Critical factors affecting the diagnostic reliability of enzyme-linked immunosorbent assay formats

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Summary
This paper aims to evaluate different formats of the enzyme-linked immunosorbent assays (ELISAs) for detection of virus-specific antibodies and focuses on factors that may influence the diagnostic reliability of such tests. Newly developed and well-established ELISAs for detection of infections of bovine herpesvirus 1 (BHV1), bovine respiratory syncytial virus (BRSV), classical swine fever virus (CSFV), Pseudorabies virus (PRV) and bovine viral diarrhoea virus (BVDV) are used as examples. Differences between competitive and non-competitive ELISAs are described, with special reference to the influence of the antigen, the conjugated antibody and the test sample on the test results. Attention is drawn to interference, which may result in false positive or false negative test results, with special emphasis on the 'bridging' phenomenon. The use of monoclonal antibodies and discriminatory tests are briefly discussed. Diagnostic reliability is described for tests that are used in monitoring or eradication programmes, emphasising the consequences of false negative and false positive test results. Finally, reducing assay-time and functional quality control for such tests are discussed.

Keywords

Introduction
In veterinary diagnostic laboratories, the use of enzyme-linked immunosorbent assays (ELISAs) is routine. The careful validation of each new ELISA to determine its usefulness for the intended goal in the target animal population (clinical diagnosis, screening, confirmation, etc.) is extremely important. The regular outbreaks of economically important and highly contagious diseases such as classical swine fever (CSF) (hog cholera) and foot and mouth disease (FMD) within and outside the European borders, the increase in international trade of animals and the subsequent increase in (inter)national control, surveillance and eradication programmes require the use of tests that are well validated, easy to perform, of high quality and internationally accepted. Likewise, trade barriers for viruses that are enzootic in many countries, such as bovine herpesvirus 1 (BHV1) and Aujeszky's disease virus (pseudorabies virus: PRV), stimulate standardisation of ELISAs and internal and external quality control (18). For many diseases, the Office International des Epizooties (OIE) has set up minimal standards to which diagnostic tests must conform (30).

This paper aims to evaluate different formats of ELISAs for antibody detection of viruses and focuses on factors that influence the diagnostic reliability of such tests. Newly developed and well-established ELISAs for detection of infections of BHV1, bovine respiratory syncytial virus (BRSV), CSF virus (CSFV), PRV and bovine viral diarrhoea virus (BVDV) are used as examples.
Test characteristics

Test formats: non-competitive and competitive or blocking enzyme-linked immunosorbent assays

Antibody-detecting ELISAs are essentially based on two formats: non-competitive ELISAs and competitive or blocking ELISAs (2, 9, 10). In a non-competitive ELISA, specific antibodies in the test sample that have been bound to the immobilised antigen are detected by enzyme-conjugated immunoglobulin-specific antibodies (Fig. 1a). Consequently, the amount of antibody bound is directly proportional to the enzyme-mediated colour development.

In a competitive or blocking ELISA, the degree to which specific antibodies in the test sample prevent binding of an agent-specific enzyme-conjugated antibody is measured. In a competitive ELISA, the test samples are mixed with conjugated virus-specific antibodies, and this mixture is then added to the antigen. Then competition for binding sites occurs between antibodies in the test sample and the conjugated antibodies. In a blocking ELISA, the test sample is first added to the antigen, and the conjugated antibodies are added subsequently, after a washing step. Thus, when antibodies in the test sample have occupied the binding sites on the antigen, the binding of the conjugated antibodies will be blocked. For competitive and blocking ELISAs, the amount

ELISA: enzyme-linked immunosorbent assay

Fig. 1
Schematic representation of non-competitive and competitive or blocking enzyme-linked immunosorbent assays (ELISA), and of the relationship between the antibody concentration (Ab) and the optical density (OD) of an ELISA
of antibody in the test sample that is bound to the antigen is inversely proportional to the intensity of the colour development (Fig. 1b).

