Quality standards and guidelines for test validation for infectious diseases in veterinary laboratories

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Summary

The World Organisation for Animal Health *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 1.1.1. summarises the most relevant governance and managerial aspects of veterinary testing laboratories, and Chapter 1.1.5. introduces quality management. Both chapters are based on the International Organization for Standardization/International Electrotechnical Commission standard ISO/IEC 17025:2005 'General requirements for the competence of testing and calibration laboratories'. This paper provides an update of standards and regulatory bodies relevant for accreditation of quality management systems (QMS), with a focus on ISO/IEC 17025:2017 for testing and calibration laboratories. Important issues and considerations that a laboratory should address in the design and maintenance of its QMS are highlighted and examples provided, in particular aspects of test validation and verification, including measurement uncertainty (MU). A QMS aims to address all aspects of the laboratory operation, including staff, organisational structure, processes, and procedures. Accreditation of a diagnostic laboratory requires three notable components: *a)* independent or third-party assessment; *b)* suitably validated tests performed by proficient laboratory operators in an adequately equipped laboratory; and *c)* ongoing internal and external quality control. Together, these components ensure a test outcome is the result of a standardised process and structured peer review, and demonstrate both competency and ability to produce technically valid diagnostic results that will meet the needs of customers – veterinarians, animal owners, regulators, organisations and industry – as well as the needs of decision-makers involved in animal health and surveillance programmes.

Keywords

Accreditation – ISO – International Organization for Standardization – OIE – Quality assurance – Quality management system – Validation – Verification – World Organisation for Animal Health.

Introduction

An accredited quality management system (QMS) enables a laboratory to identify, measure, control and improve various core processes that lead to improved performance. In simple terms:

- say what you do: document procedures
- do what you say: follow documented procedures
- prove it: audits and quality control
- improve it: corrective action processes.

'Quality' can be simply defined as 'fit for purpose' and the primary role of a 'quality standard' is to define the relationship between customer and service provider, e.g. a testing laboratory. Quality control procedures provide evidence that results are reliable, and quality assurance is the organisational framework in which a laboratory operates.

The QMS standards of the International Organization for Standardization (ISO) include ISO 9000 (QMS – Fundamentals and vocabulary) and ISO 9001 (QMS – Requirements). They are the world's most recognised QMS standards and they help organisations to meet the expectations and needs of their customers.

The ISO 9001 standard describes the requirements for a quality management system (1). In terms of testing and calibration laboratories, the ISO/IEC 17025 standard (2) is based on the laboratory's competence to perform specific activities, known as competency-based standards, together with management systems to ensure expected outcomes.

Competency-based standards are written for use by the conforming assessment body (the testing laboratory) to demonstrate to their customers that they have the competence to carry out specific conforming tasks. The testing laboratory can choose to have their conformance with ISO/IEC 17025 assessed through peer assessment programmes or they can seek formal accreditation to the standard through independent, third-party accreditation bodies.

The ISO/IEC 17025 standard forms the basis of many national standards for recognition of the competence of calibration and testing laboratories. A veterinary diagnostic testing laboratory should aim to use reputable, competency-based standards and guidelines to assist in designing its QMS. The ISO/IEC 17025 standard is the most recognised standard used in veterinary testing

laboratories around the world. For World Organisation for Animal Health (OIE) Reference Laboratories, the use of the current ISO/IEC 17025 standard is essential (3). For veterinary testing laboratories seeking accreditation, the ISO/IEC 17025 standard or equivalent is acceptable. The standard covers the required seven quality management principles (1) and is based on managerial processes, document control of processes and procedures, technical requirements, customer complaints, corrective action and improvement, internal auditing, independent assessment by accreditation bodies, and independent review of result validity by way of participation in external proficiency testing (PT). Laboratories that are assessed as compliant are regarded as competent.

Information on ISO/IEC 17025 can be obtained from national accreditation bodies, which exist in most countries. Lists of accreditation bodies and the standards they can accredit can be obtained from the International Laboratory Accreditation Cooperation (ILAC) or from ILAC-recognised regional cooperation bodies; for example, the Asia Pacific Accreditation Cooperation or the European Co-operation for Accreditation (ilac.org).

