Regulatory control of veterinary diagnostic test kits

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Summary

Veterinary diagnostic test kits are used in clinical practice and production medicine as a basis for prescribing vaccination and/or treatment regimens. These kits are also used by state and federal animal health officials to control or regulate the movement of animals. The regulation of such kits, which generally takes the form of establishing requirements for licensure, ensures that the kits conform to minimum standards for sensitivity, specificity and reproducibility. To qualify for licensing, diagnostic test kits should have consistent performance characteristics.

Keywords

Diagnostic test kits – Quality controls – Regulations – Standards.

Introduction

Diagnostic test kits are available in several test formats, e.g., agglutination, enzyme immunoassay, immunodiffusion, immunofluorescence, etc. Typically, kits may be thought of as one or more reagents used to measure an analyte. For the purposes of this article, a diagnostic test kit is defined as the reagents, hardware, instructions and other components which are used in combination to detect the existence of antigens or antibodies; wherein the detection of antigen or antibody provides some indication of the existence of, exposure or susceptibility to, a potential disease-causing agent in animals (5). The foregoing definition demonstrates a regulatory perspective as applied to most test kits licensed in the United States of America (USA) and Canada; however, it is probably applicable to test kits in general.

Regulatory authority and control

The requirements for licensing diagnostic test kits vary from country to country. In some countries, diagnostic test kits are unregulated or subject to very minimal licensing requirements. Several countries only regulate kits used to diagnose diseases of domestic livestock (food animals); test kits for companion animals are unregulated. The countries with the most extensive requirements specifically applicable to veterinary diagnostic test kits are probably the USA and Canada, which regulate all kits. In the USA, the Virus-Serum-Toxin (VST) Act and regulations published in

Title 9, Code of Federal Regulations, Parts 101-118 provide the United States Department of Agriculture (USDA) with the authority to regulate all veterinary biological products (including diagnostic components) shipped into, within or from the United States of America (2). Within the USDA, authority for administering the VST Act has been delegated to the Animal and Plant Health Inspection Service (APHIS). At APHIS, veterinary biologicals are regulated in the Center For Veterinary Biologics (CVB). In Canada, test kits are regulated by the Canadian Food Inspection Agency under the Health of Animals Act and Regulations. The following regulatory controls are described from the perspective of the USA and Canada, with comparisons to other countries when requirements are significantly different. It should be noted that both the USA and Canada operate testing laboratories for veterinary biologicals.

In both the USA and Canada, a test kit may be subject to two levels of regulatory review depending on its proposed use. All kits, regardless of proposed use, receive a primary evaluation for sensitivity, specificity and reproducibility by the governmental approving agency prior to licensing. In addition, test kits that are used by animal health authorities to regulate the interstate and/or international shipment of animals, or to certify animals for export, may also be evaluated by select animal health advisory committees appointed by animal health officials. The purpose of a secondary review by appointed special committees is to establish guidelines for using approved test kits in disease management and/or eradication applications where standardisation of processes
and continuous monitoring of the effectiveness of control measures are critical to the success of the effort. Generally, the distribution of test kits used in government-sponsored disease control programmes is restricted to authorised recipients so as to limit the unauthorised testing and/or screening of animals.

**General requirements**

All veterinary diagnostic test kits, regardless of intended use, must meet the same general licensing requirements that are applicable to most veterinary biologicals. Typically these requirements stipulate that the kits meet certain specifications, as described below.

**Outline of production**

The method of preparing kit components and reagents must be described in an 'outline of production' (OP) or 'special outline'. The specific features of the production method in need of description are methods of preparing polyclonal and/or monoclonal antibody components, antigen preparation and production, reagent and substrate preparation and reagent standardisation. Guidelines for describing the production method are given elsewhere (2, 3, 4). For each reagent/component included in the kit, the production method in use when the kit is licensed is considered to deliver optimum kit performance. Any proposal to change the production method must be evaluated for its effect on kit performance before the change is approved. Typically, such evaluations are performed by the manufacturer of the kit; the data are then submitted to licensing authorities along with a petition requesting approval of the change.