The antigen
For both non-competitive and competitive/blocking ELISAs, the way the antigen is presented is important. The antigen can either be bound directly to the wells of microtitre plates, or may be captured by antigen-specific antibodies immobilised on the plate. An advantage of the use of capturing antibodies is that the antigen is selectively captured and therefore need not be purified. Thus, crude virus preparations obtained from infected cell cultures can be used in this type of ELISA. Capturing antibodies can also be used successfully in non-competitive, (sub)class-specific ELISAs, which is of particular importance for detection of virus-specific IgM, IgA or IgG subclass antibodies. Use of (sub)class-specific capture antibodies can prevent the interference of specific antibodies of other classes that in most cases are present in excess in post-infection sera (9, 24, 41).

Detection of specific antibodies of a particular (sub)class may be useful in cases in which it is important to estimate the moment of infection of a herd. It is generally known that after primary infection, specific IgM or IgA antibodies are detected earlier than IgG antibodies. Thus, when the presence of specific IgM, IgA and IgG antibodies are determined in a statistically relevant number of animals in a herd, this may help to assess the spread of the infection in the herd and to estimate the moment of introduction of the virus into the herd. This is of particular importance when dealing with viruses, such as swine vesicular disease virus or low-virulence CSFV strains, that may cause few or no clinical symptoms and may therefore circulate on a farm unrecognised for some time. When dealing with such viruses, ELISAs detecting a specific (sub)class of antibodies may help to define the role of particular herds in spreading the infection to neighbouring farms. (Sub)class-specific ELISAs have been developed for BHV1 (24) and BRSV (47), but may be useful for other viruses as well.

The conjugated antibody
The format of an ELISA may be largely determined by the availability of specific conjugated antibodies. Competitive or blocking ELISAs can be developed when antigen-specific (monoclonal) antibodies are available. An advantage of using antigen-specific conjugated antibodies in a competitive or blocking ELISA may be that these tests can be used for detection of antibodies of various species by using the same antigen-specific conjugated antibody. A recently developed competitive panpestivirus ELISA is able to determine antibodies against pestiviruses in pigs, cattle and sheep using the same combination of pestivirus antigen and virus-specific monoclonal antibodies (19). In non-competitive ELISAs, species-specific conjugated antibodies are generally used that are directed against the immunoglobulins of a particular species, and thus the same conjugated antibody may be used in different non-competitive ELISAs within the same species. Conjugated antibodies directed against immunoglobulins of many different species are commercially available.

The test sample
An advantage of competitive and blocking ELISAs is that, in comparison with non-competitive ELISAs, low starting dilutions of test samples (sometimes even undiluted samples) are possible, because interference due to antibody binding (other than binding to the binding site of the conjugated antibody) generally does not bias the test result. For example, a recombinant BVDV-protein which is expressed in Escherichia coli as a fusion protein with β-galactosidase induced high background levels in a non-competitive ELISA, probably due to antibodies in the test sample against β-galactosidase. This problem was circumvented by using the same antigen in a competitive ELISA format (22). As a result of aspecific binding, high starting dilutions of test samples are frequently necessary in non-competitive ELISAs. For instance, in a non-competitive ELISA that detects antibodies against BRSV, in addition to the use of a control well for each test sample the starting sample dilution is as high as 1:80 (46).

The choice of the test sample may also greatly affect the diagnostic reliability of an ELISA. Milk instead of serum is increasingly used in sanitary control programmes, because samples can be obtained quickly and cheaply (11, 13, 14, 32). Although the amount of immunoglobulins in bovine milk may be 10- to 30-fold less than in serum (7), highly sensitive non-competitive and competitive ELISAs have been developed for diagnosis of BHV1 infections using milk samples (44). When bulk milk in which individual antibody-positive samples may be mixed with individual antibody-negative milk samples of herdmates is to be tested, the test must be very sensitive to detect the infection early during the transmission of the virus through the herd. It has been demonstrated that at least 10%-15% of the animals in a herd must be infected with BHV1 before the test result of the bulk milk sample will respond positively (13, 43). It is not possible to generalise about the usefulness of specific test formats for testing milk samples. Each test should be well validated for its intended use with (bulk)milk samples from the target population. Also, the internal and external quality control should be performed using milk samples from the target population.

