated sample processing procedures and bioinformatics pipelines (software) alongside the resulting data will ensure maximum yield from limited clinical material in other diagnostic laboratories or RLs.

In many cases, maintaining scientific and technical expertise relies on informal and formal collaborative links with OIE RLs and academic or industry partners. Doctoral studentships may facilitate optimum use of limited resources and enhance scientific outputs. It is highly recommended that RL research and development should be implemented following internationally recognised standards such as the International Organization for Standardization (ISO) standard ISO 9001. Together with the publication of data in peer-reviewed journals, quality assurance certification provides a high degree of confidence in the research portfolio of an RL.

### **Publications, reporting and communications, and sharing of data and materials**

Peer-reviewed publications and conference presentations remain important for enabling RLs to disseminate their findings to a broader audience. More recently, the emergence of pre-print servers has enabled scientific manuscripts to be universally accessible prior to completion of formal peer review, although both their advantages and disadvantages have attracted comment (10). Internet portals dedicated to disease-specific themes include test validation discussions, whilst social media enables rapid real-time dissemination of information.

The increased level of inter-laboratory collaboration between RLs has been invaluable, with possible conflicts of interest during the sharing of samples or information prevented thanks to materials transfer agreements. It is important that the relationship between an RL and its Competent Veterinary Authority is respected. The continuing threat of zoonotic infections also requires RLs to engage with the public health arena (11).

Public gene sequence databases are a crucial resource in understanding the molecular epidemiology of pathogens. The 2010 Nagoya Protocol (12) emphasised the international importance of transparency for the open and globally beneficial sharing of organisms' genomes.

However, some of the Protocol's recommendations have raised concerns in the public health sphere (13) and similar concerns may apply to veterinary pathogens. These questions extend to industrial collaborations if RLs source the pathogens for test development. Any potential conflicts of interest associated with intellectual property rights (14) demand ethical management to maintain the scientific credibility of RLs.

## **Training**

Training is an inherent part of any RL's remit. It incorporates both internal training to retain quality assurance accreditation and certification within the laboratory and external training to facilitate high standards in diagnosis, surveillance, biosafety and biosecurity at a regional and international level. The type of training undertaken will vary significantly from specific one-on-one training to wide-scale training and harmonisation via international meetings or workshops. Training may be organised in response to the OIE's acceptance of new diagnostic approaches; for example, in 2018, the OIE's acceptance of PCR-based assays for primary rabies diagnosis led to enhanced training by RLs and dissemination of detailed methodologies in peer-reviewed journals, including video articles (15).

Reference Laboratory staff are often requested to provide training in test protocols, test validation and quality assurance to staff at other laboratories. External training, which may be offered directly or as part of funded OIE laboratory twinning projects, is targeted at the appropriate staff and uses local reagents, equipment and facilities. Test validation training may cover diagnosis, quality assurance and/or safety. It is particularly important when providing training for a novel technique that trainees are taught how to transfer the assay to their own laboratories and how to acquire local test validation and/or equivalence data. This will ensure that ongoing training programmes at the host laboratory are locally embedded and maintained. Informal or formal assessments may be included in the training programme. For example, blind panels of previously tested samples could be used to confirm staff competency, obtain local test validation data (including test sensitivity and specificity data), ensure that local equipment and reagents are fit for purpose, and confirm the ability of laboratories to detect locally circulating strains of the pathogen. Training should include test pass/fail criteria (positive and negative controls) and establish background levels/cut-offs. The programme should also ensure the establishment of local test performance monitoring to track any trends and take corrective action as required. Host laboratories should also be encouraged to participate in EQA schemes.

