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Quality assurance/quality control of foot and mouth disease solid phase competition enzyme-linked immunosorbent assay – Part I. Quality assurance: development of secondary and working standards

N. Goris & K. De Clercq

Department of Virology, Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Brussels, Belgium

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Summary
International movement in animals and animal products has urged organisations like the World Organisation for Animal Health (OIE) to draw up guidelines to regulate and facilitate trade between Member Countries. However, as the global market continues to grow, further standardisation and harmonisation of antibody detection assays for infectious diseases are needed, especially regarding the development and use of reference materials. For OIE notifiable diseases for which primary or international reference standards are available or under development, National or Regional Reference Laboratories are encouraged to establish their own secondary and/or working standards. This paper describes the development of standards for the foot and mouth disease (FMD) solid phase competition enzyme-linked immunosorbent assay using positive serum obtained from calves vaccinated against the FMD virus. The procedure outlined in this manuscript can easily be extrapolated to similar serological assays and should lead to further international harmonisation of assays and test results.

Keywords
Accreditation – Antibody detection – Enzyme-linked immunosorbent assay – Foot and mouth disease – Primary reference standard – Quality assurance – Secondary standard – Standardisation – Validation – Working standard.

Introduction

Globalisation of international trade of livestock and livestock products has increased the risk of importing rapidly transmissible trans-boundary diseases that have severe socio-economic and/or public health consequences, such as foot and mouth disease (FMD) and highly pathogenic avian influenza. One of the key missions of the World Organisation for Animal Health (OIE) is to harmonise trade regulations between the 167 Member Countries (21). To avoid unjustified trade barriers and maintain a high level of animal health, the OIE has published standards, guidelines, and recommendations for international trade in the form of the *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*) (14). The *Terrestrial Code* constitutes an important reference document in the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (21). To qualify animals and animal products for international movement, the *Terrestrial Code* requires that exporting countries provide animal

health certificates from the national Veterinary Administration indicating that the animals are free of infection (14). However, this qualification implies that all Member Countries have confidence in the certificates and in the predictive value of the diagnostic assays used by each country (2), which reinforces the need for mutual recognition of test results.

A quality management system should enable a Reference Laboratory to assure its trading partners, among others, of the laboratory's competence and ability to generate technically valid results (13). This can be confirmed by a third party accreditation body, who could verify conformance to the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) International Standard 17025 (6). Compliance with ISO/IEC 17025 implies fulfilling numerous management requirements, as well as a series of technical criteria regarding personnel, environment and equipment, test method and method validation, measurement traceability, etc. ISO/IEC 17025 encompasses testing and calibration performed using standard, non-standard, and laboratory-developed methods. To demonstrate that the chosen test format is appropriate for the test's intended use (i.e. 'fit for purpose'), calibration using reference standards or reference materials (6) and participation in external quality control schemes (6, 7), regardless of the test status (standard versus laboratory developed) is necessary.

In the case of FMD, the solid phase competition enzyme-linked immunosorbent assay (ELISA) (SPCE) for the detection of antibodies to the FMD virus (FMDV) (10, 15) is regarded by the OIE as a prescribed test for international trade (13). Both the OIE (12, 13) and the Research Group of the Standing Technical Committee of the European Commission for the Control of FMD (EUFMD) of the Food and Agriculture Organization (FAO) (4, 5) have recommended the inclusion of reference materials in FMD diagnostic assays. One of the objectives of the FAO Collaborative Study Phases was to standardise FMD serology by harmonising test protocols and selecting primary reference sera for FMDV strains O_1 Manisa, O SKR 2000, A_{22} Iraq 24/64, A Iran 1996, C_1 Noville, and Asia 1 Shamir (8, 11, 16, 17). The consensus of a Joint FAO/International Atomic Energy Agency Meeting of Consultants (Vienna, 1992), which reviewed ELISA data expression, primary reference standards, quality assurance, and diagnostic validation, was that, at least, a strong positive (SP), a weak positive (WP), and a negative control (NC) primary standard serum should be established for antibody detection enzyme immunoassay techniques (19). International standardisation and harmonisation of the FMD SPCE is, thus, best achieved by using the developed (candidate) primary standard sera to calibrate the standard or laboratory-developed test method and reagents under local conditions.