The International Laboratory Accreditation Cooperation is the principal international cooperation for developing and harmonising accreditation bodies and operates through an established network of mutual recognition agreements (MRAs). Cooperations such as ILAC are responsible for peer evaluation and mutual acceptance of member-based accreditation bodies in accordance with ISO/IEC 17011:2017 'Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies'. A list of standards related to testing laboratories is noted below:

− testing and calibration laboratories (ISO/IEC 17025)

− medical laboratories (ISO/IEC 15189:2012 'Medical laboratories – Requirements for quality and competence')

− inspection bodies (ISO/IEC 17020:2012 'Conformity assessment – Requirements for the operation of various types of bodies performing inspection')

− proficiency testing (PT) providers (ISO/IEC 17043:2010 'Conformity assessment – General requirements for proficiency testing')

− reference material producers (ISO 17034:2016 'General requirements for the competence of reference material producers').

With the emergence of many zoonotic diseases, veterinary laboratories may become more involved in testing human samples and may, therefore, be required to conform to a medical

laboratory standard such as ISO/IEC 15189, depending on the regulatory requirements of individual countries.

The focus of this paper is on the use of ISO/IEC 17025 in veterinary testing laboratories. Examples and case studies for test validation and verification are provided and specific requirements that were introduced with the latest version of ISO 17025 (2017) are illustrated.

The quest for valid results from diagnostic tests

Valid laboratory results are essential for diagnosis, surveillance and trade. Achievement of such results requires a well-organised, functioning and maintained QMS. OIE Chapter 1.1.1. 'Management of veterinary diagnostic laboratories' and Chapter 1.1.5. 'Quality management in veterinary laboratories', describe the components of governance and management and the issues to consider when designing and maintaining a laboratory's QMS, based on the OIE's interpretations of ISO standards. These chapters were based on an old version of ISO/IEC 17025 (2005). In 2017, the following changes to ISO/IEC 17025 were introduced:

– increased emphasis on risk-based thinking and on outcomes rather than procedure, which enables some reduction in prescriptive requirements. As a consequence, the laboratory must be able to demonstrate how it minimises or eliminates the risks it identifies

– greater flexibility in the requirements for processes, procedures, documented information and organisational responsibilities

– further emphasis on complaints, confidentiality and impartiality, for example, conflict of interest, are examples of a more client-oriented approach

– regarding validation, personnel must be authorised to perform specific activities, including the development, modification, verification and validation of methods, analysis, and the interpretation and reporting of results

– appropriate comments indicating that results may be compromised need to be included in the report

– the laboratory can choose between two different management system options (see below).

Further information on a gap analysis between the old and new version of the standard can be found on the website of Australia's National Association of Testing Authorities (NATA) (4).

A valuable interpretation of ISO/IEC 17025:2017 for veterinary testing laboratories from NATA includes *ISO/IEC 17025 Application Document. Animal health – Appendix* (5).

An accredited diagnostic laboratory is based on three key components

Third-party (independent) assessment

To become a recognised ISO/IEC accredited testing and calibration laboratory (5), the following requirements should be adopted. A description has been summarised for the purpose of this paper. Full details can be found in the standard.

General requirements

− Impartiality: presence of objectivity; conflicts of interest do not exist

− Confidentiality: the laboratory shall be responsible, through legal enforcement, for the management of all information obtained or created during its laboratory activities.

Structural requirements

The laboratory must be a legal entity and legally responsible for its activities. The laboratory shall identify managerial staff that have overall responsibility for the laboratory. All laboratory activities must meet the requirements of ISO/IEC 17025:2017, customers, regulatory bodies and organisations.

Resource requirements

The laboratory shall have the personnel, facilities, equipment, systems and support services necessary to manage and perform its activities:

− *Personnel*: all staff at the laboratory shall act impartially and be competent in required laboratory activities. Staff education, qualifications, training, technical knowledge, skill and experience should be documented. Staff shall be authorised to perform specific laboratory activities, such as development, modification, verification and validation, analysis of results, reporting, reviewing and authorisation of results

− *Facilities*: the facilities and environmental conditions should be suitable for laboratory activities and not adversely affect the validity of test results

− *Equipment*: the laboratory shall have access to the equipment required for the correct performance of laboratory activities that can influence test results

− *Metrological traceability*: calibration measurements that can be related to a documented reference and contribute to calculation of measurement uncertainty (MU)

– *External providers of products and services*: only suitable externally provided products and services that affect the laboratory's activities, including test outcomes, are used.