Interference
Interference, including aspecific binding, refers to undesired antibody activity in the test leading to incorrect (false positive or false negative) results (15, 27). There are multiple sources of interference. In non-competitive ELISAs, aspecific binding of antibodies is a common problem, because all antibodies that are bound in the well, either specifically or aspecifically, will be detected by the conjugated antibody. Therefore, a disadvantage of many non-competitive ELISAs is that samples can only be tested when diluted, which reduces the detection level of the test which, as a consequence, may affect its
sensitivity. In addition, in many non-competitive ELISAs a control well in which the antigen is omitted must be included with each test sample, so that each individual test result can be corrected for an aspecific (‘background’) signal. Conversely, the ability of the non-competitive ELISAs to detect low concentrations of specific antibodies at a given sample dilution is generally considered to be better in comparison with competitive ELISAs (Table I). However, recent studies on BHV1-specific ELISAs have demonstrated that competitive ELISAs can be more sensitive than non-competitive ELISAs (31, 44).

When using competitive or blocking ELISAs, one should be aware that potentially unexpected test results may occur, which may sometimes be attributed to steric hindrance. As an example, it has been observed that in field serum samples from non-infected pigs vaccinated with a glycoprotein E (gE)-negative PRV vaccine, the percentage of inhibition (PI) (48) in a gE-specific, commercially available blocking ELISA based on one MAb was frequently higher when compared to the PI of serum samples from non-infected and non-vaccinated pigs (J.A. Kramps, unpublished observations). This phenomenon was most likely due to high levels of PRV-specific antibodies in the vaccinated animals. As a consequence, a shift in the frequency distribution of gE-blocking antibody titres was observed in the vaccinated population, which resulted in an increase in doubtful and false positive results (Fig. 2). The fact that in a gE-specific competitive ELISA based on two MAbs (38) no such increase of doubtful or false positive test results was observed as a result of the vaccinations should be noted (J.A. Kramps, unpublished observations). Clearly, careful and frequent longitudinal monitoring and statistical evaluation of test results may be crucial to detect the described problems.

Another example of unexpected interference attributable to the test sample may also be worth noting here. The authors recently observed in a BHV1-specific gB-blocking ELISA that serum samples obtained from bulls from an artificial insemination centre induced a weak positive test result when diluted at a ratio of 1:16, whereas lower or higher dilutions of the same serum samples showed clear negative results. The false-positive results at the given dilution were found to occur only when the test serum samples were diluted in the prescribed dilution buffer, whereas clear negative responses were observed when the test serum samples were diluted in negative foetal calf serum (J.A. Kramps, unpublished observations). No such phenomenon has been observed thus far with serum samples from dairy cattle. Although a clear explanation cannot be given, this result shows the need for critical test-to-test quality control.

A recently recognised source of interference is a ‘bridging’ phenomenon by so-called heterophilic antibodies (23) (Fig. 3). These antibodies include polyspecific antibodies, idiotypic antibodies and rheumatoid factors (23). ELISAs based on a capture antibody and a conjugated antibody that are both of mouse origin (MAbs) may be particularly sensitive to interference by heterophilic antibodies that bind mouse antibodies (27). In this case, heterophilic antibodies in the test sample aggregate capture antibodies and conjugated

| Table I |
| Comparison of general characteristics of non-competitive and competitive or blocking enzyme-linked immunosorbent assays |