Additionally, ISO/IEC 17025 obliges regular use of certified reference materials to continuously monitor the validity of assays used in the laboratory (6). However, internationally approved reference standards are too valuable to be used to verify daily diagnostic testing (12). To minimise usage and extend the supply of primary reference sera, it is, therefore, vital that the primary standards are used only to calibrate secondary standards (SS) produced by National or Regional Reference Laboratories (8, 12). The SS are designated as national or regional standards and are used to calibrate working or tertiary standards (WS). The WS may be synonymous with the SS (i.e. the SS may be used as a working standard) or may be calibrated against the SS and used on a routine basis (12).

This paper describes a practical method for developing SS and WS by National or Regional Reference Laboratories for use in the FMD SPCE in accordance with international guidelines for the detection of antibodies to infectious diseases (7, 12, 13, 18, 19, 20).

Materials and methods

Calibrating the solid phase competition enzyme-linked immunosorbent assay test protocol

In 2001, a SPCE protocol for measuring antibodies to FMDV was described by Mackay *et al.* (10). However, during the FMD crisis in the United Kingdom (UK) that same year, it became apparent that the original protocol using a 1:5 test serum dilution and a 30% inhibition (percent inhibition [PI]) cut-off (C/O) level had a low diagnostic specificity when used for herd-based sero-surveillance. To resolve the issue, the FAO World Reference Laboratory (WRL) for FMD (Pirbright, UK) raised the C/O threshold to 60 PI while maintaining a 1:5 serum dilution (announced at an urgent Meeting of National Reference Laboratories regarding FMD serology convened by the European Commission, Brussels, 26 March 2001). The modified version of the SPCE was subsequently validated (15) and adopted by the OIE as a prescribed test for international trade (13). However, at the Veterinary and Agrochemical Research Centre (Brussels, Belgium) the original SPCE had already been well established as the in-house test and adjusted to local conditions to account for the specificity problems. Rather than adjusting the C/O level and making several other minor changes, the SPCE standard operating procedure (SOP) was optimised by increasing the test serum dilution from 1:5 to 1:10, which circumvented the problem with low test specificity.

Briefly, ELISA plates are coated with 100 µl of an optimal dilution of rabbit anti-FMDV polyclonal antiserum in carbonate/bicarbonate buffer (pH 9.6) and held overnight

at 4°C. After washing with phosphate-buffered saline containing 0.05% Tween-20 (PBST) (pH 7.4), 100 µl of a pre-determined concentration of FMDV in PBST containing phenol red indicator is added and the plates are incubated for 1 h at 37°C. The plates are then washed a second time prior to adding 50 µl of test (or standard) serum pre-diluted 1:10 with PBST supplemented with 5% negative rabbit and 10% negative bovine serum (blocking buffer [BB]). At the same time, 50 µl of a pre-defined concentration of a competing polyclonal guinea pig anti-FMDV antiserum in BB is added. The plates are incubated at 37°C for 1 h and washed again. Subsequently, 100 µl of an optimal concentration of polyclonal rabbit anti-guinea pig immunoglobulins conjugated with horseradish peroxidase (HRPO) is added to all wells and the plates are incubated at 37°C for 1 h. A final wash is performed before adding 100 µl of substrate (0.05% H_2O_2)/chromogen (3mM orthophenylene diamine [OPD]) in citrate phosphate buffer (pH 5.2). Finally, the plates are placed in the dark at ambient temperature for 15 minutes before stopping the colormetric reaction by adding 100 µl of 1.25M sulphuric acid (H_2SO_4). The optical density (OD) of each well is read at 490 nm. Both the rabbit and guinea pig anti-FMDV polyclonal antisera are homologous to the FMDV serotype and subtype used.