Process requirements

– *Review of requests, tenders, and contracts*: the laboratory shall have a procedure in place

– *Selection and verification of fit-for-purpose methods*: use of appropriate, up-to-date methods and procedures for all laboratory activities, appropriate evaluation of MU, and statistical analysis of data. (It is recommended that laboratories choose methods that have been published in international, regional, or national standards, or peer-reviewed journals/texts)

– *Validation of methods*: the laboratory shall validate non-standard methods and standard methods outside their intended scope, or otherwise modified; performance characteristics shall be relevant to the customer's needs and consistent with specified requirements

– *Sampling*: the laboratory shall have a sampling plan and a method for when it carries out sampling of substances

– *Handling of test or calibration items*: the laboratory shall have procedures for the transportation, receipt, handling, identification, protection of integrity, storage, retention and disposal of test or calibration items

– *Use of technical records*: technical records shall have documented information to identify factors affecting the test outcome, MU and enable repetition of the method under conditions as close to the original as possible

– *Evaluation of measurement of uncertainty*: a laboratory shall identify the contributions to MU or assess when a test procedure precludes rigorous evaluation of MU

– *Ensuring validity of results*: the laboratory shall have procedures for monitoring the validity of results; where possible, statistical techniques shall be used to review and monitor results, looking for trends

– *Reporting of results*: results shall be reviewed and authorised prior to release

– *Complaints*: the laboratory shall have a documented process to receive, evaluate and make decisions on complaints

– *Non-conforming work*: the laboratory shall have a procedure that shall be implemented when any aspect of its activities or results do not conform with its own procedures or with the agreed requirements of its customer

– *Control of data and information management*: the laboratory shall have access to the data and information needed to perform its activities; information management systems used for collection, processing, recording, storage, or retrieval of data shall be validated for functionality.

Management system requirements

The laboratory must establish, document, implement and maintain a management system that is capable of supporting and demonstrating the consistent achievement of the requirements of the standard and assuring the quality of laboratory results. There are two different management system options – Option A and Option B. Option A is for laboratories that do not have accreditation to ISO 9001 and must therefore meet all the requirements of the new ISO/IEC 17025:2017, including those for management and processes. Option B is for testing laboratories that already have accreditation to ISO 9001 and are therefore exempt from the management requirements listed in the last section of the new ISO/IEC 17025:2017.

Validation or verification of a test method

Method validation is about designing and producing a test that is fit for an intended purpose. It is used to evaluate non-standard, newly developed methods by establishing test performance characteristics at an acceptable, or prescribed, level of statistical confidence. Based on ISO/IEC 17025:2017, it requires laboratory personnel to follow the methods deemed appropriate by the laboratory. It is important to note that the new version of the standard specifies that personnel must be authorised to perform validation and related activities. The complexity of regulatory requirements, coupled with an ever-changing repertoire of new and unique diagnostic reagents and many novel assay platforms and protocols, continues to precipitate discussions about how to properly validate these assays and fulfil the requirements of the standard. Some examples from international and national regulators that face the challenge of providing guidance in the interpretation of the standard for test validation are given below.

The OIE has developed a validation, certification and registration process, and 14 diagnostic test kits have thus far been approved for registration (www.oie.int/scientific-expertise/registration-of-diagnostic-kits/download-application-form/). National bodies have developed OIE-based templates and approval processes to certify diagnostic tests. In Australia, for example, the Subcommittee for Animal Health Laboratory Standards (SCAHLS) has developed separate validation templates for assays that detect fundamentally different analytes, such as antibodies and nucleic acid (6, 7). The OIE validation process uses a 'one template for all tests' approach. The Friedrich Loeffler Institute has developed a license-based marketing authorisation

application   process
(www.fli.de/fileadmin/FLI/Service/Zulassungsstelle/englisch/02_e_Zul_Mittel.pdf).