<table>
<thead>
<tr>
<th>Advantages and disadvantages</th>
<th>Non-competitive ELISA</th>
<th>Competitive and blocking ELISA</th>
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<tr>
<td>Advantages</td>
<td>High sensitivity</td>
<td>No control for each test sample</td>
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<td></td>
<td>One conjugated antibody an be used to set up different assays</td>
<td>One ELISA can be used to analyse samples of multiple species</td>
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<td></td>
<td></td>
<td>Low starting dilution of test sample</td>
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<tr>
<td>Disadvantages</td>
<td>High background</td>
<td>Low sensitivity</td>
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<td></td>
<td>Control well for each sample</td>
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<td></td>
<td>Higher starting dilution of test sample</td>
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ELISA: enzyme-linked immunosorbent assay
antibodies. First recognised in human serum samples (6), heterophilic antibodies may thus cause either false positive results (non-competitive ELISA, Fig. 3a), or false negative results (competitive/blocking ELISA, Fig. 3b). In humans, anti-mouse antibodies may be present due to administration of mouse antibodies for diagnosis and treatment of cancer. Interference by heterophilic antibodies must be seriously evaluated when ELISA results are inconsistent with the clinical representation of the disease (15, 23). The occurrence of heterophilic antibodies in veterinary diagnostic tests has recently been demonstrated in a double-antibody capture ELISA specific for antibodies against PRV in swine (J.A. Kramps, unpublished observations). For unknown reasons, anti-mouse antibody activity is present occasionally in the serum of pigs. Thus, heterophilic antibodies may lead to false negative results in competitive or blocking ELISAs. There are various ways to reduce the interference by heterophilic antibodies. A well-established method involves saturation of the binding sites of the anti-mouse immunoglobulins present in the test sample serum by addition to the test sample of normal mouse serum or of purified mouse immunoglobulins (27). This method was successfully applied to the PRV ELISA mentioned above (Fig. 3c). Use of F(ab’)2 fragments instead of whole IgGs as conjugated antibody can also reduce interference (35).

The antigen can also be a source of interference. Viral antigen preparations which may be contaminated with cellular proteins may cause high background or false positive reactions in non-competitive ELISAs. Another example is that various field virus strains that are related to each other may induce cross-reacting antibodies, which often results in decreased test specificity. Pestiviruses are notorious in this respect because they can infect more than one animal species (42) and they also show marked antigenic cross-reactivity (28, 40).

When MAbs are used for coating or as an enzyme-conjugated antibody, aspecific reactions may be limited as a result of the high specificity of the MAbs. In contrast, the use of conjugated polyclonal antibodies may result in aspecific reactions due to the presence of antibody activity directed against contaminants in the antigen preparation.

Interference may also occur by binding of antibodies to inevitable sources such as the plate surface itself. Blocking of non-coated places on the surface of coated plates by non-relevant proteins, such as bovine serum albumin or gelatine, or by non-cross-reacting serum (for instance, horse or chicken serum if the test samples are of bovine origin) and/or addition of such components to the ELISA buffer can reduce aspecific binding.

**The use of monoclonal antibodies**

The use of MAbs in ELISAs has greatly enhanced the specificity of the ELISAs, but also has its drawbacks. An ELISA for the detection of virus-specific antibodies can be based on