To calibrate the in-house FMD SPCE against the standard SPCE (13, 15) and demonstrate the validity of the assay, candidate primary reference sera originating from the FAO Collaborative Study Phases XV, XVI, and XVII (8, 11, 16, 17) and supplied by the FAO WRL for FMD were tested in duplicate. The raw OD values for each serum obtained using the in-house SPCE protocol were expressed as PI of the no serum control (Co) (20) and compared to the PI obtained by the FAO WRL using the standard SPCE protocol (13, 15). Presently, the primary reference sera supplied by the FAO WRL for FMD have been approved by the OIE as international standard sera. At the time of this study, however, the sera were still regarded as candidate primary reference sera.

Indirect sandwich enzyme-linked immunosorbent assay for foot and mouth disease

The indirect sandwich ELISA for the detection of bovine anti-FMDV antibodies in cattle serum is a slightly modified version of the protocol described by McCullough *et al*. (9).

Very briefly, ELISA plates are coated with 50 µl of rabbit anti-FMDV polyclonal antiserum (homologous to the FMDV used in the following step) and held overnight at 4°C. Fifty microlitres of FMDV is then added to each well, followed by the addition of 50 µl of test bovine serum and 50 µl of rabbit anti-bovine immunoglobulins conjugated with HRPO. Finally, 50 µl of substrate (H_2O_2)/chromogen (OPD) is added and the colormetric reaction is stopped by the addition of 50 µl of H_2SO_4 per well. The OD of each well is read at 490 nm. The corresponding dilution reagents and the washing and incubation conditions are identical to those described for the SPCE.

Development of secondary and working standards

To obtain antibody positive sera against five FMDV strains (O_1 Manisa, A_{22} Iraq 24/64, A Iran 1996, C_1 Noville, and Asia 1 Shamir), for the preparation of SS and WS, two calves per strain (aged between six to eight months at the time of vaccination) were vaccinated intramuscularly using homologous monovalent oil emulsion vaccines (Merial, Pirbright, UK). One calf received a single cattle vaccine dose as prescribed by the manufacturer; the other was given a booster injection at five months post initial vaccination. Blood was collected from the monovaccinated calves five to six months post vaccination; whereas, blood from the calves given the booster injection was collected 21 to 28 days after the second injection. Sera that were free from haemolysis and excessive lipaemia were retained (12), filtered with a 0.2 µm microfilter, and stored at –20°C until use.

Subsequently, each serum was serially titrated in BB and tested in duplicate on two different plates using the in-house homologous SPCE. The positive titration series consisted of neat (undiluted) serum plus 19 two-fold dilutions (1:2 to 1:524288). The titrations were repeated on five different days with a fresh set of reagents, resulting in ten individual titration curves that were used to estimate repeatability. The mean PI of the duplicates for each plate was calculated and the average PI for each dilution was determined based on the values from all ten runs and used to establish a dose-response curve. Serum dilutions with a PI that was nearly identical to the PI, obtained in direct comparison on the test plate, of the C/O, WP, and SP FAO WRL candidate primary reference sera were selected as the corresponding in-house C/O, WP, and SP candidate SS. A commercially available adult bovine serum batch was chosen as a candidate NC standard and results were compared to the candidate primary negative reference (8, 16, 17).

The in-house SP, WP, and C/O candidate secondary standards, diluted once in the candidate NC standard, were tested in parallel with the candidate primary reference sera three additional times. Candidate secondary standards with a PI that closely matched the PI of the candidate PS were used to prepare (11, 12) single-use aliquots for each of the four candidate SS (SP, WP, C/O and NC). The aliquots were stored at –20°C, thawed prior to use, and pre-diluted in the same manner as routine samples. After thawing, the PIs of the aliquots of candidate

SS were compared five times (on average) to the PI of the candidate primary reference sera to exclude any bias due to an additional freeze/thaw cycle.

