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A review of diagnostic tests recommended by the World Organisation for Animal Health *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*

A. Cullinane & M. Garvey

Virology Unit, Irish Equine Centre, Johnstown, Naas, Co. Kildare, W91 RH93, Ireland

*Corresponding author: acullinane@irishequinecentre.ie

Summary

To provide a standardised approach to the diagnosis of diseases and to facilitate health certification for trade, the World Organisation for Animal Health (OIE) standards, described in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*), include internationally agreed laboratory diagnostic techniques. This review examines the type of tests recommended in the disease-specific chapters of the *Terrestrial Manual* for the six most common purposes of diagnostic techniques, including certification for movement, confirmation of clinical cases and disease surveillance. The most frequently recommended tests for all six purposes are enzyme-linked immunosorbent assay and/or polymerase chain reaction, for which there are detailed validation guidelines in the OIE *Terrestrial Manual*. This is true for all species and no species-specific barriers to validation related to test type were identified. Classical techniques continue to be well represented in the *Terrestrial Manual* recommendations whereas novel technologies are slow to gain acceptance. These classical tests can present challenges for validation as there may be a dearth of international standard reagents and harmonised protocols.

Keywords

Agent detection – Diagnostic tests – Immune response – *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* – Purposes – *Terrestrial Manual* – Validation – World Organisation for Animal Health.

Introduction

The Office International des Épizooties (OIE), renamed the World Organisation for Animal Health in 2003, is the reference organisation to the World Trade Organization (WTO) for standards relating to animal health and the trade of live animals and animal products. The standards are published within the OIE *Terrestrial Animal Health Code* (*Terrestrial Code*) (1) and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*)

(2). The *Terrestrial Code* standards are employed by the Veterinary Authorities of importing and exporting countries to prevent the transfer of infectious agents via international trade. In order to provide a standardised approach to the diagnosis of diseases and to facilitate health certification for trade, the *Terrestrial Manual* standards include internationally agreed laboratory diagnostic techniques. The OIE recognises the six most common purposes for diagnostic techniques as:

− demonstration of freedom from infection in a defined population (country/zone/compartment/herd)

− certification of freedom from infection or the presence of the agent in individual animals or products for trade/movement

− eradication of disease or elimination of infection from defined populations

− confirmation of a diagnosis of suspect or clinical cases

− estimation of the prevalence of infection or exposure to assist risk analysis

− determination of the immune status of individual animals or populations (post-vaccination).

In 2011, the OIE recognised the need to identify the purpose of each of the diagnostic methods described in the disease-specific chapters of the *Terrestrial Manual*. To give a concise guide as to which tests are suitable for which purpose, it was agreed to substitute the traditional identification of tests as 'prescribed tests for international trade' and 'alternative tests', with a table in each chapter grading the diagnostic tests available against the six purposes. Ideally, the relative merits of the different methods should be linked to the validation parameters, fitness for purpose, and also the practicability of the test. The aim of the current review is to describe the type of tests recommended in the disease-specific chapters of the *Terrestrial Manual* for each of the six purposes across species, to identify any potential barriers to validation, and to determine any species-specific challenges.

Materials and methods

A database was compiled of the test methods and their purposes tabulated in the disease-specific chapters of the OIE *Terrestrial Manual*. Seventeen chapters that did not include a table were excluded from the analysis. Only tests that scored +++ = 'recommended' or ++ = 'suitable' by the OIE disease experts were included. Those that scored + = 'may be used in some situations but cost, reliability or other factors severely limit its application' were not included.

Throughout this review, the term 'recommended' includes tests scored as recommended or suitable in the *Terrestrial Manual.* The test categories used in the *Terrestrial Manual,* i.e. agent

identification and detection of immune response, were retained. The tests were then grouped for agent identification as:

- polymerase chain reaction (PCR)
- culture (includes *in vitro* and *in vivo)*
- visualisation (direct and microscopy with or without chemical staining)
- immunolabelling (includes immunofluorescence and immunohistochemistry)
- antigen enzyme-linked immunosorbent assay (ELISA).

For the detection of immune responses, tests were grouped as:
- antibody ELISA
- neutralisation
- immunolabelling
- precipitation
- agglutination
- haemagglutination
- complement fixation.

Less frequently recommended tests were grouped as miscellaneous.

