Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis

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Summary: Enzyme-linked immunosorbent assay (ELISA) techniques for the detection of antibodies are now widely used throughout the world for the diagnosis of infectious diseases in veterinary medicine. Although many laboratories have independently developed ELISA techniques for their own purposes, little progress has been made with respect to the international standardisation and validation of these techniques. This lack of international conformity is of major concern to organisations such as the Office International des Epizooties (OIE), the United Nations Food and Agriculture Organisation (FAO), the World Health Organisation (WHO) and the International Atomic Energy Agency (IAEA) which are involved in the establishment of international guidelines and programmes for the control, surveillance and/or eradication of infectious diseases. In this regard, a Joint FAO/IAEA Meeting of Consultants was convened in Vienna in January 1992 to review aspects of ELISA data expression, primary reference standards, quality assurance and diagnostic validation. Based on the consensus derived from this meeting, the authors describe procedures which are recommended as a platform on which to build definitive guidelines for international standardisation of ELISA protocols and reagents, in cooperation with the OIE and the OIE Reference Laboratories.


INTRODUCTION

The definitive diagnosis of infectious disease has traditionally been accomplished through the direct demonstration and identification of the causative agent(s) by culture and isolation procedures. Unfortunately, this may be beyond the expertise and capabilities of many diagnostic laboratories, particularly in developing countries. However, accurate diagnosis can be achieved when serological techniques are used in combination with clinical observation and epidemiological history.

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Although classical serological techniques (i.e. agglutination, precipitation, complement fixation and virus neutralisation tests) have proved useful, they suffer from a number of drawbacks. In general terms, these drawbacks relate to a combination of inadequate diagnostic performance, lack of standardisation and/or poor cost efficiency. Because enzyme-linked immunosorbent assay (ELISA) techniques potentially resolve all of these problems, a great deal of emphasis has been placed on research, development and application of these techniques in the diagnosis of infectious diseases of veterinary importance.

The development of ELISA techniques for the diagnosis of infectious diseases and the transfer of these techniques to counterpart laboratories in developing countries is part of the work within the Joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) Division's Programme of Nuclear Techniques in Food and Agriculture. The animal health component of the programme involves close co-operation between two groups within the IAEA. The Animal Production and Health Section of the Joint FAO/IAEA Division is responsible for the establishment of scientific and technological expertise in counterpart laboratories so that researchers in these countries may identify and resolve problems related to infectious diseases in domestic livestock. The Animal Production Unit of the Agency's Laboratory, Seibersdorf, provides technical support to these activities through the development and provision of standardised and quality-assured ELISA kits for some of the major epizootic diseases of veterinary importance. These kits are used in epidemiological studies, in vaccination monitoring programmes and in control and eradication programmes in over seventy-five counterpart laboratories in Africa, Asia and Latin America.

Through collaboration with more than fifteen of the major veterinary research laboratories throughout the world, ELISA techniques and reagents have been adapted to a kit format which is suitable for distribution to and use in counterpart laboratories. Diagnostic kits have been developed for rinderpest, brucellosis, trypanosomiasis, babesiosis, foot and mouth disease, infectious bovine rhinotracheitis and haemorrhagic septicemia; while various stages of planning and development have been reached for kits for bluetongue, bovine leukosis, contagious bovine pleuropneumonia, Aujeszky's disease and Newcastle disease. The development of kits on such a large scale for international use requires a high degree of standardisation of both test procedures and reagents.

A Joint FAO/IAEA Meeting of Consultants was convened in Vienna in January 1992 to review aspects of ELISA data expression, primary reference standards, quality assurance and diagnostic validation. Experts attending this meeting included Dr S. Edwards (Central Veterinary Laboratory, Weybridge, United Kingdom, and Member of the Office International des Epizooties [OIE] Standards Commission), Dr R.J. Jacobson (New York State College of Veterinary Medicine, Cornell University, Ithaca, New York) and Dr T. Spencer (Regional Veterinary Laboratory, Benalla, Australia). The following summarises the consensus of opinions reached at this meeting with regard to diagnostic ELISA techniques for the detection of antibody.