To assess the difference between the candidate primary standards and the respective candidate SS the parametric bootstrap was used (3). Based on the assumption that the data was normally distributed, the maximum likelihood estimate of mean and variance were used to parameterise a random variable for each of the standards. New datasets (with five values for each standard) were simulated by the Monte Carlo simulation (3), and for each iteration the PS and candidate SS were compared to determine if the mean of the candidate SS was higher than or equal to the mean of the primary standard. The proportion of simulations that fulfilled the criterion (i.e. the PS and candidate SS were equal) represented a measure of confidence. Tail area values (close to a value of one or zero) were suspicious and indicated a difference between the means: a value of one indicated with absolute certainty that the candidate SS was higher than the primary standard and vice versa for a value of zero. If no tail values were obtained using the Monte Carlo simulation, the candidate SS were accepted as definite SS.

In a similar manner, single-use aliquots of WS (SP, WP, C/O, and NC) were prepared and standardised against the SS (12, 18). The SP, WP, C/O, and NC WS sera were subsequently used in daily laboratory diagnostics. On each plate, four replicates of the C/O and WP WS were incorporated (19). However, due to a lower coefficient of variance (two to three times) only two replicates of the SP WS were included. The NC WS was also tested in duplicate, and four replicates of a no serum control (Co) were used to calculate the PI (the PI is based on the ratio between the median OD of the test or standard serum and the median OD of the Co).

Results

Calibrating the solid phase competition enzyme-linked immunosorbent assay test protocol

The FAO Collaborative Study Phases XV, XVI, and XVII resulted in the distribution to several FMD Reference Laboratories of candidate primary reference standards against FMDV strains O_1 Manisa, O SKR 2000, A_{22} Iraq 24/64, A Iran 1996, C_1 Noville, and Asia 1 Shamir (8, 11, 16, 17). Each standard was tested following in-house SPCE SOP. Table I summarises the results for the Asia 1 Shamir SPCE. The PI obtained were highly similar to the corresponding PI provided by the FAO FMD WRL (16, 17), indicating that the in-house assay was calibrated very closely to the standard SPCE.

Table I
Comparison of the solid phase competition enzyme-linked immunosorbent assay (SPCE) type Asia 1 Shamir test protocol results obtained using the in-house test and the World Reference Laboratory test, based on candidate primary reference standards from the Food and Agriculture Organization (FAO) Collaborative Study (Phase XVII part 1 and 2)

Standards	WRL SPCE [a]	In-house SPCE [b]
SP candidate primary standard part 2	98	90
WP1 candidate primary standard part 2	92	89
WP2 candidate primary standard part 2	90	89
WP3 candidate primary standard part 2	69	73
WP4 candidate primary standard part 2	63	69
C/O candidate primary standard part 1	42	35

a) Results obtained by the FAO World Reference Laboratory (WRL) for foot and mouth disease (FMD) using the World Organisation for Animal Health (OIE) prescribed SPCE (13, 15). Values are expressed as percent inhibition (PI) of the no serum control
b) Results obtained by the Belgium Reference Laboratory for FMD using the in-house SPCE based on the SPCE described by Mackay *et al.* (10), but with a 1:10 serum dilution. Values are expressed as PI of the Co
C/O:cut-off
SP: strong positive
WP: weak positive

For the SPCE type O_1 Manisa, A_{22} Iraq 24/64, A Iran 1996, and C_1 Noville a similar approach was taken. If necessary, the analytical sensitivity of the in-house test was adjusted to equal that of the standard test through checkerboard titration of the FMDV and the homologous guinea pig anti-FMDV antiserum, which involved adapting the level of competition.

Development of secondary and working standards

The SS and WS for the SPCE type O_1 Manisa, A_{22} Iraq 24/64, and C_1 Noville were prepared by direct comparison to the candidate primary reference standards originating from the FAO Collaborative Study Phases XV and XVI (8, 11). For the A Iran 1996 and Asia 1 Shamir SPCE, homologous SS and WS were derived based on the FAO Study Phase XVII candidate primary reference standards (16, 17). Development of SS and WS for the SPCE type Asia 1 Shamir will be discussed in depth.

The FAO Collaborative Study Phase XVII part 1 and 2 resulted in the distribution to nine FMD Reference Laboratories of a SP, a WP1, a WP2, a WP3, a WP4, a C/O, and an NC candidate primary standard against FMDV Asia 1 Shamir (16, 17). The primary standards served as reference values against which the SS and WS under development could be compared.