Analysis of tables

The test methods for each purpose are tabulated according to: *(i)* species as listed in Part 3 of the *Terrestrial Manual* and *(ii)* pathogen type (3).

Purpose 1: population freedom from infection

The popularity of antibody ELISA tests for establishing that a population is free from infection (31%) is consistent with their sensitivity and relative technical simplicity, in addition to the fact that they are easily automated for high-throughput screening (see Tables I and II). Furthermore, ELISA tests to differentiate infected from vaccinated animals (DIVA) are used in conjunction with genetically engineered marker vaccines for diseases such as infectious bovine rhinotracheitis, classical swine fever and Aujeszky's disease (pseudorabies). Where neutralising antibodies tend to persist for long periods, virus-neutralising and plaque-reduction tests are considered to be sensitive for evidence of prior infection and consequently represent 10% of the recommended tests. They are also very specific, which is useful for differentiating infection with different subspecies and serotypes. They are more time-consuming and technically complex than

ELISAs, but are recommended for establishing freedom from certain economically important diseases, for example, foot and mouth disease, peste des petits ruminants and bluetongue.

When the emphasis is on detecting the agent, for example, on completing an eradication programme after the incursion of an exotic agent, PCR is frequently used to detect nucleic acid in both live and dead animals. As a result of its higher sensitivity, it is more often recommended (21%) than the antigen ELISA test (5%). Since antibody detection assays are not applicable to bees, there is a reliance on agent detection (for example, PCR, microscopy, antigen ELISA and culture) to establish freedom from disease for a population of this species. For other species, culture is recommended (6%) for specific bacteria and mycoplasmas, but not for viruses. Microscopy is recommended (5%) for several parasitic diseases, e.g. trichomonosis, infestation with *Aethina tumida* (small hive beetle) and New World screwworm.

Purpose 2: freedom from infection in individual animals

To certify individual animals for international movement, PCR is the test of choice (27%) for establishing freedom from infection (see Tables III and IV). Less popular methods of agent detection are culture (13%), antigen detection by ELISA (6%) and microscopy (4%), reflecting their inferior sensitivity. Serology indicates exposure to the infectious agent, while antibody ELISA tests (20%) and, to a lesser extent, virus neutralisation (10%) are recommended for some agents. Such antibody detection tests are particularly relevant for agents associated with persistent infection and to provide assurance of lack of exposure when animals are travelling from a country, zone or compartment that is free from a specific pathogen. Positive immunoglobulin M (IgM) ELISA results are suggestive of recent exposure, and antibody testing of paired sera during pre-export quarantine may play a role in establishing that animals are not acutely infected.

Purpose 3: contributing to eradication policies

The tests recommended for eradication policies (see Tables V and VI) are similar to those recommended for establishing population freedom from infection, i.e. antibody ELISA (25%) and PCR (23%) tests. Other tests include antigen ELISA (8%), culture (8%) and neutralisation (7%).

Purpose 4: confirmation of clinical cases

Polymerase chain reaction, which has very high analytical specificity and sensitivity, in addition to a fast turnaround time, represents the majority of OIE-recommended tests (30%) for confirmation of clinical cases in all species, and for all types of infectious agents examined in this

review (see Tables VII and VIII). Culture (19%), particularly for viruses and bacteria, and microscopy (7%), mainly for parasites, are also preferred methods. Antigen detection by ELISA (7%) is considered an appropriate alternative in all species for selected viruses and bacteria. Antibody tests are recommended for indirect confirmation of clinical cases in all species except bees. Such antibody tests include ELISA (10%), virus neutralisation (4%), complement fixation (4%), agglutination (2%) and haemagglutination (2%). For most agents, confirming a clinical case by serology ideally requires demonstration of seroconversion in two samples, taken at least 14 days apart.

Purpose 5: prevalence of infection

Antibody detection by ELISA (26%), as a measure of exposure, and agent detection by PCR (22%), as a measure of infection, comprise the majority of OIE-recommended tests to assist in risk analysis and monitoring disease control programmes (see Tables IX and X). Both are suitable for high-throughput testing and their cut-offs can be modified, based on the prevalence of infection in the target population. Measuring exposure by virus neutralisation (9%) or immunolabelling (6%) is also recommended for some agents, as is detection of infection by culture (7%) or antigen ELISA (6%). Less commonly recommended tests include microscopy, the complement fixation test (CFT), agglutination and haemagglutination.