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**Fig. 3**

**Interference caused by heterophilic antibodies**

In a non-competitive enzyme-linked immunosorbent assay (ELISA), heterophilic antibodies may cause a false positive test result (a); in a competitive ELISA heterophilic antibodies may cause a false negative test result (b). Based on a practical experience with a discriminatory complex-trapping blocking ELISA for the detection of Aujeszky’s disease (pseudorabies) virus-specific antibodies, seropositive pig serum samples occasionally respond negatively in the test due to the presence of heterophilic antibodies. By pre-incubation of these test samples with mouse immunoglobulins, the binding sites of the heterophilic antibodies for mouse immunoglobulins may be saturated, which prevents false negative results (c).
only one MAb, which recognises a particular epitope on a viral protein. In such a case it is vital to know whether this epitope is stable in field strains and vaccine strains of the virus. In case antigenic variation of that epitope occurs in the field, antibodies are induced in the infected animals that are not able to recognise the original epitope in the test and consequently are not able to compete with the MAb in the test. False negative test results may occur under such circumstances. To minimise this potential risk, two MAbs directed against two different epitopes on the viral protein may be used in the test. Wensvoort et al. used this approach in a CSFV-specific complex-trapping blocking (CTB)-ELISA (45). In this assay, antibodies in a test serum against one epitope block the antigen from being captured by MAb 1, and antibodies against the second epitope will block binding of the conjugated MAb 2 (Fig. 4). Thus, change in antibody specificity due to antigenic drift of virus in the field will still result in a positive CTB-ELISA. No or minimal risk exists under such circumstances of false negative test results. Likewise, van Oirschot et al. have implemented use of two MAbs directed against two different epitopes on PRV (38) instead of one MAb (37), in a discriminatory test against the gE protein of PRV. In discriminatory tests used in eradication programmes, reducing the risk of false negative results is of particular importance, because undetected spread of a mutant virus may severely impede such programmes.

If an assay used for the detection of virus-specific antibodies is based for any reason on the use of only one MAb, care should be taken to ensure that the MAb is directed against a highly conserved epitope in order to avoid false negative results. For instance, a blocking ELISA has been developed for the detection of BHV1-specific antibodies using one MAb directed against an epitope on the gB of BHV1 (gB-blocking ELISA) (17). The stability of this epitope in the field was demonstrated by specific binding of the gB-specific MAb to all of 160 BHV1 isolates originating from ten countries.

Discriminatory tests

In discriminatory tests, infected animals must be distinguished from non-infected animals in a population in which vaccination is practised (39). Such tests must therefore be able to do the following:

a) detect antibodies induced after infection with any of the virus wild-types, preferably both shortly after infection and during the lifespan of the animal

b) give no response with antibodies induced by vaccination(s).

Extensive studies on herpesviruses have demonstrated that the membrane gE can be deleted successfully from the genome (39): this resulted in the development of an efficacious marker vaccine while at the same time the deleted viral protein could act as the discriminatory antigen in gE-specific tests. Thus, deletion of non-essential genes may provide a basis for development of discriminatory tests.

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**Fig. 4**

Schematic representation of the advantage of using monoclonal antibodies against two different epitopes (A and B) in a complex-trapping blocking enzyme-linked immunosorbent assay

When antibodies in the test sample are present against both of the epitopes A and B (a), the test result correctly identifies infected animals. Likewise, when antibodies against one of the epitopes are missing in test samples (b and c), the test result still correctly identifies infected animals.
However, genes that can be successfully deleted have not been identified for all viruses, and another approach is to detect antibodies against non-structural proteins. Discriminatory tests are being developed based on antibodies against non-structural proteins, in combination with inactivated vaccines, for FMDV (3, 33), equine influenza virus (4) and bluetongue virus (1). Another approach is the development of subunit marker vaccines, where different virus proteins are selected for use in the vaccine and in the discriminatory test. The following stand as examples:

a) a subunit vaccine against BHV1 based on gD has been described that can be used in conjuction with an ELISA based on another glycoprotein of BHV1, such as gB (36)

b) a subunit vaccine against CSFV based on structural protein E2 that can be used in conjunction with a discriminatory ELISA based on the structural protein E™ has been developed (29).

**Diagnostic reliability**

To guarantee the quality of an ELISA, careful validation and standardisation of the test should be performed (9, 20, 48). During a meeting of specialists of the Food and Agricultural Organisation (FAO), the International Atomic Energy Agency (IAEA) and the OIE in 1992, useful guidelines that apply to international standardisation and validation of ELISA techniques were established (48). Validation refers to every aspect of the ELISA that has effect on the intended purpose of the ELISA (Table II), and must demonstrate the diagnostic reliability of a test. Reliability refers to the sensitivity and specificity of the test. The sensitivity of a test refers to the fraction of infected animals with a positive response in the test. In the same way, the specificity is the fraction of non-infected animals with a negative response in the test.