The dose-response curve for the Asia 1 Shamir monovaccinated calf serum titrated in the homologous SPCE exhibited a steep sigmoidal pattern, and, although,

the linear portion of the curve spanned only four dilutions (1/32 to 1/256), the results were highly reproducible (the coefficient of variance was less than 10%) (Fig 1). Candidate SP, WP, and C/O secondary standards were prepared by using the dose-response curve to determine serum dilutions with a PI that were similar to the PI of the candidate primary reference standards.

The candidate SP SS should be diluted to a final concentration of 1:10, the WP to 1:50, and the C/O to 1:140. The performance characteristics of the SS and the corresponding PS should match (i.e. the SP SS to the SP candidate PS part 2, the WP to the WP4 candidate PS part 2, and the C/O to the candidate C/O PS part 1). To confirm the final dilutions chosen, the candidate SP, WP, C/O, and NC SS were tested three times in parallel with the PS (data not shown). Therefore, a stock of SP, WP, and C/O candidate SS was prepared at respective dilutions of 1:1, 1:5 and 1:14. This was calculated in accordance with the in-house SPCE, in which samples have to be diluted 1:10 in BB prior to testing. Consequently, bias introduced by any special pre-dilution or preparation was prevented.

Once it was confirmed that the PI values of the candidate secondary and primary standards closely matched, single-use aliquots of the candidate SS were prepared by the same methods used to prepare the one-time dilutions. The NC SS was prepared in the same manner. The single-use aliquots were stored at –20°C and thawed prior to use. The results of the five tests (after an additional freeze/thaw cycle) conducted in parallel with the candidate primary reference sera are summarised in Table II. The parametric bootstrap resulted in a confidence value for the C/O, WP, SP, and NC standards of 0.743, 0.046, 0.129 and 0.581, respectively.

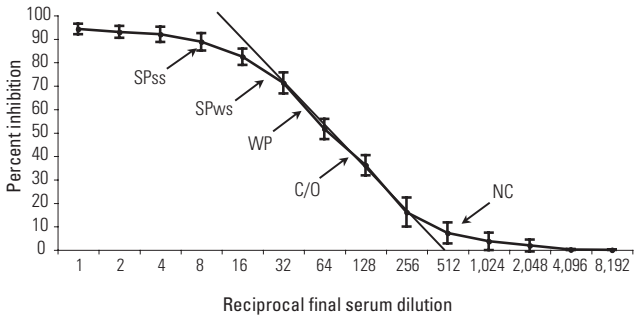

Fig. 1

Dose-response curve determined with the in-house solid phase competition enzyme-linked immunosorbent assay type Asia 1 Shamir using serum collected from a calf vaccinated with a single dose of the FMD vaccine

The curve represents the average ± one standard deviation of individual titration series (n = 10). The arrows indicate the percent inhibition obtained for the selected strong positive (SP), weak positive (WP), negative (NC), and cut-off (C/O) secondary standard (SS) and working standard (WS). The SS and WS are identical for the WP, C/O, and NC standards

Table II

Comparison of primary, secondary and working standards for the in-house solid phase competition enzyme-linked immunosorbent assay (SPCE) type Asia 1 Shamir

Standards	Primary standard [a]	Secondary standard [a]	Working standard [a]
Cut-off standard	35 ± 5.4	39 ± 4.8	39 ± 4.8
Weak positive standard	69 ± 5.1	58 ± 3.9	58 ± 3.9
Strong positive standard	90 ± 1.7	87 ± 2.1	75 ± 3.3
Negative standard	6 ± 2.9	7 ± 3.9	7 ± 3.9

a) results obtained by the Belgium Reference Laboratory for foot and mouth disease using the in-house SPCE based on the SPCE described by Mackay et al. (10), but with a 1:10 serum dilution. Values are expressed as mean percent inhibition of the no serum control ± one standard deviation (n = 5)