**DATA EXPRESSION**

The vast majority of diagnostic ELISA techniques utilise a chromogenic enzyme/substrate system. When read photometrically, the optical density (OD) or absorbance values are the raw data generated in the test system. Several methods have
been employed for the expression of antibody activity but no single method has been universally adopted.

**Indirect ELISA techniques**

In the indirect ELISA, there is a positive relationship between the intensity of colour and the amount of antibody bound in the test system. A number of qualitative and quantitative methods have been applied to the expression of antibody activity in the indirect ELISA and these have been compared in a number of reviews (4, 8). The methods commented on below represent those most frequently used in the veterinary diagnostic laboratory.

- **a) Raw OD values**
  
  This is the simplest form of data expression, often written as a decimal or multiplied by 1,000 to give a whole number. Raw OD values are of little diagnostic use without an in-depth knowledge of assay performance, nor are these values useful for intra- or inter-laboratory comparisons.

- **b) Corrected OD values**
  
  These values are commonly used in virological systems where the production of pure antigen can be technically difficult. The subtraction of a control antigen OD value (to give the “corrected” OD value) compensates for binding of antibody to host cell components in the viral antigen preparation and gives a more uniform base line to the assay. Corrected OD values suffer from the same drawbacks as raw OD values.

- **c) End-point titration**
  
  This approach is derived from other classical serological assays. The end-point titre is expressed as the reciprocal of the highest serial dilution which demonstrates a minimum of antibody activity. Although this method has the advantage of familiarity, it has no diagnostic advantage over single dilution assays, except in cases where more quantitative data are required.

- **d) Standard curve**
  
  The OD of each sample tested at a single dilution is read against a standard curve to obtain an estimated end-point titre. This method assumes a parallel dose/response curve for all samples. The standard curve must be established for every run of the assay. For inter-laboratory comparison, the same set of standards must be available to and used by all laboratories.

- **e) Normalised OD values**
  
  With the inclusion of a defined positive reference standard in the test, a correction factor is determined on the basis of “expected” versus “observed” OD values for this reference at a single dilution. All sample OD values for a given run are normalised with this correction factor. Diagnostic interpretation using normalised OD values suffers from the same drawbacks as using raw OD values.

- **f) Signal to noise ratio**
  
  Also referred to as positive:negative ratio, the OD for the test sample is expressed as a ratio relative to a negative reference standard. This method assumes that the negative reference standard is truly representative of the normal population.
g) Percent positivity

The OD of each sample tested is expressed as a percentage of a high positive reference standard. This relative measurement, based on the inclusion of a single positive reference standard, results in a uniform and continuous scale of 0-100 percent positivity (PP) for all indirect ELISAs.

Consensus on indirect ELISA data expression

The following points were agreed by participants at the Meeting of Consultants:

- Under most circumstances, quantitative data are not required for diagnostic purposes.
- Raw, corrected or normalised OD values are absolute measurements which are influenced by test parameters and photometric instrumentation, and cannot be used for inter-laboratory standardisation.
- End-point titrations are labour-intensive, costly and impractical for most routine diagnostic purposes.
- Signal to noise ratios assume that all normal populations generate a uniform level of background activity (noise) and therefore this method cannot be used for inter-laboratory standardisation.
- When samples are tested at a single dilution, indirect ELISA techniques provide a semi-quantitative measure of antibody activity.
- Single dilution estimates of titre from standard curves, although quantitative, assume parallelism in dose/response for all test samples and can be misleading.
- Percent positivity relative to a reference standard, although semi-quantitative, has a number of distinct advantages: it requires only a single dilution, is expressed on a continuous scale of 0-100 PP and does not assume parallelism or uniform background activity. It may therefore be used for inter-laboratory standardisation.
- If more quantitative data are required, an algorithm may be established to convert PP values to units which are directly proportional to antibody activity.