To conclude that an animal is infected, the test response of a sample under investigation must surpass a certain threshold. How can such a threshold be defined? It can easily be understood that a very low threshold leads to a high sensitivity, but the disadvantage is that the incidence of false positive results in most instances will rise (low specificity). The threshold can best be determined by testing a large number of positive and negative serum samples from the target population. Negative and positive samples should ideally be defined using a well-established 'gold standard' test. When a gold standard is available, the optimum threshold for a new ELISA can then be determined by constructing and analysing receiver operation characteristics curves. The principle of this method is to compare test results of a new ELISA, determined at different threshold values, relative to those of a gold standard (16). Using this method, it was demonstrated that detection of antibodies against gE of PRV in a single droplet of whole blood gave a better performance than the detection of antibodies against gE in colostrum (34). When a gold standard test is not available, the optimal threshold can be estimated from the frequency distribution diagram of a randomised panel of samples taken from infected and non-infected local animal population, in a sufficient number to allow a statistically relevant 95% confidence (48). It is important to use samples from the target population, because genetically and/or other geographically determined

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Explanation</th>
<th>Method</th>
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<tr>
<td>Reliability</td>
<td>Defined in terms of sensitivity and specificity</td>
<td>Use carefully defined positive and negative samples/compare to gold standard test</td>
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<tr>
<td>Sensitivity</td>
<td>Proportion of known infected animals that test positive in the test</td>
<td>Calculate from agreement with known positive and negative samples</td>
</tr>
<tr>
<td>Specificity</td>
<td>Proportion of known uninfected animals that test negative in the test</td>
<td>Calculate from known positive and negative samples</td>
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<td>Predictive value (likelihood ratio) of:</td>
<td></td>
<td>Determine by the prevalence of the disease using the sensitivity and specificity data of the test</td>
</tr>
<tr>
<td>a positive test result</td>
<td>Probability that an animal with:</td>
<td>Compare results between tests in the same laboratory</td>
</tr>
<tr>
<td>a negative test result</td>
<td>Probability that an animal with:</td>
<td>Compare test results between laboratories</td>
</tr>
<tr>
<td>Repeatability ('within test')</td>
<td>Agreement of test results between replicates within and between mutually independent runs of the same assay under similar circumstances within the same laboratory</td>
<td>Determine:</td>
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<td></td>
<td></td>
<td>- percent positivity (PP)</td>
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<tr>
<td>Reproducibility</td>
<td>The ability of a test to provide constant test results when applied to aliquots of the sample at different laboratories</td>
<td>Determine from frequency distribution of positive and negative samples</td>
</tr>
<tr>
<td>Data expression</td>
<td>Expression of the test result</td>
<td>Vary conditions of reaction steps</td>
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<td>non-competitive ELISA</td>
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<tr>
<td>competitive/Blocking ELISA</td>
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<tr>
<td>Cut-off/threshold</td>
<td>The test result value selected for distinguishing between negative and positive test results</td>
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<tr>
<td>Robustness</td>
<td>Insensibility to (inadvertent) changes in performance of the test procedure</td>
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ELISA: enzyme-linked immunosorbent assay
factors may affect the frequency distribution, and the use of one and the same threshold for geographically different animal populations may result in different sensitivity and specificity (48). Furthermore, intensive vaccinations may change the frequency distribution as mentioned earlier (Fig. 2).

In eradication programmes, the requirements for the accompanying ELISA may change during the advancement of the programme. During an eradication programme and at the final stage, it is important to detect every infected animal, and thus a sensitive ELISA must be employed. False negative results will impede the removal of infected animals, and will seriously prevent a successful outcome of the programme. When eradication is completed, the ELISA must be highly specific because each (false) positive result may lead to (incorrect) extensive measures with dramatic consequences. For example, in the case of CSFV or FMDV, positive results will lead to the destruction of the total herd. Consequently, false positive results must be maximally prevented. To compensate for the loss of sensitivity on the individual animal in highly specific ELISAs, increasing the number of test samples from a population in veterinary medicine than in human medicine, this possibility should be exploited where needed. If a highly specific test is not available and false positive results are unwanted, positive results should be confirmed by an independent diagnostic test of high sensitivity.