The WS in our case, incorporated in daily diagnostic runs, are identical to the SS, except for the SP standard. To minimise the amount of reagent needed to prepare the SP working standard, the concentration of the SP serum was adjusted to fall within the linear portion of the dose-response curve as described for the indirect ELISA (20), resulting in a final 1:20 dilution of the neat serum (a 1:2 dilution in candidate NC serum was used for preparation of the aliquots). Due to the steep transition from positive to negative in dose-response curves often obtained in competitive ELISA tests (12), it is believed that titration results are not reproducible. However, the in-house results showed that the approach was justified by comparing the dose-response curve of the FMD SPCE to the dose-response curve of the FMD indirect sandwich ELISA. Notably, the linear portion of the indirect anti-bovine ELISA dose-response curve spans six dilutions, which is only two more than the number of dilutions spanned by the linear portion of the SPCE dose-response curve, resulting in very similar curves for both assays (Fig. 2).

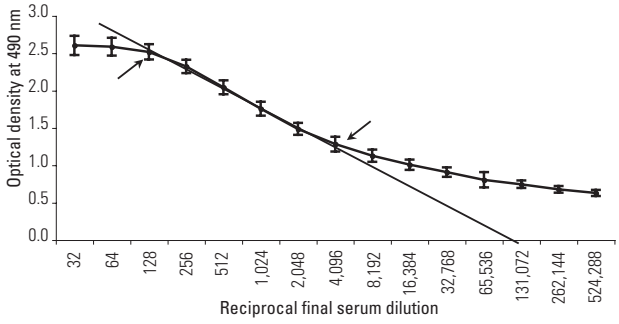

Fig. 2

Dose-response curve determined with the in-house solid phase competition enzyme-linked immunosorbent assay type Asia 1 Shamir using serum collected from a calf vaccinated with a single dose of the FMD vaccine

The curve represents the average ± one standard deviation of the individual titration series (n = 10). The arrows indicate the beginning and the end of the linear portion of the curve

Table II summarises the values of primary, secondary, and tertiary standards for the SPCE type Asia 1 Shamir. Similar dilutions were determined for the SPCE type O_1 Manisa, A_{22} Iraq 24/64, A Iran 1996, and C_1 Noville, and, as per type Asia I Sharmir, SS and WS aliquots were prepared from sera collected from monovaccinated calves and/or calves given a primary vaccination followed with a booster injection.

Discussion

Assay characteristics, such as repeatability and analytical and diagnostic sensitivity and specificity, are essential for validation (7). However, these criteria are no longer sufficient when faced with globalisation of trade. To facilitate international movement of animals and animal products, there is a pressing need for assay validation at the global level and test quality assurance under local conditions (1). In addition to external quality assurance based on proficiency testing (7), which remains indispensable, test validation and quality assurance can be achieved through the development of international standards for antibody detection assays. Since serological diagnostic techniques are widely used in veterinary medicine throughout the world and have replaced traditional culture and isolation-based diagnosis for trade purposes (19), international test standards would allow for global harmonisation of assays and test results.

Primary reference standards are synonymous with international standards (12) and are used to harmonise the detection range and the analytical sensitivity of the assay methods used by different international and National Reference Laboratories (18). In addition, the PS also represents the 'gold standard' for the preparation of SS or national standards (19). In turn, the SS serve as reference values for the production of WS or tertiary standards (12, 18), which should be incorporated in daily diagnostics to continuously assure the quality of the test results (8, 12).

Positive reference standards, primary or secondary and tertiary standards, should be selected from animals that exhibit a 'typical' humoral (antibody) response to the organism in question. Serum from naturally or experimentally infected animals is suitable as well as serum from animals that have been immunised by vaccination (19, 20). However, obtaining sera from animals that have sero-converted due to natural infection is extremely difficult for most former OIE List A diseases, especially in countries that have been disease-free for a long period of time. Furthermore, experimental infection is a more controlled method to elicit the humoral immune response in animals, and sera collected from animals that were naturally infected should only be used in exceptional circumstances (12). On the other hand, infecting animals experimentally requires expensive high-containment animal facilities (P3). Therefore, and because some of the primary reference sera supplied by the WRL for FMD originated from vaccinated animals (16, 17), this paper describes an experimental vaccination protocol for obtaining antibody positive sera to be used for the development of SS and WS for the FMD SPCE. Vaccination combines the controlled conditions of an experimental infection with a significant reduction in operating costs because no P3 animal facilities are required to conduct the experiments. However, proper authorisation must be given by the competent authorities.