Competitive ELISA techniques

In the competitive ELISA, there is a negative relationship between the intensity of colour and the amount of test sample antibody bound in the test system. The results are expressed relative to the OD of a standard competing antibody. Some laboratories express results as a percentage of the OD of this standard (7). Percent inhibition (PI) of the OD produced by the standard competing antibody is more widely used (1). Quantitative expression of antibody activity is difficult from a single dilution because the dose/response curve is so steep. Hence, development of consistent standard curves for these assays is extremely difficult.

Consensus on competitive ELISA data expression

The following points were agreed at the Meeting:

- Expressing data as a percentage of the OD of the competing antibody results in a scale which is inversely proportional to antibody activity in the test sample and this may cause confusion.
- Data expressed as PI of the competing antibody results in a continuous scale of 0-100 PI, which is directly proportional to antibody activity.
PRIMAR Y REFERENCE STANDARDS

Reference standards are necessary to ensure that a given ELISA technique is capable of measuring antibody activity to a specified level of diagnostic sensitivity. Irrespective of the type of ELISA system used, this measurement is a function of both antibody concentration and avidity (2). Avidity is the overall binding strength of all the antibodies in the test sample. In the establishment of reference standards for any ELISA, consideration must be given to the selection of standards which represent, on average, what would be expected of an immune response to the organism in question. Consideration must be given to antibody isotypes, concentration and avidity, as well as to antigenic specificity.

Primary reference standards represent the "gold standard". All other standards are compared and calibrated against the primary reference. These other standards may include national (secondary) standards or working (tertiary) standards which are in routine diagnostic use (5).

Consensus on primary reference standards

The following points were agreed at the Meeting:

- For each ELISA, three primary reference standards should be established: a strong positive, a weak positive and a negative standard. These standards should be characterised using standard reference protocols for reagent production and assay procedures.

- Positive reference standards should be selected from animals which exhibit a typical humoral (antibody) immune response to the organism in question. The immune response may be elicited by experimental infection or by immunisation with vaccines, preferably in specific pathogen-free animals. The positive standard may be derived from a single serum or a pool of sera.

- Negative reference standards should be selected from animals which have never been exposed to or vaccinated against the organism in question. There should be no evidence of cross-reacting antibodies or other non-specific factors which interfere with the interpretation of assay results. The negative standard may be derived from a single serum or a pool of sera.

- The reactivity of the standards should be stable upon freeze-drying and reconstitution. The standard should be free from any infectious materials.

- A primary reference standard should not require any special predilution by the recipient laboratory prior to use in the ELISA. The standard should be tested in the same way as any field sample under routine diagnostic conditions; this prevents the introduction of bias related to special handling or preparation. The amount of antibody activity in a positive reference standard should be within the accurate detection limits of the ELISA.

- In preparing positive (strong or weak) reference standards, it may be necessary to make a single dilution of the positive stock in the negative reference serum in order to achieve a defined reactivity which is within the accurate detection limits of the ELISA.

- In the indirect ELISA, the strong positive reference standard should exhibit an antibody activity which lies on the linear portion of the dose/response curve, just below the plateau phase (Fig. 1).
Reciprocal of dilution (in negative reference serum)

FIG. 1

Selection of positive reference standards for the indirect enzyme-linked immunosorbent assay (ELISA)

Hypothetical indirect ELISA titration curve of a "typical" serum from an infected animal. C++ represents the dilution (in negative reference serum) which would be chosen to prepare the strong positive reference standard, representing 100 percent positivity (PP). C+ represents the dilution (in negative reference serum) which would be chosen to prepare the weak positive reference standard. In this example, the weak positive (about 50 PP) should consistently demonstrate antibody activity which is greater than the established positive/negative threshold of 20 PP. The negative reference serum used as diluent should consistently demonstrate activity below this threshold.