## Surveillance

Reference Laboratories play a vital role in the surveillance and reporting of animal diseases. National surveillance systems monitor endemic disease and, when they are well targeted and have effective control plans, can help to reduce prevalence of important production and zoonotic disease. Active surveillance programmes provide valuable access to ‘field samples’, which are critical for diagnostic assay development and validation. The impact and utility of programmes that use well-validated tests can be seen in the example of national *Salmonella* control plans in Europe, which have resulted in a fall in the prevalence of targeted *Salmonella* serovars in laying hens across the continent, from 3.7% in 2008 to 1.1% in 2018 (16). National surveillance networks established to monitor and control endemic disease can additionally be utilised to improve sensitivity of detection for exotic and emerging disease threats. Reference Laboratories have a responsibility to be aware of the threat of disease incursion via international horizon scanning. Some exotic epizootic diseases with potential public health consequences, such as highly pathogenic avian influenza, have continued to evolve over the last 25 years and, consequently, fit-for-purpose tests need to be updated if such surveillance is to be reliably carried out. Additionally, African swine fever has been spreading in Asia and Europe in recent years and vector-borne diseases have become more widespread due to changing climatic factors. Effective surveillance systems require well-characterised, validated tests for effective monitoring.

Antibiotic-resistant bacteria emerging from extensive usage of antibiotics in livestock production pose an increasing threat to veterinary public health (17). Surveillance of antimicrobial resistance in bacteria in livestock populations, using validated tests, will be vital for ensuring that antibiotics are used safely in the veterinary sector and for protecting public health.

New and emerging disease threats have always been a concern to livestock industries and the emergence of SARS-CoV-2 (COVID-19) in 2019 via a presumed wildlife source (18) has highlighted the impact that a new zoonotic disease can have on public health and the economy. The international community looks to RLs for responsive development of relevant validated tests.

## Characterisation of infectious agents and maintenance of culture collections

Reference Laboratories employ a range of approaches for pathogen characterisation, essentially centred on: *a)* long-established bacteriological and virological techniques, which include pathogen isolation as a key requisite; and *b)* molecular methods, including real-time PCR and gene sequencing. Major investment over the past 15 years has seen the widespread

implementation of real-time PCR technologies in veterinary RLs globally (19, 20), both at the national RL level and now increasingly in regional RL front-line diagnostic settings. While validated real-time PCR techniques demonstrate many advantages, e.g. high sensitivity and specificity and rapid throughput in the laboratory, it remains prudent for RLs to maintain their long-standing culture and isolation facilities. Reference Laboratory archives should contain a mixture of quality-controlled derivatives (e.g. titrated isolated virus or bacteria, as reference strains), nucleic acid, serum and fixed tissues. Clinical samples are crucial to ensure that tests are validated using the appropriate sample matrices. The choice of tools and appropriate clinical samples is dependent on the pathogen, disease and test purpose.

The OIE generally continues to recommend isolation or culture from the index case of any new outbreak, and the propagated pathogens require secure curation in long-term pathogen collections and bio-banks, ideally with their accompanying genetic and epidemiological data. However, this is dependent on it being possible to isolate the virus in cell culture (not possible for viruses of crustaceans and molluscs) and on there being appropriate containment facilities for biosafety level 4 zoonotic pathogens. For bacterial characterisation, the process is at least simpler and dependent on an initial pathogen isolate. Traditional approaches include examination of phenotypic and biochemical properties alongside serological and phage typing reactions, but these have been increasingly supplemented by molecular approaches (21), which continue to be developed for many bacterial pathogens.

Appropriate tools validated to international standards are a key component of disease outbreak management, including surveillance, and they have an impact on longer-term animal disease policies, both nationally and internationally. For example, the rapid and effective implementation of control measures to combat incursions of highly pathogenic AIV (H5Nx subtypes) in Europe, the Middle East and Central Eurasia between 2014 and 2021 was made possible by earlier validation of appropriate AIV real-time PCR assays. The assays were shared out amongst national and international RLs (20) to control major epizootics during 2016–2017 and 2020–2021 (22). Detailed validation of the assays showed utility for a diverse group of contemporaneously circulating viruses ahead of their substantive global spread (23). Detailed validation of the assays showed utility for a diverse group of contemporaneously circulating viruses ahead of their substantive global spread (23). These tests were used for statutory interventions and actions, including: *a*) confirming disease in different avian hosts; *b*) monitoring the spread of infection to susceptible populations and, importantly, providing unequivocal evidence for lack of spread to contact farms; *c*) performing pre-movement testing of birds to prove they were non-infected; *d*) testing and monitoring farms following restocking with birds; and *e*) providing proof of freedom from infection in apparently healthy birds in control zones

(later enabling countries to regain OIE disease-free status). All of these AIV outbreak experiences have not only informed the ongoing continuous validation of the relevant real-time PCR assays, but have also provided additional insights into diverse but important aspects of AIV ecology, as has the monitoring of the AIV natural reservoir in wild birds (24).