The highly reproducible sigmoidal dose-response curve for the FMDV Asia 1 Shamir SPCE exhibited a pattern similar to that described for the indirect ELISA (12, 20). This demonstrates that the vaccination procedure outlined in this paper can be used to stimulate a humoral response in calves and, thus, obtain anti-FMDV antibody positive sera that reflect the characteristics of field samples collected from naturally infected animals.

However, before any attempt is undertaken to produce SP, WP, and C/O SS and/or WS from the sera, it should first be demonstrated that the assay method is appropriate for the intended use of the test. Owing to the availability of (candidate) primary reference sera (8, 11, 16, 17), resulting from several international collaborative studies sponsored by the FAO-EUFMD, different SPCE protocols can be calibrated against the standard method for FMD antibody detection described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (13). The data presented in this study clearly indicate that the in-house modified version of the SPCE has a similar analytical sensitivity to the standard method and can, therefore, successfully be used to qualify animals and animal products for international trade.

Having established a technically valid FMD SPCE, preparation of SS and WS is the next step in assuring continuous quality of the test results provided to trading partners. A model for the selection of reference standards based on dose-response curves for the indirect ELISA for bovine brucellosis has been previously described (20), and a similar approach should be (18) taken, as demonstrated in the present study, to select and define SS and WS for the competitive ELISA.

In the SPCE type Asia 1 Shamir, the candidate SS were prepared by direct comparison to the candidate primary reference standards used in the same assay (Table II). Since the results of the parametric bootstrap indicated no significant difference between the calculated PI of the chosen dilution for each candidate SS and the PI obtained for the corresponding candidate primary reference standard (although the value of the WP standard was close to the tail area), it was concluded that the SS were

homologous to the PS. The single-use aliquots were thus accepted as SS for the SPCE. It should be noted, however, that the primary C/O standard was not chosen according to the generally accepted definition (negative in 50% of the cases and positive in 50% of the cases). Rather, serum that had a doubtful or low positive FMD antibody status in the majority of laboratories was selected as the primary C/O standard (11). This view is supported by the authors as it is impossible to foresee whether or not the C/O serum will meet this criterion at the moment of test interpretation, and, therefore, it is impractical to maintain the 50%/50% definition when using an assay on a routine basis. Consequently, in this study, the serum that was expected to score borderline to low positive in approximately 97.5% of the cases (assuming a Gaussian distribution) was selected as the C/O SS.

To reduce the use of different standards (primary, secondary, and tertiary), and because the National Reference Laboratory is the only Reference Laboratory for FMD diagnosis in Belgium, the developed SS were also used as WS, with the noted exception of the SP standard. Although a SP reference serum should result in maximum inhibition (100%) of the competing antibody in the competitive ELISA (19), the in-house SP WS was slightly adjusted to fall within the linear portion of the dose-response curve, just below the plateau phase (19). This adjustment was done to minimise the amount of positive serum needed to prepare large batches of WS and justified based on the highly reproducible and comparable dose-response curves for both the competitive and indirect FMD ELISA.

While reference standards are used to evaluate test accuracy (19), carefully selected and defined WS, when used as an internal quality control (IQC) to continuously monitor the performance of an assay, can also provide data on the precision and repeatability of an assay. Mutual recognition of test results can only be achieved through sharing and transparency of IQC data. Therefore, based on the results of the present study, the authors promote not only the development of SS and WS by all Reference Laboratories, but also the use of the WS as an internal quality control.