- In the competitive ELISA, the strong positive reference standard should exhibit sufficient antibody activity to consistently result in maximum inhibition of the standard competing antibody (Fig. 2).

- For both indirect and competitive ELISAs, the weak positive reference standard should exhibit an antibody activity which lies on the linear portion of the dose/response curve above the positive/negative threshold (Figs 1 and 2).

- The negative reference standard should exhibit an activity which is below the positive/negative threshold.

QUALITY ASSURANCE

Quality assurance (QA) encompasses all the check and control activities necessary to ensure that the results of a given ELISA are accurate, precise and reproducible (3, 9). Quality control (QC) refers to defined activities within the context of QA. Internal QC...
Selection of positive reference standards for the competitive enzyme-linked immunosorbent assay (ELISA)

Hypothetical competitive ELISA titration curve of a “typical” serum from an infected animal. C+ represents the dilution (in negative reference serum) which would be chosen to prepare the strong positive reference standard, representing 100 percent inhibition (PI). C+ represents the dilution (in negative reference serum) which would be chosen to prepare the weak positive reference standard. In this example, the dilution of the weak positive (about 75 PI) should consistently demonstrate antibody activity which is greater than the established positive/negative threshold of 50 PI. The negative reference serum used as diluent should consistently demonstrate minimal inhibition of the competing antibody.

One aspect of internal QC involves the monitoring of variables which, if not controlled, will adversely affect assay performance. Good laboratory practice (proper preparation and use of reagents, strict attention to physical/chemical parameters, maintenance and calibration of instruments, etc.) will address the majority of these variables.

A second aspect of internal QC involves the use of control samples. In the case of an ELISA for the detection of antibody, a set of control samples is used which represent defined activities within the assay system. Included in every run, they are used to determine whether an assay is operating within acceptable limits and to monitor variability within and between assays.

While control sera are used to monitor precision and reproducibility, it should not be inferred that they constitute a measure of accuracy. For the latter measure, reference...
standards must be used in the assay. Reference or working (tertiary) standards may also function as controls for internal QC but not vice versa. When used for dual purposes in an assay, “standard” and “control” are functional terms applied to the same reference material.

**Indirect ELISA techniques**

When using PP as the method of data expression, the strong positive control (reference standard) represents 100 PP and is used to define the antibody activity of the other controls and all test samples. All OD values are mathematically expressed as a percentage of the strong positive control standard. The activities of the weak positive and negative controls (reference standards) are measures of assay accuracy relative to the strong positive control.

**Consensus on indirect ELISA internal quality control**

The following points were agreed at the Meeting:

- Four replicates of strong and weak positive and negative controls should be included on each ELISA plate. These controls should be treated in the same way as any test sample with respect to dilution and test procedures.

- In addition to these three controls, four replicates of a conjugate control should be included on every plate. This control is used to monitor background activity in the system in the absence of internal controls or test samples (when diluent buffer alone is present).

- In the first step of assay acceptance, the two intermediate raw OD values of the strong positive control replicates must fall within an acceptable operating range. If not, the assay (ELISA plate) is rejected as out of control, compromising the ability of the test to discriminate between positive and negative samples.

- All control and sample data (raw OD values) from an accepted ELISA plate should be expressed as PP relative to the average of the intermediate OD values (median OD value) of the strong positive control.

- Upper and lower control limits (UCL and LCLs) for PP values of all controls should be statistically established by repeated testing under routine diagnostic conditions.

- In the second step of assay acceptance, at least three of the four replicate PP values for all of the controls must fall within the respective UCL and LCL. If not, the corresponding ELISA plate is rejected.

**Competitive ELISA techniques**

When using PI as the method of data expression, the standard competing antibody, by definition, represents zero PI and all control and test sample OD values are mathematically expressed as PI of the standard competing antibody.