Another useful interpretation of ISO/IEC 17025 for method validation in testing laboratories (including veterinary) can be found in the *NATA General Accreditation Guidance (GAG) for the Validation and Verification of Quantitative and Qualitative Test Methods* (8).

Method verification, as noted in Chapter 2.2.8. of the OIE *Terrestrial Manual* (9), provides evidence that the test performance characteristics of an already validated or standard method are comparable when used in another laboratory. The ISO/IEC 17025:2017 standard (2) states, 'The laboratory shall verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance. If the method is revised by the issuing body, verification shall be repeated to the extent necessary'.

The NATA GAG (8) states, 'Verification is the process of demonstrating the performance criteria included in the method can be met by the facility prior to introducing them for routine use'.

In ISO 9001 terms, verification is simply defined as ensuring a test is performing correctly and remains fit for purpose. Interpretations of ISO standards can be fraught with inconsistencies. Confusion about what actually constitutes verification of a test in terms of the veterinary laboratory is likely to be attributed to inconsistent interpretations of the testing laboratory standards.

Although standards and guidelines for validation are available to reference, guidelines for verification of established tests are less so. Table I gives an example interpretation of ISO/IEC 10725:2017 (2), OIE *Terrestrial Manual* Chapter 1.1.6. (10) and NATA GAG (8) to provide some clarification of what test performance characteristics may be required for suitable validation and verification exercises in a veterinary testing laboratory. See also Table II for an example of a qualitative test reported using numerical values for calculation of MU.

Verification studies have been referred to as 'taking an already established test and using it in a different laboratory'. Verification of commercially available 'off-the-shelf' kits is not always straight forward because the level of validation information provided with the kit varies. Even the most well-designed validation dossier will likely not have all validation required to be suitable for all intended purposes of a particular testing laboratory.

The roles and responsibilities of kit producer and end-user need to be more clearly defined to avoid gaps which have the potential to allow the use of kits which are not fit for purpose (13).

Although kit producers should provide supporting data for intended purposes, some uses may not be covered, e.g. use of the test in remote geographical target populations. In such circumstances, the testing laboratory must take responsibility for filling the gaps in the validation process when using the test for the purpose they intend.

Chapter 2.2.8. of the *Terrestrial Manual* discusses comparability studies, which are carried out to ensure that, if minor changes are made to an established test, the analytical performance characteristics of the modified test are as good as those of the validated test within statistical defined limits. The chapter notes that important variables 'outside of the assay' may require verification. These include the nature of the target population, the species, and the specimen. Such changes would require a verification study 'to validate the performance characteristics of the test under the new circumstances' (9).

Terrestrial Manual Chapter 1.1.6. (section 5.4) considers a process of limited validation of commercial kits and candidate assays with published validation. After assessment to ensure expected analytical test performance, 'a limited stage-two validation [diagnostic performance] should be considered in the context of the intended application and target population' (10). After this approach, if the test does not have the required fit for purpose, a full validation will be required.

A related paper (Kirkland & Newberry, this volume [14]), includes a flowchart that provides a pictorial description of what may be required when a standard test has variables 'outside of the assay' that have changed from the initial validation, and when a full validation is required.

The following example illustrates what performance characteristics may be investigated when a commercially sourced test is used outside the scope of its intended application. A commercially available porcine epidemic diarrhoea virus (PEDV) antibody enzyme-linked immunosorbent assay (ELISA) (or 'standard' test) was found not to be fit for the intended purpose of serosurveillance in a population that was geographically different from the one the manufacturer used to designate its cut-off. To be fit for the purpose of serosurveillance of pig populations in the country where the kit was manufactured, there was a need to reduce the test's diagnostic sensitivity (DSe) to balance diagnostic specificity (DSp) due to the presence of a closely related virus, transmissible gastroenteritis (TGE). During the manufacturer's validation process, the ELISA was shown to cross react with TGE antibodies present in local populations. The ELISA was purchased for serosurveillance and estimation of prevalence of antibodies against PEDV in a geographical area that was expected to have an extremely low prevalence of PEDV or related viruses. Due to the low prevalence of PEDV in the area of interest and the limited number of

samples able to be assessed as part of the planned surveillance study, the test required a high level of DSp and a reasonable level of DSe to be suitable for the customer's requirements.