As the amount of antibodies, their (sub)class (24) and affinity (26) vary most drastically shortly after primary infection, it is important to incorporate samples that are obtained during this period in the evaluation of the reliability of the test.

After the basic test format and aim of the test have been clearly defined, the ultimate goal is to develop a reliable ELISA that is easy to produce at a constant level of quality, and that is easy and quick to perform. The growing number of mandatory eradication and control programmes demand such a high number of samples to be tested that there is increasing pressure to develop reliable easy-to-use kits. For instance, in The Netherlands, the number of serum samples that were tested for antibodies against CSFV during the CSFV outbreak of 1997 has exceeded 2 million.

In an ELISA, the test procedure frequently includes step-by-step addition of the reagents with in-between washing steps. The antigen (sometimes preceded by a capturing antibody), test sample, conjugated antibody and substrate are generally added successively. To avoid time-consuming step-by-step addition of the reagents and washings between each incubation step, pre-coated plates and simultaneous addition of antigen, test sample and conjugated antibody can sometimes be used (8, 43). In a recently developed ELISA for the detection of antibodies against CSFV, the test serum sample, conjugated antibodies and the antigen are simultaneously added to a capturing antibody-coated microtiter plate (8). Combining assay-steps greatly reduces the assay-time and facilitates the practical use of the test. Combining reaction steps without in-between washings may also help to reduce the handling and the time required for performing an assay and allow the use of automatic equipment. However, such a format puts heavy constraints on the optimal tuning of the reagents during production of the test. For instance, in the newly developed CSFV-antibody ELISA, the coated antibodies, the conjugated antibodies and the antibodies in the test sample simultaneously compete for the antigen. Consequently, the adjustment of the coating of the plates, the antigen and the conjugated antibody must be carefully performed. Conversely, in most assays conjugated antibody is added after previous incubation of reagents and washings, and excess conjugated antibody is then much less critical.

In many cases, end-point titrations for diagnostic purposes have to be performed by serial dilution of paired samples. This may be necessary in areas where an infectious disease is endemic. The serodiagnosis can then be obtained by demonstration of a fourfold increase in virus-specific antibody titre. To circumvent the need to serially dilute the samples, single-dilution quantitative assays have been described, which reduce the time to perform the assay. However, this requires careful standardisation of the ELISAs employed (12).

The batch-to-batch uniformity of the biological reagents (antibody and antigen), and their stability during storage is obviously of utmost importance. In this respect, monoclonal antibodies and synthetic antigens may have advantages over polyclonal antibodies and natural antigens because of the biological variation of the latter. As an example, ELISAs based on small peptides derived from the conserved central hydrophobic regions of the attachment protein G of bovine respiratory syncytial virus and human respiratory syncytial virus proved to be highly sensitive and even virus subtype-specific (21).

The robustness of an ELISA reflects the insensibility of a test to changes in the performance of the test, such as different laboratory workers and differences in equipment, water quality and variations in incubation times and temperature, etc. It is a factor of increasing importance because there is an increase in exchange of tests, reagents and reference sera as a result of the growing need for standardisation. By implementing quality control (QC) programmes in which test results of identical test samples are compared on a routine basis between test runs within the laboratory (internal QC) or between tests performed by different laboratories (external QC), the robustness of an ELISA can be determined (5, 20). A
simple test procedure, with a minimum of reaction steps, can help to achieve a higher robustness, and consequently has a positive effect on the test repeatability and reproducibility (Table II).