Conclusions

Serum from susceptible animals immunised through vaccination can be used as a source of SP, WP, and C/O SS and WS. A typical sigmoidal dose-response curve produced with results from a well-calibrated test can be used for the selection of serum dilutions that mimic the characteristics of the (candidate) primary standards tested in parallel. Negative standards can be obtained from animals that have never been exposed to or have been vaccinated against the disease in question and whose sera exhibit neither cross-reacting nor non-specific results in the assay (19). Preparation of SS and WS, according to the method described in this paper, and incorporation of the WS in daily diagnostic runs will improve the reliability of the accuracy and diagnostic sensitivity range of the assay. In addition, the authors promote the use of the same WS for IQC procedures. Precision and repeatability over time can be estimated through the use of control charts (7). Transparency between laboratories regarding the accuracy, precision, and reproducibility of data will help to improve assay standardisation and will eventually lead to mutual recognition of test results between trading partners.

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L'assurance qualité et le contrôle de la qualité appliqués à l'épreuve immuno-enzymatique de compétition en phase solide pour le diagnostic de la fièvre aphteuse – I. Assurance qualité : mise au point d'étalons secondaires et de travail

N. Goris & K. De Clercq

Résumé
Les échanges internationaux d'animaux et de produits d'origine animale ont incité les organisations telles que l'Organisation mondiale de la santé animale (OIE) à élaborer des normes visant à réglementer et à faciliter le commerce international entre Pays membres. Toutefois, face à la croissance ininterrompue du marché mondial, il convient d'avancer dans le domaine de la normalisation et de l'harmonisation des épreuves de diagnostic basées sur la détection d'anticorps vis-à-vis des maladies infectieuses, notamment en ce qui a trait à la mise au point et à l'utilisation des matériels de référence. S'agissant des maladies à déclaration obligatoire figurant sur la liste de l'OIE pour lesquelles des étalons de référence primaires ou internationaux sont disponibles ou en cours d'élaboration, il est recommandé aux Laboratoires de référence régionaux ou nationaux de procéder à la mise au point de leurs propres étalons secondaires ou de travail. Les auteurs décrivent l'élaboration d'étalons de référence pour l'épreuve immuno-enzymatique de compétition en phase solide pour le diagnostic de la fièvre aphteuse à partir de sérums positifs issus de veaux vaccinés contre la fièvre aphteuse. La procédure décrite dans ce document est aisément transposable à d'autres épreuves sérologiques du même type, et devrait permettre d'avancer vers l'harmonisation des résultats des épreuves et des tests de diagnostic.

Mots-clés
Accréditation – Assurance qualité – Détection d'anticorps – Épreuve immuno-enzymatique – Étalon de référence primaire – Étalon de travail – Étalon secondaire – Fièvre aphteuse – Normalisation – Validation.

Garantía y control de calidad del ensayo inmunoenzimático de competición en fase sólida para la fiebre aftosa. Parte I. Garantía de calidad: elaboración de patrones secundarios y de trabajo

N. Goris & K. De Clercq

Resumen
Ante el creciente movimiento internacional de animales y productos de origen animal, instancias como la Organización Mundial de Sanidad Animal (OIE) han respondido sin tardanza elaborando directrices que reglamenten y faciliten el comercio entre los Países Miembros. No obstante, a medida que el mercado planetario se desarrolla más y más, se hace necesario un mayor grado de normalización y armonización de las pruebas de detección de anticuerpos, sobre todo en lo tocante a la elaboración y utilización de productos de

referencia. En el caso de enfermedades de notificación obligatoria a la OIE para las que existan o estén en vías de elaboración patrones primarios o de referencia internacional, se alienta a los laboratorios de referencia nacional o regional a elaborar sus propios patrones secundarios y/o de trabajo. Los autores describen la creación de patrones para el ELISA de competición en fase sólida que se aplica a la fiebre aftosa, con utilización de suero positivo procedente de terneras vacunadas contra la enfermedad. El procedimiento aquí descrito puede extrapolarse fácilmente a otros ensayos serológicos similares, y debería inducir un mayor grado de armonización internacional de estos ensayos y de la interpretación de sus resultados.

Palabras clave
Acreditación – Detección de anticuerpos – Ensayo inmunoenzimático – Estandarización – Fiebre aftosa – Garantía de calidad – Patrón de referencia primario – Patrón secundario – Patrón de trabajo – Validación.

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