**Consensus on competitive ELISA internal quality control**

The following points were agreed at the Meeting:

- Four replicates of the standard competing antibody should be included on each ELISA plate. This competing antibody control should not be influenced by any extraneous factors related to other internal controls or test samples.
Four replicates of the strong positive, weak positive and negative controls should be included on each ELISA plate. These controls should be treated in the same way as any test sample with respect to dilution and test procedures.

In addition to these four controls, a conjugate control should be included on every plate if the standard competing antibody is not itself conjugated with an enzyme. This control is used to monitor background activity in the system in the absence of competing antibody and internal controls or test samples (when diluent buffer alone is present).

In the first step of assay acceptance, the two intermediate raw OD values of the competing antibody control replicates must fall within an acceptable operating range. If not, the assay (ELISA plate) is rejected as out of control, compromising the ability of the test to discriminate between positive and negative samples.

All control and sample data (raw OD values) from an accepted ELISA plate should be expressed as PI relative to the average of the intermediate OD values (median OD value) of the competing antibody control.

UCL and LCLs for PI values of all controls should be statistically established by repeated testing under routine diagnostic conditions.

In the second step of assay acceptance, at least three of the four replicate PI values for all of the controls must fall between the respective UCL and LCL. If not, the corresponding ELISA plate is rejected.

Test sample acceptance

If an indirect or competitive ELISA plate meets all of the acceptance criteria outlined above, it must still be determined whether or not individual test serum results are acceptable. Confidence in results increases with the number of replicates which are tested. The acceptable limits of variability between replicates will depend on the precision required of the assay.

Consensus on test sample acceptance

The following points were agreed at the Meeting:

- In general, two replicates of a test sample should be adequate for interpretation in diagnostic assays.

- For indirect and competitive ELISAs, acceptance criteria may be defined relative to the “cut-off” value between positive and negative (see below). Both replicate values for any test sample should fall either above or below the cut-off in order to be accepted. If the replicate values fall on both sides of the cut-off, the sample should be retested.

DIAGNOSTIC VALIDATION

As mentioned in the “Introduction”, most ELISA techniques have been developed to counteract drawbacks in the use of classical serological tests which may be currently prescribed. However, in order to replace an existing test, the new technique must be validated and shown to be equal or superior in terms of diagnostic performance.
Cut-off determination

Central to any serological assay is the determination of the diagnostic threshold or "cut-off" (8). The cut-off is defined as the level of antibody activity which determines negative or positive reactor status for a given animal. The selection of a cut-off value for a given test will depend on the intended application, as the selected cut-off will determine the diagnostic sensitivity and specificity and the predictive value of the test (6). The most appropriate method for determining a diagnostic cut-off is to test samples from known negative and known positive animals.

Consensus on cut-off determination

The following points were agreed at the Meeting:

- The herd/flock from which samples from negative animals are derived should have no epidemiological, clinical or other serological evidence of the disease in question. No new animals should be introduced into the herd/flock during the period under study.

- Ideally, this herd/flock should not have been vaccinated against the disease in question, but it is recognised that this may not be possible or desirable in areas where vaccination of all animals is a routine or mandatory practice. Under these circumstances, it will be necessary to select animals of a certain age post-vaccination.

- The herd/flock from which positive samples are derived should demonstrate epidemiological, clinical and/or other serological evidence of the disease in question.

- Ideally, a procedure capable of definitive diagnosis (e.g. bacterial culture) should yield at least one positive result from an animal in this herd/flock.

- Experimentally-infected or recently immunised animals may also be used as a source of positive samples.

- Sequential sampling of potentially positive animals should yield at least one positive test result in a standard serological test other than the ELISA, in order to provide confidence that a given animal has seroconverted.

- The numbers of both negative and positive samples to be tested should be statistically relevant to the anticipated diagnostic sensitivity and specificity of the assay. A sufficient number of samples should be tested to provide a 95% confidence in the estimate of the diagnostic sensitivity and specificity.