**Purity of products of animal origin**

Master seeds and ingredients of animal origin used to prepare antigens and reagents included in the kit must be free of extraneous bacterial, viral and fungal contaminants. Fluid thioglycollate medium with 0.5% beef extract is used to test for bacteria. Heart infusion broth and agar are used to test for mycoplasma, and soybean-casein digest medium is used to test for fungi. Inoculation of monolayer cultures of cells with examination for cytopathogenic and/or haemadsorbing agents and fluorescent antibody techniques are used to test for extraneous virus. Tests are performed by the manufacturer. Testing laboratories for veterinary biologicals in the USA and Canada confirm the purity of seeds and ingredients using samples provided by the manufacturer. Other countries conduct confirmatory testing on a selective basis. Acceptable test methods are described elsewhere (2, 6). Extracted, synthetic and recombinant-derived antigens must be characterised using laboratory procedures appropriate to the confirmation of a claimed distinguishing feature. For example, if the antigen is described as a specific extracted protein, the manufacturer should describe laboratory procedures which can be used to confirm the identity of the protein in supplemental protocols. As is the case for the production method, antigen production/extraction procedures cannot be altered without prior approval from licensing authorities.

**Purity of cell cultures**

Cell cultures (primary cells and cell lines) used for antigen production must be established at specific passage levels and tested for bacteria, viruses, fungi and extraneous agents, using acceptable test methods as described elsewhere (1, 6). Hybridomas used for monoclonal antibody production should be tested for purity, and for mycoplasma and viral contaminants. Monoclonal antibodies should be characterised as to isotype and specificity. Typically, protocols and samples are supplied by the manufacturer and confirmatory testing is performed by the licensing authority.

**Purity of imported products**

Test kits, ingredients of animal origin and reagents imported into the USA and Canada are required to meet the same standards for quality and purity specified above for domestically produced kits. Special treatments and tests may be prescribed if a risk assessment determines that the ingredients of animal origin pose an increased risk for introducing diseases not known to exist in either country. In most countries, diagnostic test kits are considered to be of low risk for introducing exotic diseases and restrictions, therefore, are considered only under extreme circumstances.

The requirements for diagnostic test kits in most countries stress quality ingredients, consistent production methods and validation of procedures and performance. The most significant difference appears to be the pre-approval confirmatory testing requirement stipulated by the USA and Canada. However, it should be noted that other countries may conduct supplementary testing on an individual case basis.

**Data requirements**

Label claims determine which data are required to support the issuance of a license for diagnostic test kits. In some countries, the requirements may be kit-specific. From a regulatory perspective, however, the primary data considerations are sensitivity, specificity, reproducibility and suitability. In most countries, licensing authorities specify that these parameters should be assessed by comparison to a recognised reference procedure, i.e., 'gold standard', which unequivocally classifies an animal as infected or uninfected. In the absence of a gold standard, and as a matter of policy, some countries allow comparison to lesser standards. In many instances a manufacturer may only be required to demonstrate performance equivalent to that of previously approved tests (1); although this is not the preferred method, it is sometimes the only method available. The discussion of sensitivity, specificity, reproducibility and suitability that follows is brief; however, a more thorough discussion can be found elsewhere (6, 7).
Sensitivity

Sensitivity is defined as the probability of obtaining a positive test result in randomly selected individuals of known infected status. Typically, the sensitivity of a kit is established by comparing the test result obtained using the kit with the true result as determined by a reference gold-standard test method. Virus isolation, bacterial culture, haemagglutination inhibition, serum neutralisation, complement fixation and necropsy are examples of laboratory procedures that are used as reference methods. Strong positive, medium positive and weak positive samples should be evaluated. With the exception of test kits used in government-sponsored animal disease control and/or eradication programmes, most licensing authorities do not mandate minimum levels of sensitivity. Cut-off values which serve to distinguish between positive and negative samples are determined by the manufacturer. However, where test kits are used in government-sponsored disease control programmes, most licensing authorities specify the use of known, highly characterised samples to ensure that kits do not have unacceptably high false-positive or false-negative rates. As far as possible, the same set of samples (panels) should be used to evaluate all kits of a given type. Thus, while sensitivity levels are not specified in licensing requirements, the use of reference panels ensures similar levels of sensitivity for most kits purporting to detect antigen or antibody associated with a given disease condition.

Specificity

Specificity is defined as the probability of obtaining a negative test result in randomly selected individuals that are of known non-infected status. Typically, the emphasis is on ruling out cross-reactivity, and hundreds of samples may be tested. Kits for the detection of antibody should be tested against immune sera to related antigens to detect cross-reactivity. Most often these are samples taken from animals that have received commonly used immunising products. Kits for the detection of antigens should be tested against other related antigens to detect false-positive reactions.