During initial verification, analytical specificity (ASp) was established using local negative pig populations. Testing of a small number of well-characterised, outbreak-sourced field sera from endemic areas and Day 14 post-infection experimental serum showed evidence of lower than expected DSe (Fig. 1a). Notably, the manufacturer's validation file did acknowledge the limitations of their validation process (data not shown).

Additional verification of the ELISA was performed by testing sera from 25 PEDV-infected and 243 PEDV non-infected pigs. Changing the cut-off and using a confirmatory test – a PEDV indirect immunofluorescence assay (IFA) – on sera with ELISA reactivity in the 40%–60% range resulted in an increased DSe (Fig. 1b). Subsequent testing of a larger number of PEDV-positive field sera from PEDV-endemic pig populations and antibody assessment of closely related viruses confirmed results. Although a full assessment of DSe and DSp is not normally thought to be a requirement of test 'verification', 'off-the-shelf' diagnostic kits developed in different geographical parts of the world should have some level of validation to ensure that its DSe and DSp are sufficient for the new purpose of the testing laboratory. The example also illustrates the advantage of a serial testing approach, or 'testing algorithm' – first testing samples using a screening test with high DSe, then testing the positive samples using a confirmatory test with a high DSp – to optimise overall specificity and decrease the chance of false-positive results.

Proficiency testing

The role of PT is vital for any laboratory that is accredited or seeking accreditation and the purpose of such programmes is to evaluate the laboratory's performance. It allows results with assigned (or expected) values to be compared between laboratories, which is important to implement quality control measures. There are many PT schemes available worldwide and programmes that are accredited for ISO/IEC 17043 are best used, as they operate under recognised QMS. Organisations such as ILAC have listings of accredited PT providers, with information about contact details and scope of accreditation on their web pages. Proficiency testing providers found on the database of ILAC MRA signatories have been peer-evaluated in accordance with the requirements of ISO/IEC 17011 to demonstrate their competence. It is important that the choice of PT provider is appropriate and is fit for the laboratory's intended purpose. For further details on PT schemes, the authors refer readers to PT and reproducibility papers in this volume (Johnson & Cabuang and Waugh & Clark [15, 16]).

Conclusions

Good laboratory practice for obtaining valid laboratory results consists of three important components: *i)* independent, third-party accreditation, *ii)* method validation/verification, and *iii)* ongoing quality control/quality assurance in the form of intra-laboratory and inter-laboratory assessment.

A sound laboratory QMS that includes well-structured validation and verification processes will ensure that the testing laboratory can transparently demonstrate its competency and its ability to produce consistent, technically valid results that will meet the needs of its customers.

Validation of standard tests is not always as comprehensive as expected by the laboratory (and, by extension, the customer) for intended purposes. There are instances when extended assessment of parameters such as DSp and DSe will be required. It is important to note that the new version of ISO 17025:2017 specifies that personnel must be authorised to perform validation and related activities, which means that training in validation and verification methods, including results interpretation, is likely to become more important to prove competence.

National and international organisations, accreditation bodies and PT providers have processes that can ensure a laboratory's QMS is peer reviewed and shown to be competent, giving the customer, regulatory bodies or stakeholders increased confidence in testing outcomes.

Regardless of whether a laboratory is accredited or not, the steps leading to the implementation of a quality system remain the same. Early discussions with the accreditation body are useful to get a realistic assessment of operational and financial requirements. Some regulatory bodies offer cost-free pre-assessment, which helps to define the scope of accreditation. The OIE Reference Laboratories, and international organisations such as the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency, provide training and offer enrolment in proficiency test rounds to facilitate development of a culture of quality within a testing laboratory (17).