- After testing a statistically relevant number of both negative and positive samples, a graph of the frequency distribution should be plotted to demonstrate the modality of both groups. The cut-off should be defined on the basis of the acceptable percentage of false-positive and false-negative results (diagnostic sensitivity and specificity) for a given application (Figs 3 and 4).

- It is recognised that genetic and/or other differences related to different regions or countries may affect the frequency distribution of the negative group in terms of position and/or profile. For purposes other than international trade, such as regional or national programmes for the control, surveillance or eradication of animal disease, the cut-off may need to be refined.
Selection of the positive/negative threshold in the indirect enzyme-linked immunosorbent assay (ELISA)

Hypothetical frequency distribution of samples from healthy and infected animal groups as tested in the indirect ELISA. The positive/negative threshold (cut-off) should be chosen on the basis of the percentage of false-positive and false-negative results which are acceptable for a given diagnostic application. In this example, at a threshold of 20 percent positivity (PP) the diagnostic sensitivity of this assay would be greater than the diagnostic specificity.

Selection of the positive/negative threshold in the competitive enzyme-linked immunosorbent assay (ELISA)

Hypothetical frequency distribution of samples from healthy and infected animal groups as tested in the competitive ELISA. The positive/negative threshold (cut-off) should be chosen on the basis of the percentage of false-positive and false-negative results which are acceptable for a given diagnostic application. In this example, at a threshold of 50 percent inhibition (PI) the diagnostic sensitivity and specificity of this assay would be equal (theoretically 100%).
Validation

Validation refers to comparison of the diagnostic performance of a new assay with the tests in current use. Diagnostic sensitivity and specificity may be used for such comparisons. It is important that all results be derived from the same set of defined samples (10). The test samples should not be chosen using any of the tests involved in the comparison, nor should test samples be chosen from herds/flocks from which animals have been recently culled based on any test in the comparison. Such practices would create a bias in favour of the performance of the tests currently in use, especially when comparing diagnostic specificities. Caution is advised in the comparison of diagnostic sensitivities when using positive samples derived from naturally-infected animals. By the time clinical signs appear, or when there are sufficient numbers of organisms for reliable isolation, the immune response will already be well-established and estimates of diagnostic sensitivity will be biased and may even reach 100% for all assays in the comparison.

Consensus on validation

The following points were agreed at the Meeting:

- The same negative and positive test samples used to determine the ELISA cut-off may be used to compare the diagnostic sensitivity and specificity of the ELISA with other assays, provided that the other assays are not used in the selection of individual samples.

- In some cases, it may be appropriate to compare diagnostic specificities in both vaccinated and non-vaccinated groups.

- Comparisons of diagnostic sensitivity using samples from experimentally-infected or vaccinated animals are preferable to comparisons using samples from naturally-infected animals. This relates to the ability to define exposure or infection under controlled conditions.

- In cases where diagnostic sensitivities cannot be compared under the conditions outlined above, relative sensitivities may be compared using samples from infected herds/flocks. Selection of infected herds/flocks may be based on epidemiological, clinical and serological evidence, but samples from all animals in the herd/flock should be used in the comparison.

- If validation is to be performed in more than one laboratory, each laboratory must use the same assay reagents and protocol for the new assay, and the same selection criteria for test samples should be applied. Alternatively, samples may be exchanged between laboratories.

- Where serological cross-reactions between organisms are known to occur, evidence should be provided to indicate the extent to which the new technique is prone to such false-positive reactions.

ADOPTED PROCEDURES

All participants in this Joint FAO/IAEA Meeting of Consultants agreed on the need to define uniform guidelines for the standardisation and validation of ELISA techniques for the detection of antibody in infectious disease diagnosis. Although a
universally-accepted consensus may be impossible, the opinions expressed at the Meeting were formulated on the basis of professional experience and an in-depth knowledge of the literature. An international perspective was maintained throughout the Meeting.