Purpose 6: immune status

To determine the immune status of individual animals or populations post vaccination, antibody ELISAs are by far the most popular recommended test (43%), followed by neutralisation (28%) (see Tables XI and XII). Other tests, such as haemagglutination (6%) and immunolabelling (6%), are less widely recommended. Virus neutralisation for rabies in dogs and single radial haemolysis (SRH) for equine influenza are examples of tests that have been internationally harmonised to monitor antibody response to vaccination.

Discussion

Since 2018, the OIE has required that OIE Reference Laboratories implement a quality standard, and are accredited to Standard 17025 of the International Organization for Standardization (ISO), or similar (4). Notwithstanding this requirement, it does not necessarily follow that all tests carried out in these laboratories are accredited. The OIE Reference Laboratories must list in their annual report the accredited tests that relate to their OIE designation. Such accreditation demonstrates the competency of the laboratory to perform the tests and promotes international acceptance of the results.

However, quality assurance is not particularly useful if the test is inappropriate for the purpose. Thus, the OIE standard, as documented in the *Terrestrial Manual*, is an interpretation of the generally stated requirements of the ISO standard, which places particular emphasis on the evaluation of a test to determine its fitness for purpose. The OIE has defined a chronological validation pathway with four stages: analytical performance characteristics, diagnostic performance of the assay, reproducibility and programme implementation. Initial validation is followed by ongoing monitoring to ensure that the assay maintains its fitness for purpose. Certain factors, such as repeatability and reproducibility, are always important but the balance between sensitivity and specificity may vary for screening and confirmatory tests (5, 6). Shortcomings in sensitivity and/or specificity can be overcome by using tests in combination. For example, due to its high sensitivity, the CFT remains the test of choice for routine testing for glanders but the Western blot assay has a markedly higher diagnostic specificity than the CFT, and is suitable for use as a confirmatory test to avoid false-positive diagnoses (7). In the case of international trade, highly sensitive tests are required to avoid incursions of pathogenic organisms into disease-free populations, but lack of specificity and the resultant false positives can result in significant financial losses for producers and exporters and damage to their reputations, while posing difficult problems for veterinary authorities. Variations in disease incidence may also influence decisions on setting the optimum sensitivity and specificity for a particular assay. The same assay may be validated for more than one purpose by optimising its sensitivity and specificity for each purpose. For quantitative tests, the balance may be achieved by changing the cut-off value used to designate a result as positive or negative.

The inclusion of tables in the disease-specific chapters of the OIE *Terrestrial Manual*, which classify tests according to their fitness for assessing freedom from infection and other purposes, is undoubtedly useful (3). However, it is unclear if the majority of the tests recommended are validated to the OIE standard. The recommended diagnostic methods are usually referred to as 'validated for the purpose shown', while the suitable methods are described as 'possibly needing further validation'. A recent methodological review of test validation studies for OIE-listed diseases in wild mammals revealed many deficiencies (8).

Resources permitting, barriers to engagement with the OIE validation process are primarily related to the onerous investigation of diagnostic performance in target populations in their natural environment. Challenges include the lack of availability of well-characterised clinical samples, in particular, a sufficient number of true-positive and/or true-negative samples from geographically diverse regions. To encourage validation of tests to the OIE standard, it may be necessary to introduce some flexibility to the inclusion criteria. A recent investigation of the merits of different serological assays for glanders benefited from such a pragmatic approach (7).

Similarly, assays developed for use in emergency or outbreak situations may qualify for 'provisional recognition', based on their analytical sensitivity and specificity, repeatability, and an estimate for reproducibility.

Countries that are free from a specific disease face particular challenges in investigating diagnostic performance and depend on collaboration with endemic countries for access to clinical samples (9). When dealing with tests for minor species, and diseases for which there is only a single OIE Reference Laboratory, countries may have difficulty identifying other laboratories for reproducibility estimates and proficiency testing. The lack of international reference standards can also be a problem for internal quality control.