The application of molecular approaches, including next-generation sequencing (NGS), in RL work provides increased opportunities for more refined research activities and improved diagnosis. The value of NGS has been demonstrated in the *de novo* identification of novel viral agents (25) and the detection of minority variants and antibiotic/antiviral-resistant forms of bacteria and viruses. The whole-genome sequencing of bacteria provides the ultimate level of resolution (26). However, significant capital investment is required for both NGS instrumentation and sufficient computing power, plus trained bioinformaticians to analyse the considerable viral and bacterial genomic metadata. As a result, it is largely only international or national RLs with research-oriented programmes that can use the technology. Combining both genetic and serological data in antigenic cartography serves to characterise new and evolving pathogens, which include antigenic variants (27, 28). Standards to describe the level of confidence in the quality of assembled genomes, particularly for novel or new pathogens, still need to be refined.

Further tools for detection of historical exposure to infection in RL surveillance need to be developed using platform technologies, such as enzyme-linked immunosorbent assay. An important application of this technology is where serology is critical during outbreak or endemic disease eradication programmes, to demonstrate the absence of infection in livestock (29). Several RLs have been replacing *in vivo* assays with pathogen detection and characterisation in order to respect the 3Rs principle (6); for example, intravenous inoculation of embryonated chicken eggs has been used for the isolation of bluetongue virus (30). Acceptance of alternative *in vitro* assays has, however, been slow in some areas. The value of bio-archives for the development of diagnostic tests for prion diseases (which included a possible zoonotic element) was demonstrated at the time of the bovine spongiform encephalopathy (BSE) epidemic. Although subsequently controlled by epidemiologically driven interventions and management practices (31), BSE precipitated test development for prion detection in cloven-hoofed animals. Validation of many of these diagnostic tools relied on the availability of well-characterised sample material from RLs, which provided access to extensive global bio-archive collections of tissues and fluids from both field (naturally infected) and experimentally infected cases. The precedent of BSE underlines the importance of well-provenanced bio-archives and highlights their role in enabling use to be prepared for any future recurrence of an animal and/or zoonotic prion disease.

## **Evaluation and definition of reference methods**

Evaluation and description of reference methods remains a central activity undertaken by RLs. The main drivers for method development and review are the dynamic nature of infectious disease and the advent of new technology. Pathogens are constantly evolving; therefore, diagnostic tests have to be regularly updated to provide the required levels of analytical and diagnostic sensitivity (DSe) and specificity (DSp). Reference Laboratories have an obligation to provide diagnostic tools that reflect the current national and international disease profiles for their particular reference designations, as well as providing unequivocal diagnosis.

The development and modification of RL reference methods may impact the wider scientific community, and it is an RL responsibility to disseminate this information in a timely manner at both national and international level through scientific networks and publication. Communicating and engaging with the scientific community and key bodies such as the OIE, World Health Organization and FAO also serves to identify challenges with methodology and helps to progress test harmonisation at an international level, which is a vital element of sustaining meaningful RL activity and developing capability for responding to new and emerging diseases.

There are circumstances where established reference methods may have limited value. Their worth is determined by undertaking thorough test validation which will provide key information about their comparative performance. Additionally, the context within which the test is applied determines the applicability of a test result. If a test is used as a preliminary screen at herd level, then lower test sensitivity may be acceptable; by comparison, a test used for confirmatory purposes may have a requirement for different sensitivity. Increased or appropriate knowledge and understanding of the pathogen and disease biology helps to identified circumstances in which an otherwise limited test could be applied.