Reproducibility or precision

The coefficient of variation (CV) is the most commonly used measure of precision for kits that have antigen or antibody fixed to a plate, or some other solid phase; for example, the enzyme-linked immunosorbent assay kit (7). Quadruplicate testing of negative, positive, weak positive, and strong positive samples on at least five different solid phase units should be used to establish the CV of the test (3, 9). The CV must be within the acceptable limits for the planned application of the test (7, 9). While repetitive testing may provide an indication of variation, such determinations may be of limited value, depending upon the samples used to make the determination, and the fact that the test may be used in a manner not envisioned by the manufacturer when the test was developed.

Suitability

Licensing authorities in the USA and Canada recommend that diagnostic test kits be evaluated in multiple laboratory settings prior to licensing approval. Samples tested should include routine laboratory submissions as well as samples supplied by the manufacturer of the kit. Test kit results should be compared to results obtained using the reference method routinely utilised by the collaborating laboratory. Adequacy of directions for use and interpretation of results should also be determined. In addition to providing a broader database for reaching conclusions regarding the reproducibility of test results, multiple independent locations are considered to result in a more representative cross-section of husbandry and laboratory procedures. These data are sometimes used to make adjustments to the test before licensing.

While each of the above parameters provide valuable data, sensitivity and specificity best characterise test accuracy and are thus the most important evaluation elements in licensing considerations (7).

Serial release testing: purity, safety and potency

To ensure consistent performance characteristics, licensing authorities in both the USA and Canada require manufacturers to submit a report of the test results for each batch or lot of kits produced. Batch lots with satisfactory test results are approved for marketing. The licensing authority, however, may select random samples for confirmatory testing. In other countries, marketing is allowed upon certification that a batch was produced and monitored in accordance with a 'good manufacturing practice' marketing authorisation. As in the USA and Canada, animal health authorities may require additional testing of kits used in disease control programmes sponsored by the government. Generally, the purpose of such testing is to ensure that the performance of the kit is appropriate for the proposed use. Most countries require retention of samples from each batch of kits for future examination should problems arise.

Purity and safety testing

Except for the previously discussed requirement to test antigens, cell lines and other ingredients of animal origin for extraneous viral, bacterial and fungal contaminants, most countries exempt the components of diagnostic test kits from the requirement to test finished veterinary biologicals for purity and safety. The pre-approval requirement to establish starting ingredients as free of extraneous micro-organisms, and a post-approval restriction requiring revalidation of the test prior to substituting any starting materials or altering the production method specified in the OP or marketing authorisation, are considered adequate to ensure the purity of finished reagents. Exemptions to safety testing are justified on the basis that the product will not be administered to animals.
Potency

Potency may be defined as the relative strength of a component as compared to a reference standard. For diagnostic test kits, potency tests provide confirmation that the reagents or components included in the kit were produced, assembled and standardised in a manner consistent with the achievement of a test result which accurately reflects the status of the animal relative to the disease. Each batch or lot of assembled kits should be tested for potency by the manufacturer, regardless of whether such testing is required by the licensing authority.

Prior to the release of a batch lot of kits for general distribution and sale, the manufacturer should evaluate the assembled components to ensure that the performance of the test meets product specifications or marketing authorisations. Typically, potency tests are sensitivity, specificity and reproducibility evaluations of the assembled components using reference samples supplied by the manufacturer and/or the licensing authority. Positive and negative quality control samples included in the kit, reference antigens or standards used to standardise the kit and previously characterised sensitivity/specificity panels are the basis of most potency tests. Potency testing may be thought of as the final quality control procedure applied to the assembled components before marketing. A discussion of the importance of quality control can be found in the ‘Manual of Standards for Diagnostic Tests and Vaccines’ of the Office International des Epizooties (6).

Diagnostic kits used in regulatory programmes

Regulatory requirements for diagnostic kits used in co-operative State/Federal/industry animal disease control and/or eradication programmes in the USA can serve as a model for those used elsewhere.

Generally, control programmes may be defined as a set of extraordinary procedures used by animal health officials to prevent the spread of animal diseases. The following example discusses procedures used in the USA. It is not intended, however, to suggest that the methods used originated in the USA or that they are unique. This is just one example of how diagnostic test kits may be used in programmes designed to control the spread of diseases of livestock.

In the USA, the Administrator of APHIS, in consultation with State and industry animal health officials, has the authority to prescribe requirements for the approval of diagnostic test kits used in control and/or eradication programmes. Such tests must be:

a) licensed in accordance with the provisions of the VST Act as specified in Title 9, Code of Federal Regulations, Parts 101-118, and

b) designated in the codified regulations as ‘official’ or ‘approved’ tests for the diagnosis of specified disease conditions when conducted in approved laboratories.