Analysis of the tables of recommended tests in the *Terrestrial Manual* clearly demonstrates the ever-increasing replacement of agent detection employing traditional methods, such as bacterial culture, virus isolation, microscopy and antigen detection, with PCR. This is now the case across species, not only to diagnose clinical cases and for pre-movement testing but also for surveillance and other purposes. Real-time PCR is extremely popular but it has not totally superseded conventional PCR and nested PCR, which are the recommended assays for several infectious agents (data not shown). Polymerase chain reaction tests are exquisitely sensitive but do not distinguish between living agents and residual nucleic acid, which can be present long after an animal is no longer considered to be infectious or even as a result of contamination during vaccination (10, 11). Results may be generated within the working day but all PCRs require strict laboratory protocols to avoid contamination, technical expertise, thermocycling equipment, enzymes and other reagents (12).

The importance of a reliable supply chain was demonstrated during the initial months of the COVID pandemic when there was a global shortage of PCR reagents, particularly those used for nucleic acid extraction (13). If the reagent supply chain is interrupted, over-reliance on a single technology results in diminished testing capacity and the potential failure of laboratories to meet their obligations. Therefore, it is important that veterinary laboratories are encouraged to retain their traditional assays, even though these may not lend themselves to automation as easily as newer technologies, and the use of in-house sera, cell lines and other reagents, along with different protocols, may present challenges for international standardisation and validation to OIE standards. Virus isolation and bacterial culture are recommended to confirm clinical cases in many of the disease-specific chapters. This is essential for agent characterisation, which should not focus entirely on nucleotide sequencing but should also include antigenicity and biotyping (14).

In aquatic animals, histopathology is commonly used across species and is the mainstay of diagnostic methods. However, the need for histopathology as a diagnostic test for terrestrial animals has decreased as molecular methods are faster, more sensitive and can be performed directly from accessible, minimally invasive, clinical samples. Conventional microscopy and immunofluorescence retain their place as standard methods for agent detection but can be difficult to standardise between laboratories, due to differences in staining procedures and the individual subjective judgement of microscopists. Microscopy is a simple and cheap test and is particularly useful in rural laboratories that cannot afford the equipment necessary for molecular diagnostics. Microscopy also represents the majority of OIE-recommended tests for parasites, along with PCR to confirm clinical cases. Furthermore, a slide can be examined for different species, whereas molecular assays are frequently very specific. In fact, the specificity of probe-based PCR tests can lead to reduced sensitivity with highly mutable pathogens when there is a mismatch between the genotype of the pathogen in the field and those used to design the assay. Micro-array technology overcomes this shortcoming by allowing simultaneous testing for a variety of pathogen species and strains (15, 16). However, at present, although they are recommended in the *Terrestrial Manual* for henipaviruses and avian chlamydiosis, micro-arrays are not widely used in routine veterinary diagnosis.

Similarly, loop-mediated isothermal amplification (LAMP) is an alternative to PCR that requires no DNA purification (17). Although it is considered to be cheaper, simpler and faster than PCR and well suited to point-of-care use in rural settings (18), LAMP is not as well established in the field as PCR and is only recommended for a small number of agents.

The majority of recommended tests in the OIE *Terrestrial Manual* for detection of the immune response are ELISA tests, which are readily automated. For instance, ELISAs are used as the example for the validation of antibody detection assays in the *Terrestrial Manual*. However, in some instances, the sensitivity of classical techniques continues to exceed that of ELISA. For example, the CFT for glanders may have specificity problems, but is still the recommended test for the international movement of horses, due to its unparalleled sensitivity (7). Many long-established immune detection systems, such as the agar gel immunodiffusion test for equine infectious anaemia and immunoblotting for bovine spongiform encephalopathy, have served the animal health industry well in eradication programmes worldwide. Other common antibody detection assays include virus neutralisation, complement fixation and haemagglutination inhibition. These classical tests often use in-house antigens, require interpretive skills, are more laborious to perform and have longer turnaround times than ELISA tests. They can also present challenges to validation, as there is a dearth of international standard reference sera and harmonised protocols. However, even in the absence of formal validation, many traditional

assays have earned their place as recommended tests, due to their routine nature and their proven track record over many decades.

The characteristics of the disease and the agent may influence the selection of the test method. Persistent infections may be more easily identified by detecting the immune response rather than the agent. For example, infected equids remain viraemic carriers of equine infectious anaemia virus for life and, with very rare exceptions, yield a positive serological test result. In contrast, virus isolation is difficult and the high mutation rate of the virus may generate false-negative PCR results (19). Serological tests are also recommended for the diagnosis of latent infections with agents such as Aujeszky's disease virus, especially in eradication programmes and determination of the health status of animals for international trade. Cross-reacting antibody may affect the usefulness of serological methods. For example, ELISA tests are recommended for detecting antibodies against the bluetongue virus (BTV) serogroup (20) but neutralisation tests are employed to identify specific antibodies against the 26 recognised BTV serotypes. The latter are very useful in endemic areas where multiple serotypes are likely to be present.