The valuable role RLs play in test validation is demonstrated when analysing the important factors to consider when planning a test validation exercise. These include availability of appropriate control materials with known performance in internationally recognised tests. Further, the inclusion of recent field strains in a test validation exercise is imperative, as this provides evidence that the test is developed to perform in 'field' circumstances. Many of these materials can often only be sourced via an RL bio-archive. Emergence of new pathogens creates challenges for test validations undertaken with no available field samples or standard established reference controls. However, it is important to recognise that other challenges can also arise when undertaking test validation, e.g. the health status of the population being tested may be unknown, or there may be no validated methodology against which to undertake comparative test

performance analysis. Test validations are traditionally based around animal populations with recognised disease status. The challenge arises when such tests are applied to different populations, such as wildlife populations (32), or for a different purpose, such as demonstration of freedom from infection. In those circumstances, pre-determined DSe and DS<sub>p</sub> of a test are less likely to have meaningful context. The emergence and application of Bayesian latent class analysis, a probability-based statistical modelling approach (33), has provided an alternative approach for estimation of DSe and DS<sub>p</sub> in the absence of established methods, or for the application of a test to a population of unknown disease status.

### **Preparation of standard reference materials**

An international standard reagent cannot be designated as OIE-approved unless it has been endorsed by the OIE Biological Standards Commission. The commission designates OIE RLs to prepare, validate and distribute international standard reagents to be used in diagnostic assays for infectious diseases of animals. International standard reagents must be prepared in accordance with established OIE guidelines for antibody, antigen and PCR standards (34, 35, 36), with the same principles for diagnostic standards also applying to human infections (37). The standards distributed are classed as either a primary reference standard, which is synonymous with 'international standard reagent', or secondary reference standards. Primary reference standards are for use in conjunction with tests described in the OIE *Terrestrial Manual* (7) or the *Manual of Diagnostic Tests for Aquatic Animals* (38). They enable the scientific community to harmonise diagnostic testing and encourage the mutual recognition of test results used for international trade. They represent the standard by which all other reagents are compared and calibrated against. Secondary standards may include national reference standards or locally generated standards that are in daily or routine use at the diagnostic laboratory level.

The primary reference standards are key for new assay validation. For most diagnostic assays, three reference standards are available (weak positive, strong positive and negative). The weak positive standard is the most critical standard, as it is used to provide assurance of the analytical sensitivity of the test. For quantitative tests, the standards must be assigned a reference status (e.g. in international units [IU]). Standards can be obtained from a pool of clinical samples or from individual samples. It is recommended that OIE RLs producing international standard reagents organise an inter-laboratory trial with other OIE RLs to improve the acceptance and consistency of results, particularly when first assigning the reference value in IU. Any distribution of subsequent batches of reference standards should also be preceded by inter-laboratory testing to determine the new IU value observed at different laboratories and, in relation to the previous batch, to ensure new reagents meet the 'fit for purpose' checks required

under ISO 17025 accreditation. Inter-laboratory testing also allows laboratories to ensure that testing outputs are not unduly influenced by the change in reference standard. This inter-laboratory testing was used in the establishment of a new batch of equine influenza virus reference antiserum for vaccine potency testing (39) and in the production of rabies virus reference antiserum for pet travel serological testing (40).

In addition to standardisation, the RL must also ensure that the standards undergo appropriate quality control in order to ensure that they are free from other infectious agents before being distributed (i.e. are safe and specific); are obtained from the appropriate natural host (i.e. are relevant); are free from confounding entities (e.g. cross-reacting antibodies, mycoplasma or cytotoxins); have been evaluated for preferred storage conditions (i.e. have known stability) and are in sufficient quantities for wide distribution (i.e. one single batch with enough stock to fulfil demand for at least five years). Approved standards must be appropriately labelled with the OIE logo, name of the standard, batch number and reconstitution method/storage conditions. New batches must be distributed along with a comprehensive data sheet. Where relevant, genome sequence data must be published for reference strains.

### **Organisation of inter-laboratory comparisons**

The organisation of inter-laboratory comparisons is a key element in the portfolio of activities for which an RL is responsible, and it is critical for adoption and maintenance of newly validated methods. In the simplest terms, inter-laboratory comparisons or EQA exercises/ring trials/PT (see [Waugh & Clarke and Johnson & Cabuang, this volume \[41, 42\]](#)) present an ideal opportunity for a testing laboratory to review several key elements of their performance. Inter-laboratory comparisons are a valuable opportunity to compare testing performance across a broad testing group, in many instances at a national or international level. There may be an opportunity for laboratories to test isolates/tissues which are not commonly seen in that region or country. Inter-laboratory comparisons also provide an opportunity to test the EQA panel using methods that are similar to those used by other participants or to check alternative/secondary methodology which is not in frequent use. Laboratories may also use results of participation in a PT programme to justify increased expenditure for capability improvements (e.g. change from conventional PCR to real-time PCR).