By declaring particular diagnostic procedures (tests) to be official tests or approved tests, regulatory officials are able to standardise the diagnostic procedures used to determine the disease status of an animal. In this manner, animal health officials are able to ensure that only the most efficacious tests are used in national and international disease control programmes.

In addition to the basic requirements for licensure under the VST Act, test kits which are candidates for designation as official or approved tests are subjected to an additional, more extensive field evaluation for sensitivity, specificity and suitability. Such evaluations may be conducted in specifically selected locations (States and/or laboratories) that allow the tests to be conducted in previously characterised populations of animals, i.e., infected herds, high-risk herds or animals imported from high-risk States or countries. For example, prior to being recognised as an official or approved test, a test kit for the diagnosis of bovine tuberculosis may be evaluated in five or six quarantined infected herds that have a history of Mycobacterium bovis infection and at least one positive M. bovis isolation within the previous year. Test kit results may be compared to results obtained using the standard tuberculin test, histopathology and/or culture. These supplemental evaluations allow animal health officials to identify specific uses for the test in control programmes. Typically, these data determine whether a test kit receives the official or approved test designation. Concurrence of the appropriate animal health officials is required before the test can be altered or modified, or the components changed.

In addition, official or approved tests must be conducted in an approved laboratory. Approval of a laboratory is accorded after the following preconditions are satisfied (8):

a) personnel responsible for conducting the tests shall have completed training in proper techniques at the National Veterinary Services Laboratories (NVSL)

b) laboratory personnel agree to conduct USDA-licensed tests according to label instructions or in accordance with official protocols provided by the NVSL
c) laboratory personnel agree to test only samples submitted by accredited veterinarians or other officials designated by proper animal health officials
d) laboratory personnel agree to report test results according to specified procedures within 48 hours of completion
e) laboratory personnel agree to laboratory inspections by Federal or State animal health officials
Laboratory personnel agree to demonstrate proficiency in conducting official tests by passing a proficiency test administered by the NVSL at least once every eighteen months.

Additional information regarding proficiency testing and the approval of laboratories can be found elsewhere (6, 9).

Conclusions

Whether manufactured in accordance with regulatory procedures used by the USA and Canada or in accordance with the good manufacturing practices prescribed in other countries which have regulatory programmes, the production and validation requirements for diagnostic test kits are remarkably similar. Sensitivity, specificity and reproducibility are the most important performance characteristics, with special emphasis placed on documentation of production procedures, quality control and validation of the effects of changing reagents or production methods. In combination, these procedures serve to standardise the testing of animals for communicable diseases in laboratories throughout the world.

Contrôle officiel des trousses vétérinaires de diagnostic

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Résumé
Les trousses vétérinaires de diagnostic constituent, dans la pratique clinique et dans la médecine vétérinaire des animaux de rente, l'outil sur lequel se fondent les décisions concernant vaccinations ou traitements. Ces trousses sont également utilisés par les responsables de la santé animale (à l'échelle nationale ou de l'État fédéré) pour contrôler ou réglementer les déplacements d'animaux. La réglementation applicable à ces trousses, visant généralement à définir les critères de leur agrément, garantit la conformité des diagnostics ainsi obtenus à des normes minimales de sensibilité, de spécificité et de reproductibilité. Pour être agréées, les trousses de diagnostic doivent présenter des caractéristiques de performance régulières.

Mots-clés
Contrôles de qualité - Normes - Réglementation - Trousses de diagnostic.

Control reglamentario de los kits veterinarios de diagnóstico

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Resumen
En el ejercicio clínico y la medicina de los animales de producción, los kits veterinarios de diagnóstico sirven para fundamentar las vacunaciones y/o tratamientos que puedan prescribirse. Las autoridades estatales o federales responsables de la sanidad animal recurren asimismo a esos kits para controlar o reglamentar el traslado de animales. El control normativo de los kits de
diagnóstico, que suele concretarse en requisitos a los que se condiciona la obtención de licencia de comercialización, garantiza que esos kits respondan a determinados criterios mínimos de sensibilidad, especificidad y reproductibilidad. Para que le sea concedida la licencia, es preciso que un kit de diagnóstico exhiba características constantes en la obtención de resultados.

Palabras clave
Controles de calidad – Kits de diagnóstico – Normas – Reglamentos.

References