Normes et lignes directrices relatives à la qualité applicables à la validation des tests de diagnostic pour les maladies infectieuses dans les laboratoires vétérinaires

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Résumé

Le chapitre 1.1.1 du *Manuel des tests de diagnostic et des vaccins pour les animaux terrestres* de l'Organisation mondiale pour la santé animale donne une vue d'ensemble des principaux aspects de la gouvernance et de la gestion d'un laboratoire de diagnostic vétérinaire tandis que le chapitre 1.1.5 introduit aux principes de la gestion de la qualité dans les laboratoires. Les deux chapitres reposent sur la norme de l'Organisation internationale de normalisation/Commission électrotechnique internationale ISO/IEC 17025:2005, « Exigences générales concernant la compétence des laboratoires d'étalonnages et d'essais ». Les auteurs font le point sur l'état actuel des normes et des organismes de réglementation pertinents en matière d'accréditation des systèmes de gestion de la qualité , en mettant l'accent sur la norme ISO/IEC 17025:2017 qui est plus précisément axée sur les laboratoires d'essais et d'étalonnage. Les auteurs mettent en avant un certain nombre de questions et de considérations importantes qu'un laboratoire devrait prendre en compte lors de la conception et de la mise en œuvre continue de son système de gestion de la qualité et les illustrent d'exemples relatifs à des aspects particuliers de la validation et du contrôle des performances d'un test, notamment l'incertitude des mesures. Un système de gestion de la qualité doit courir tous les aspects opérationnels d'un laboratoire, y compris le personnel, la structure organisationnelle, les processus et les procédures. L'accréditation d'un laboratoire de diagnostic repose sur trois composantes majeures : *a*) l'évaluation, qui doit être conduite de manière indépendante ou par des tiers ; *b*) des tests validés de manière appropriée et utilisés par des opérateurs de laboratoire qualifiés dans un laboratoire correctement équipé; *c*) un contrôle de la qualité continu, à la fois interne et externe. Prises ensemble, ces composantes garantissent que les résultats d'un test sont le fruit d'un processus normalisé et d'un examen structuré et conduit par des pairs, et démontrent aussi bien la compétence que la capacité à produire des résultats diagnostiques robustes sur le plan technique et répondant aux besoins des clients – vétérinaires, propriétaires d'animaux, régulateurs, organisations et secteur privé – ainsi qu'aux besoins des décideurs en charge des programmes de santé animale et de surveillance.

Mots-clés

Accréditation – Assurance qualité – Contrôle des performances d'un test – ISO – OIE – Organisation internationale de normalisation – Organisation mondiale de la santé animale – Système de gestion de la qualité – Validation.

Normas de calidad y directrices de validación de pruebas para enfermedades infecciosas en los laboratorios veterinarios

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Resumen

En el capítulo 1.1.1 del *Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres* de la Organización Mundial de Sanidad Animal (OIE) se resumen los aspectos más importantes de la dirección y la gestión de laboratorios de análisis veterinarios, mientras que en el capítulo 1.1.5 se aborda el tema de la gestión de la calidad. Ambos capítulos están basados en la norma ISO/IEC 17025:2005, "Requisitos generales para la competencia de los laboratorios de ensayo y de calibración" de la Organización Internacional de Normalización y la Comisión Electrotécnica Internacional. Los autores ofrecen información actualizada sobre las normas aplicables y los organismos de reglamentación competentes por lo que respecta a la certificación de sistemas de gestión de la calidad, partiendo básicamente de las disposiciones de la norma ISO/IEC 17025:2017 que se aplican a los laboratorios de ensayo y de calibración. También destacan las cuestiones y consideraciones importantes que un laboratorio debe tener en cuenta para concebir y mantener su sistema de gestión de la calidad, en particular los aspectos relativos a la validación y verificación de pruebas, incluida la incertidumbre de medición, y ofrecen ejemplos al respecto. Un sistema de gestión de la calidad tiene por objetivo cubrir todos los aspectos del funcionamiento de un laboratorio, lo que comprende su personal, su estructura organizativa y sus procesos y protocolos. La acreditación de un laboratorio de diagnóstico consta de tres componentes fundamentales: a) una evaluación por parte de un tercero independiente; b) la realización de pruebas debidamente validadas, a cargo de técnicos de laboratorio competentes, en un laboratorio convenientemente equipado; y c) controles continuos de la calidad, tanto internos como externos. La suma de estos componentes garantiza que los resultados de un ensayo sean fruto de un proceso normalizado y de una revisión por homólogos estructurada y demuestra que el laboratorio posee tanto la competencia como la capacidad necesarias para obtener resultados de diagnóstico técnicamente válidos, que respondan a las necesidades tanto de los clientes (veterinarios, propietarios de animales, organismos de reglamentación, organizaciones y entidades industriales) como de las instancias decisorias que intervienen en los programas de sanidad animal y vigilancia zoosanitaria.