Testing recommendations may be influenced by the ease of sample collection. Ear-notch testing by PCR or antigen ELISA to identify persistently infected cattle has become the cornerstone of recent bovine viral diarrhoea eradication programmes, with samples frequently submitted directly to the laboratory by the farmer (21). Both assays are suitable for automation and high-throughput testing, and the sensitivity of PCR enables pooling of the samples for screening, thus reducing costs for the producer. In the case of zoonotic agents, safety is a priority and laboratories may choose to restrict agent detection to molecular tests, rather than attempting agent propagation, and avoid serological tests, such as plaque reduction and neutralisation tests, that use live virus.

Analysis of the test method tables in the disease-specific chapters of the OIE *Terrestrial Manual* indicates that a wide variety of tests are recommended for different purposes but there is a preponderance of PCR and ELISA tests. This is true for all species and no species-specific barriers to validation related to test type were identified. At present, classical techniques continue to be well represented while novel technologies appear slow to gain acceptance. That said, the small number of novel technologies recommended may − at least in part − reflect the time lag in revising chapters. To assist those who consult the *Terrestrial Manual*, consideration could be given to including, in each chapter, a section devoted to the rationale behind the selection of tests and their suitability for different purposes. For example, the recommended test for determining immune status in individual animals or populations for equine influenza, post vaccination, is the SRH test, for which OIE-approved reference antisera are available from the European Directorate for the Quality of Medicines. The correlation between post-vaccination SRH antibodies and

protection has been observed in experimental challenge studies (22, 23, 24), as well as in the field during outbreak investigations (25, 26). Furthermore, the technique has been employed in a global study to establish, within the context of existing OIE standards, a science-based rationale to identify the ideal time period for equine influenza vaccination before shipment (27).

The recommended tests for freedom from infection with African horse sickness virus, for individual animals prior to movement, are the real-time, reverse transcription polymerase chain reaction (RT−PCR) methods of Agüero *et al.* (28) and Guthrie *et al*. (29). In an international ring trial, these methods correctly detected all the representative strains with high sensitivity in the analysis of field samples and are validated for certifying individual animals before movement. Such justifications for test recommendations, supported by reference to peer-reviewed published papers, would be a useful accompaniment to the tables. Moreover, they would assist laboratories in their selection when two or more assays have the same score or in circumstances not covered by the current tables. Unique circumstances, not envisaged by the authors of *Terrestrial Manual* chapters, may arise from time to time. An explanation of the utility of different assays for different purposes may assist laboratory workers when deciding how to approach new situations.

Nucleic acid detection and ELISA tests to detect antigen and antibody are the subjects of detailed validation guidelines in the OIE *Terrestrial Manual*. Furthermore, the OIE accepts Bayesian mixture (latent class) models when reference standards are imperfect (30). To encourage the validation of tests to the OIE standard and the wider use of such validated tests, a list of OIE-validated tests could be published on the OIE website, similar to the Register of Diagnostic Kits certified by the OIE as validated fit for purpose. Links to the validation reports for such tests would inform laboratories of different interpretations of the OIE validation recommendations, provide templates and encourage more collaborative discussion. Similarly, a standard template capturing relevant validation parameters in the Standards for Reporting Diagnostic Accuracy (STARD) statement − for example, source population, sample size, specimen, reference standard, statistical methods, diagnostic sensitivity and diagnostic specificity, etc. − would inform decision-making by end users (31).

Conclusions

To conclude, the following recommendations are proposed for consideration:

1. the retention of traditional assays by veterinary laboratories to avoid over-reliance on any single technology and any associated risk of interruption to service

2. the ongoing integration of agent isolation within the laboratory work programme to facilitate comprehensive characterisation of pathogens

3. the inclusion of a section devoted to the rationale behind the selection of tests for different purposes in each disease-specific chapter of the OIE *Terrestrial Manual*

4. the inclusion of links within OIE *Terrestrial Manual* disease-specific chapters to the validation reports for tests validated to the OIE standard

5. the adoption of a flexible approach to the inclusion criteria for clinical samples for validation of diagnostic performance to the OIE standard, when necessary

6. the publication of a list of tests validated to the OIE standard on the OIE website.