The composition of an EQA panel needs careful consideration and is reliant on some fundamental questions. The panel should represent key targets that are likely to be found relatively commonly globally and should include representative positive samples. It can also include more challenging samples that participants may not encounter commonly (e.g. new or emerging pathogens, or multiple pathogens in the same sample). Potential limits of detection of

the different tests used by the different participants should be considered when putting a panel of samples together whose detectable signal may reside around test thresholds. Additionally, an RL needs to have considered carefully how it proceeds if the panel of samples comprises difficult or poorly selected representative samples. A high failure rate can result in temporary suspension of designated laboratories, which may have serious repercussions for maintenance and compliance of statutory testing requirements in some countries. For this reason, homogeneity and stability of panel samples are critical considerations when undertaking quality assurance of EQA panels.

Proficiency testing use inter-laboratory comparisons is underpinned by internationally recognised quality standards such as the ISO/IEC 17043:2010 standard, which specifies the general requirements for the standards of providers of PT schemes and also for the development and operation of PT schemes. The ISO/IEC 17043:2010 standard covers all aspects of sample production, including sample stability, homogeneity, uniformity of sample labelling, traceability, data systems management, confidentiality and provision of competent assessors. Accreditation of PT providers to these internationally recognised standards provides assurances that the providers offer effective PT schemes and assures customers that they can be confident in the performance of the laboratories that take part in the schemes.

The impact of EQA performance cannot be overstated, particularly when laboratories have to provide assurance of the quality of testing intended to underpin international trade requirements. External quality assurance is equally valuable for RLs, where the confirmatory methods are used to endorse positive test results from laboratories within a national network.

## **Conclusions/recommendations**

- Current global farming practices and pathogen divergence and emergence continue to drive the need for RLs to continually monitor existing test performance and evaluation, validation and implementation of new and improved test methodologies.
- Provision of demonstrated expertise in diagnostic test validation through RLs needs to be maintained.
- Reference Laboratories play a key role in assessing ongoing performance of existing tests whilst developing and robustly validating new assays to international standards. Continued vigilance by RLs is required to ensure appropriate tools for diagnosis and control of animal and aquatic diseases.
- Reference Laboratories are invaluable and unique resources which should be recognised and maintained to ensure high standards of test evaluation/validation and technology transfer

including: facilities, expertise (i.e. people), collections of materials (i.e. reference standards and bio-archives), quality assurance systems and training capability.

- Rapid development of test capability needs RL leadership through collaborative networks in order to respond to new and evolving infectious agents.
- Reference Laboratories should continue to fulfil important functions in translating new innovations and approaches from research and development into validated tests available for wider use.
- This review has highlighted the importance of key reference activities and may guide individual laboratories, and the national and international authorities responsible for them, in making key decisions which will both respect and maintain the necessary OIE standards.

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## Le rôle des laboratoires vétérinaires nationaux et internationaux

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### Résumé

Dans le domaine de la validation des tests de diagnostic, les Laboratoires de référence de l'Organisation mondiale de la santé animale (OIE) jouent un rôle central et fournissent à la communauté internationale des conseils impartiaux ainsi qu'un soutien pour la sélection, la mise au point et la validation des tests de diagnostic utilisés pour la détection des maladies correspondant à leur domaine de spécialisation. Les Laboratoires de référence nationaux remplissent une fonction inestimable en facilitant l'introduction, la validation continue et l'application de tests de diagnostic validés conformément aux normes internationales. Ces laboratoires sont dotés de personnels expérimentés possédant une connaissance approfondie de ces systèmes et qui ont accès à des installations spécialisées pour mener à bien leurs opérations et suivre de près les changements ou les avancées technologiques. Ils peuvent ainsi examiner leur