Palabras clave
Acreditación – Certificación – Garantía de calidad – ISO – Organización Internacional de Normalización – Organización Mundial de Sanidad Animal – OIE – Sistema de gestión de la calidad – Validación – Verificación.

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Table I

Alignment of validation and verification performance criteria for diagnostic assays

Validation criteria for non-standard diagr	Verification criteria for standard (established diagnostic assay
Definition of intended purpose	Similarly defined fit for intended purpose
Optimisation of test	Not required
Standardisation	Not required
Robustness–Ruggedness (test developmer	Not required
Repeatability	Required
Analytical sensitivity – quantitative tests onl	Required
Analytical specificity, or selectivity	Some degree of validation will be required if previo similar testing populations, species, required purpc
Diagnostic sensitivity	Some degree of validation will be required if previo similar testing populations, species, required purpc
Diagnostic specificity, or selectivity	Some degree of validation will be required if previo similar testing populations, species, required purpc
Defined threshold (cut-off) values	Verify the defined threshold suits the laboratory's r validation required
Reproducibility: Precision. Inter- and intra-la	Required
Reproducibility: Accuracy. Inter- and intra-l	Required
Designation of intended fit for purpose after determined	Designation of intended fit for purpose after cut-off
Measurement of uncertainty (MU) for quant	Ongoing assessment required for quantitative tests

* ISO/IEC 17025:2017 notes that qualitative tests are those whose test outcomes are not numerically based. However, if a qualitative test is reported using a numerical value, it should be regarded in the same way as for quantitative test procedures (MU only)

Table II

Top-down or control sample approach for an equine influenza TaqMan A assay

Number of test runs	Weak positive control sample (Results expressed as Ct value)
1	33.60
2	33.20
3	33.96
4	33.18
5	33.96
6	32.72
7	33.57
8	33.45
9	32.80
10	33.20
Mean	33.36
Standard Deviation (STD)	0.43
Relative Standard Deviation (RSD) = STD/Mean	0.0128
Expanded Uncertainty U$_{(95\%CI)}$ = 2 X RSD	0.0255
Apply Expanded Uncertainty at cut-off (37*0.025)	0.94
Lower limit (Cut-off - expanded uncertainty) 37 - 0.94 =	36
Upper limit (Cut-off + expanded uncertainty) 37 + 0.94 =	38

Ct: cycle threshold

This example uses data from replicate runs of a weak positive control sample (10 runs) of an equine influenza TaqMan A assay. The mean cycle threshold (Ct) value after 10 runs is 33.36 and the standard deviation is 0.43. The relative standard deviation is 0.0128. The expanded uncertainty (95% CI) is 2 × the relative standard deviation = 0.0255. Measurement of uncertainty (MU) is most relevant at the cut-off (Ct = 37) and can be applied by multiplication (37 × 0.0255 = 0.94). Subtraction from the threshold provides the lower 95% CI limit (Ct = 36) and in addition the upper 95% CI limit (Ct = 38).

Interpretation of results

Any positive result (Ct < 37) that is higher than 36 Ct is not positive with 95% confidence. Similarly, any negative result (Ct > 37) that is less than 38 is not negative with 95% confidence.