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Un examen des tests de diagnostic recommandés par le Manuel des tests de diagnostic et des vaccins pour les animaux terrestres de l'Organisation mondiale de la santé animale

Résumé

[FR to follow] Con objeto de instaurar métodos normalizados de diagnóstico de enfermedades y de facilitar la expedición de certificados sanitarios para el comercio, la Organización Mundial de Sanidad Animal (OIE), en las normas que establece en su *Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres* (el *Manual Terrestre*), incluye técnicas de diagnóstico en laboratorio que suscitan consenso a nivel internacional. Los autores pasan revista a los tipos de prueba recomendados en los capítulos del *Manual Terrestre* relativos específicamente a una determinada enfermedad en relación con los seis propósitos con los que más comúnmente se utilizan las técnicas de diagnóstico, entre ellos la expedición de certificados para el desplazamiento de animales, la confirmación de casos clínicos y la vigilancia de enfermedades. Las pruebas recomendadas con más frecuencia para este conjunto de seis propósitos son el ensayo inmunoenzimático y/o la reacción en cadena de la polimerasa, para cuya validación se ofrecen detalladas indicaciones en el *Manual Terrestre* de la OIE. Esto se aplica a todas las especies, pues no se ha observado ninguna barrera a la validación asociada a una u otra

especie que tenga que ver con el tipo de prueba. En las recomendaciones del *Manual Terrestre* siguen estando bien representadas las técnicas clásicas, a la par que las tecnologías novedosas van cobrando aceptación con lentitud. A veces la validación de estas pruebas clásicas presenta dificultades por la escasez de protocolos armonizados y de reactivos de referencia a nivel internacional.

Mots-clés

Détection d'agents – Finalités – Manuel des tests de diagnostic et des vaccins pour les animaux terrestres – Manuel terrestre – Organisation mondiale de la santé animale – Réponse immunitaire – Tests diagnostiques – Validation.

Repaso de las pruebas de diagnóstico recomendadas en el Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres de la Organización Mundial de Sanidad Animal

A. Cullinane & M. Garvey

Resumen

Con objeto de instaurar métodos normalizados de diagnóstico de enfermedades y de facilitar la expedición de certificados sanitarios para el comercio, la Organización Mundial de Sanidad Animal (OIE), en las normas que establece en su *Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres* (el *Manual Terrestre*), incluye técnicas de diagnóstico en laboratorio que suscitan consenso a nivel internacional. Los autores pasan revista a los tipos de prueba recomendados en los capítulos del *Manual Terrestre* relativos específicamente a una determinada enfermedad en relación con los seis propósitos con los que más comúnmente se utilizan las técnicas de diagnóstico, entre ellos la expedición de certificados para el desplazamiento de animales, la confirmación de casos clínicos y la vigilancia de enfermedades. Las pruebas recomendadas con más frecuencia para este conjunto de seis propósitos son el ensayo inmunoenzimático y/o la reacción en cadena de la polimerasa, para cuya validación se ofrecen detalladas indicaciones en el *Manual Terrestre* de la OIE. Esto se aplica a todas las especies, pues no se ha observado ninguna barrera a la validación asociada a una u otra especie que tenga que ver con el tipo de prueba. En las recomendaciones del *Manual Terrestre* siguen estando bien representadas las técnicas clásicas, a la par que las tecnologías novedosas van cobrando aceptación con lentitud. A veces la validación de estas pruebas clásicas presenta dificultades por la escasez de protocolos armonizados y de reactivos de referencia a nivel internacional.

Palabras clave

Detección de agentes infecciosos – *Manual de las Pruebas de Diagnóstico y de las Vacunas para los Animales Terrestres – Manual Terrestre* – Organización Mundial de Sanidad Animal – Propósitos – Pruebas de diagnóstico – Respuesta inmunitaria – Validación.