pertinence et intérêt au regard de l'évolution des exigences relatives aux tests de diagnostic. Le mandat des Laboratoires de référence recouvre souvent un large éventail d'activités reliant les programmes de recherche ou développement et les activités de surveillance, ce qui permet de réaliser une évaluation continue des outils diagnostiques et, si besoin, de procéder à leur amélioration. Les Laboratoires de référence entretiennent ou ont accès à des banques de matériels biologiques uniques (panels d'échantillons positifs et négatifs connus) et produisent des réactifs de référence internationale, deux catégories de matériels essentielles pour procéder à la validation point par point d'un test diagnostique suivant les critères requis. Les Laboratoires de référence interviennent individuellement ou en partenariat avec d'autres Laboratoires de référence ou instituts scientifiques, mais aussi, lorsque c'est nécessaire et dans le respect des règles d'impartialité, avec le secteur privé, afin de s'assurer que les nouveaux tests sont validés conformément aux normes de l'OIE. Ils soutiennent et appliquent des programmes officiels d'assurance de la qualité (y compris en participant à des programmes d'essais d'aptitude inter-laboratoires) pour les tests nouvellement validés et garantissent leur suivi continu ainsi que leur conformité avec les normes, ou, suivant les cas, définissent les limites ou le niveau d'incertitude à prendre en considération. Les Laboratoires de référence publient les données relatives à la validation des tests dans des journaux scientifique et sur les sites Web pertinents et diffusent également des informations sur le sujet lors d'ateliers et de conférences internationales. En outre, ils peuvent proposer des formations sur les procédures et les systèmes qui sous-tendent la validation des tests.

### **Mots-clés**

Banques biologiques – Conformité aux normes – Exactitude d'un test – Impartialité – Laboratoire de référence – Prestations de conseil.

## **Función de los laboratorios veterinarios nacionales e internacionales**

I.H. Brown, M.J. Slomka, C.A. Cassar, L.M. McElhinney & A. Brouwer

### **Resumen**

En el terreno de la validación de pruebas de diagnóstico, los Laboratorios de Referencia de la Organización Mundial de Sanidad Animal (OIE) cumplen una función central y proporcionan a la comunidad internacional servicios de apoyo y asesoramiento imparcial para la selección, el desarrollo y la validación de pruebas de diagnóstico, que pueden aplicarse a la enfermedad para

la que cada laboratorio esté designado. Los laboratorios de referencia nacionales cumplen una inestimable función de apoyo a la implantación, la continua validación y la utilización de pruebas de diagnóstico validadas con arreglo a las normas internacionales. Disponen de personal experimentado y muy buen conocedor de estos sistemas y de acceso a instalaciones especializadas de trabajo, lo que les permite seguir de cerca los cambios o adelantos tecnológicos y estudiar su utilidad o interés en relación con la evolución de los requisitos de las pruebas de diagnóstico. Los Laboratorios de Referencia suelen tener un mandato amplio, que a los programas de investigación y desarrollo aúna actividades de vigilancia, en aras de la continua evaluación y, en caso necesario, mejora de las herramientas de diagnóstico. Estos laboratorios poseen (o tienen acceso a) archivos biológicos únicos (conjuntos de muestras probadamente positivas y negativas) y elaboran patrones de referencia internacional, elementos ambos indispensables para llevar a buen fin la necesaria validación detallada de toda prueba de diagnóstico. Los Laboratorios de Referencia pueden trabajar en solitario o en colaboración con otros Laboratorios de Referencia, con institutos científicos e incluso, cuando hace falta, y procediendo con imparcialidad, con entidades del sector privado, a fin de garantizar que toda nueva prueba sea validada con arreglo a las normas de la OIE. También promueven y llevan adelante programas oficiales de garantía de la calidad de pruebas recién validadas (incluidos programas de pruebas de competencia), lo que asegura un seguimiento continuo y el cumplimiento de la normativa en todo momento, o fijan, cuando es necesario, limitaciones o niveles de incertidumbre. Asimismo, estos laboratorios publican datos sobre la validación de pruebas en revistas científicas y sitios web conexos y difunden información al respecto en talleres y conferencias internacionales. Además, pueden impartir formación sobre los procesos y sistemas que fundamentan la validación de pruebas de diagnóstico.

### **Palabras clave**

Archivos biológicos – Asesoramiento – Cumplimiento de la normativa – Exactitud de prueba – Imparcialidad – Laboratorio de Referencia.

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