Conclusion

The format of an ELISA, the choice of the reagents and the number of reaction steps has important consequences for the quality of a test. There is no general rule about which test format should preferentially be used for a specific sanitary programme. However, in order of priority, one should evaluate the intended use of the test, the availability of the reagents (whole virus preparations, recombinant proteins, peptides, MAbs), and the scale on which the test will be used. A test must be carefully validated using samples from the target population before being approved for use in a sanitary programme. Furthermore, test results must be monitored continuously to verify that a change in the prevalence of the disease, the vaccination status of the animals or other changes in the target animal population does not have an impact on the diagnostic reliability of the test. Careful selection and subsequent monitoring of a test to be used in an animal health programme may enhance the success of such a programme, and this will enable successful prevention and control of infectious diseases of animals.

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Facteurs critiques affectant la fiabilité diagnostique des épreuves immuno-enzymatiques

R.S. Schrijver & J.A. Kramps

Résumé

Les auteurs évaluent les différents types d'épreuves immuno-enzymatiques (enzyme-linked immunosorbent assay: ELISA) appliquées à la recherche d'anticorps spécifiques d'un virus, et étudient en particulier les facteurs susceptibles d'influencer la fiabilité diagnostique de ces techniques. Des tests ELISA récemment mis au point et ayant fait leurs preuves pour la recherche du virus de la rhinotrachéite infectieuse bovine (herpès-virus 1 des bovins), du virus syncytial respiratoire des bovins, du virus de la peste porcine classique, du virus de la maladie d'Aujeszky et du virus de la diarrhée virale bovine ont été pris comme exemples. Les auteurs présentent les différences entre les épreuves ELISA de compétition et ELISA classiques, en montrant notamment comment les résultats du test peuvent être influencés par l'antigène, l'anticorps conjugué et le prélèvement analysé. Ils attirent également l'attention sur d'éventuelles interférences pouvant induire des résultats faussement positifs ou faussement négatifs, en particulier les interférences dues à des anticorps hétérophiles (phénomène d'agrégation ou bridging). L'utilisation d'anticorps monoclonaux et d'épreuves discriminatoires fait l'objet d'une brève discussion. Les auteurs évoquent la question de la fiabilité diagnostique des épreuves utilisées dans les programmes de surveillance et d'éradication, et mettent l'accent sur les conséquences de résultats faussement négatifs ou faussement positifs. Enfin, ils discutent de la réduction de la durée des épreuves et des contrôles de qualité fonctionnels de ces tests.

Mots-clés
Factores críticos que inciden en la fiabilidad diagnóstica de los diversos modelos de ensayo inmunoenzimático

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Resumen
Los autores evalúan los distintos modelos de ensayo inmunoenzimático (enzyme-linked immunosorbent assay: ELISA) utilizados para detectar anticuerpos específicos de un virus determinado, centrándose básicamente en los factores que pueden influir en la fiabilidad del diagnóstico. A modo de ejemplo se utilizan algunas recientes pruebas ELISA y otras ya consolidadas para la detección de infecciones por el virus de la rinotraqueítis infecciosa bovina (herpesvirus bovino 1), el virus sincicial respiratorio bovino, el virus de la peste porcina clásica, el virus de la enfermedad de Aujeszky y el virus de la diarrea viral bovina. Se describen las diferencias existentes entre las pruebas ELISA de competición y las demás, atendiendo especialmente a la influencia que sobre los resultados de la prueba tienen el antígeno, el anticuerpo del conjugado y la muestra utilizada. También se contempla la posibilidad de interferencias, que pueden dar lugar a resultados falsos positivos o falsos negativos, sobre todo las interferencias causadas por anticuerpos heterofilos (fenómeno de agregación o bridging). Se examina sucesivamente el uso de anticuerpos monoclonales y de ensayos discriminantes. Se valora después la fiabilidad diagnóstica que ofrecen una serie de pruebas utilizadas en programas de monitoreo (seguimiento) o de erradicación, haciendo especial hincapié en las posibles consecuencias de resultados falsos positivos o falsos negativos. Por último, los autores hacen algunas consideraciones en torno a la reducción del tiempo de ensayo y el control de calidad funcional en este tipo de pruebas.

Palabras clave

References