Scope and limitations

Methods for quantifying uncertainty (addressing MU) for tests vary. When estimating MU for quantitative, biologically based diagnostic tests, where variations in the substrate or matrix have large and unpredictable effects, a top-down approach is recommended (11). The advantage of this method is that quality control data are generated during normal test runs and can be used to estimate the precision of the assay and express it at the cut-off. The application at the cut-off level depends on the performance of the test at different analyte concentrations, e.g. variation is likely to increase at higher Ct values. The top-down approach does not identify individual contributors to measurement uncertainty but rather provides an overall estimate (12). Measurement of uncertainty does not replace test validation; however, the validation process includes assessments of repeatability through quality control samples which facilitate calculation of MU.
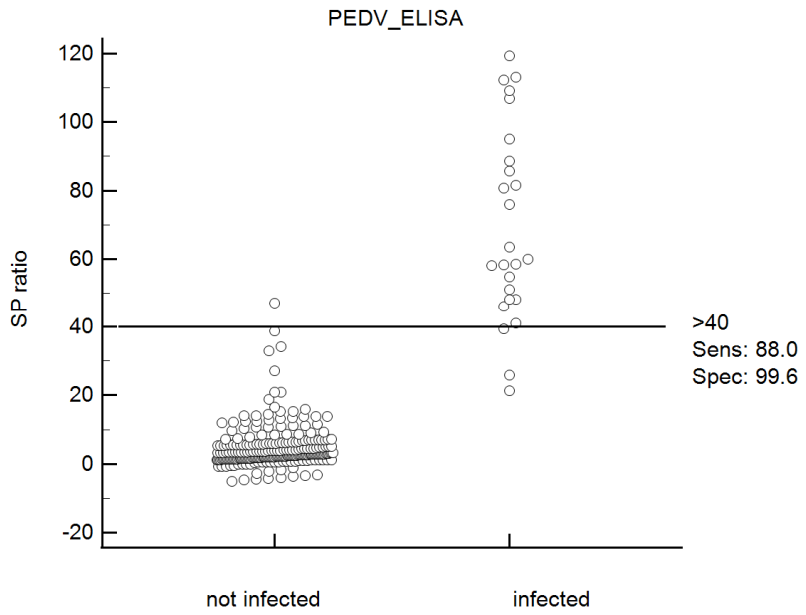
Fig. 1

Diagnostic sensitivity and diagnostic specificity of porcine epidemic diarrhoea virus enzyme-linked immunosorbent assay

a) 60% cut-off as provided by kit producer
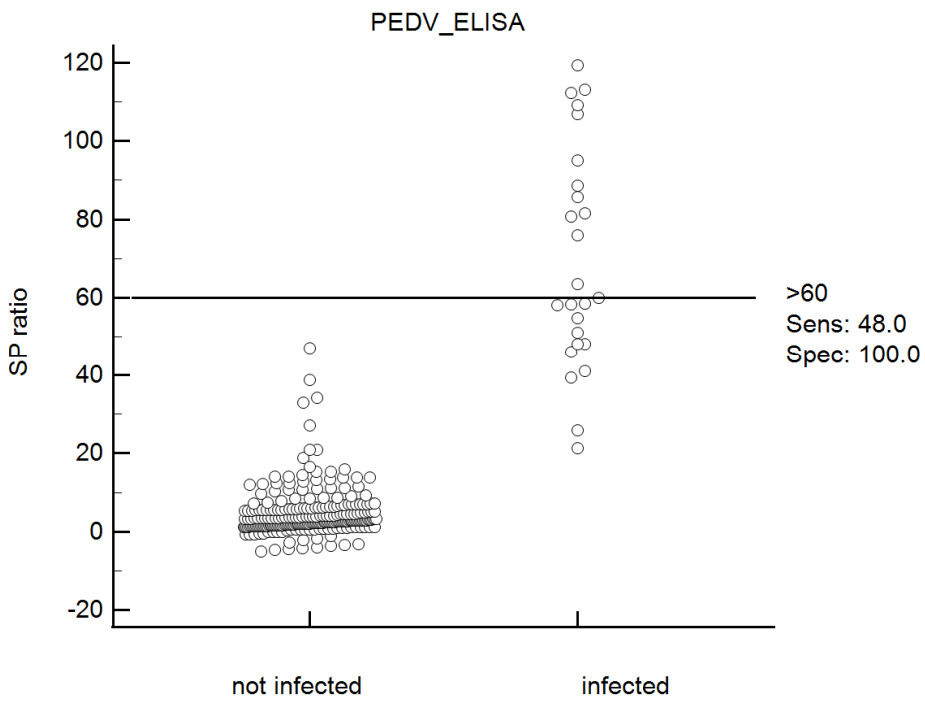
b) 40% cut-off as obtained through verification study

PEDV_ELISA

>40
Sens: 88.0
Spec: 99.6