Based on the consensus of opinions from this Meeting, the following procedures related to the standardisation and validation of diagnostic ELISA techniques for antibody detection have been incorporated into the development and use of Joint FAO/IAEA ELISA kits:

- Data in indirect ELISAs are expressed as PP.
- Data in competitive ELISAs are expressed as PI.
- ELISA protocols and reference standards have been developed in co-operation with collaborating laboratories, as no uniformity of protocols or primary reference standards exists at present.
  - Strong positive, weak positive and negative reference standards (controls) are included on every ELISA plate, as well as a conjugate control, for both types of ELISA.
  - In indirect ELISAs, the strong positive control represents 100 PP.
  - In competitive ELISAs, the standard competing antibody control is reacted in diluent buffer only and represents 0 PI.
- Internal QC procedures have been incorporated into ELISA protocols and are supported by custom software programmes.
- Guidelines for cut-off determination and validation under specific local conditions and for specific applications have been incorporated into ELISA protocols.

INTERNATIONAL GUIDELINES

ELISA techniques for the detection of antibody are now widely used throughout the world for infectious disease diagnosis in veterinary medicine. Many laboratories have independently developed ELISA techniques for their own purposes. However, little progress has been made with respect to the international standardisation and validation of ELISA techniques. This lack of international conformity is of major concern to organisations such as the OIE, FAO, World Health Organisation (WHO) and IAEA which are involved in the establishment of international guidelines and programmes for the control, surveillance and/or eradication of infectious diseases.

The major emphasis of the Joint FAO/IAEA Programme in animal health is the development of ELISA techniques and the transfer of these techniques to counterpart laboratories throughout the developing world. Because of the international nature of this programme, it is essential that these ELISA kits and protocols represent an acceptable standard in terms of quality and performance. To achieve an internationally-acceptable standard, links have been established between the IAEA programme and those of the FAO, WHO and OIE. Because of the importance of international standardisation, the FAO/IAEA Central Laboratory for ELISA and Molecular Techniques in the Diagnosis of Animal Diseases has been formally established at the Agency’s Laboratory, Seibersdorf, Austria. The OIE has also officially designated this
laboratory as an OIE Collaborating Centre for ELISA and Molecular Techniques in the Diagnosis of Animal Diseases. In collaboration with the OIE and the OIE Reference Laboratories, work will commence on the development of definitive guidelines for the international standardisation of ELISA protocols and reagents. Based on the consensus derived from the Meeting of Consultants, the procedures described in the present article are recommended as a platform on which to build these definitive guidelines.
muchos laboratorios desarrollaron independientemente pruebas ELISA para su uso propio, no se ha progresado demasiado en cuanto a la estandarización y validación de estas técnicas. La falta de criterios internacionales al respecto se cuenta entre los temas que preocupan a organismos internacionales tales como la Oficina internacional de epizootias (OIE), la Organización de las Naciones Unidas para la alimentación y la agricultura (FAO), la Organización mundial de la salud (OMS) y el Organismo internacional de energía atómica (OIEA). En efecto, estos organismos se ocupan de establecer directivas y programas internacionales de control, vigilancia y/o erradicación de enfermedades contagiosas. Una reunión de consulta conjunta FAO/OIEA se celebró en este sentido en la ciudad de Viena (Austria) en enero de 1992, donde se trataron los asuntos siguientes: formulación de los datos de las pruebas ELISA, principales estándares de referencia, garantías de calidad y validación de los diagnósticos. A partir del consenso que pudo establecerse en esta reunión, los autores describen los procedimientos recomendados como plataforma para la elaboración de directivas que puedan considerarse definitivas sobre la estandarización internacional de los protocolos y los reactivos biológicos ELISA, en cooperación con la OIE y sus laboratorios de referencia.


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REFERENCES