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Table I

Tests to establish a population's freedom from infection in different species

Test	Ab ELISA	PCR	VN/PRNT	Culture	Ag ELISA	Immunolabelling	Visualisation	Agglutination	CFT	Precipitation	Haemagglutination	Immunolabelling	Immunoblot	Miscellaneous [a]
Species	**Immune**	**Agent**	**Immune**	**Agent**	**Agent**	**Immune**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Agent**	**Immune**	**Both**
Apinae	0	6	0	2	2	0	4	0	0	0	0	0	0	0
Aves	7	8	3	1	2	0	0	2	0	1	1	0	1	2
Bovinae	8	6	2	2	2	1	2	0	1	1	0	1	1	0
Caprinae	7	4	3	1	1	0	0	0	3	1	0	0	0	0
Equidae	5	3	1	1	0	2	0	0	1	1	1	0	0	1
Leporidae	3	1	0	0	0	0	0	0	0	0	1	0	0	0
Multiple species	23	6	9	1	0	3	2	6	1	1	1	2	0	4
Other diseases	2	1	1	2	1	0	1	1	0	0	0	0	0	0

Suidae	6	6	0	1	2	4	0	0	0	0	0	0	1	0
Total	61	41	19	11	10	10	9	9	6	5	4	3	3	7
%	31%	21%	10%	6%	5%	5%	5%	5%	3%	3%	2%	2%	2%	4%

a) Miscellaneous tests include loop-mediated isothermal amplification and skin tests. Miscellaneous tests for detection of immune response include micro-array, skin tests and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table II

Tests to establish a population's freedom from infection, for different types of infectious agents

Test	Ab ELISA	PCR	VN/PRNT	Culture	Ag ELISA	Immunolabelling	Visualisation	Agglutination	CFT	Precipitation	Haemagglutination	Immunolabelling	Immunoblot	Miscellaneous [a]
Type	Immune	Agent	Immune	Agent	Agent	Immune	Agent	Immune	Immune	Immune	Immune	Agent	Immune	Both
Bacterium	12	12	0	9	5	2	2	7	3	2	0	1	0	6
Fungus	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mycoplasma	3	2	0	2	0	0	0	0	3	0	0	0	1	0
Parasite	6	6	0	0	0	3	7	1	0	0	0	0	0	0
Protozoan	1	1	0	0	0	1	0	0	0	0	0	0	0	0
Virus	39	19	19	0	5	4	0	1	0	3	4	2	1	3
Total	61	41	19	11	10	10	9	9	6	5	4	3	2	9
%	31%	21%	10%	6%	5%	5%	5%	5%	3%	3%	2%	2%	1%	5%

a) Miscellaneous includes loop-mediated isothermal amplification and skin tests. Miscellaneous tests for detection of immune response include micro-array, skin tests and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table III

Tests to demonstrate an individual animal's freedom from infection prior to movement, in different species

Test	PCR	Ab ELISA	Culture	VN/PRNT	Ag ELISA	Visualisation	Immunolabelling	Agglutination	CFT	Haemagglutination	Precipitation	Immunoblot	Miscellaneous [a]
Species	**Agent**	**Immune**	**Agent**	**Immune**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Both**
Apinae	6	0	2	0	2	4	0	0	0	0	0	0	0
Aves	9	3	6	4	4	0	0	0	0	1	1	0	3
Bovinae	11	6	3	3	2	3	2	0	1	0	1	1	2
Caprinae	6	6	3	3	2	0	0	0	2	0	2	1	0
Equidae	8	4	5	1	1	0	1	0	2	2	1	1	1
Leporidae	1	3	0	0	0	0	0	0	0	1	0	0	1
Multiple species	12	14	5	8	0	1	2	6	1	1	0	0	3
Other diseases	0	1	1	1	0	1	0	0	0	0	0	0	0

Suidae	7	7	4	2	2	0	4	0	0	0	0	1	0
Total	60	44	29	22	13	9	9	6	6	5	5	4	10
%	27%	20%	13%	10%	6%	4%	4%	3%	3%	2%	2%	2%	5%

a) Miscellaneous includes immunolabelling, haemagglutination, loop-mediated isothermal amplification, rodent inoculation, skin tests, micro-arrays and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table IV

Tests for different types of infectious agents, to demonstrate an individual animal's freedom from infection prior to movement

Test	PCR	Ab ELISA	Culture	VN/PRNT	Ag ELISA	Visualisation	Immunolabelling	Agglutination	CFT	Haemagglutination	Precipitation	Immunoblot	Miscellaneous [a]
Type	**Agent**	**Immune**	**Agent**	**Immune**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Both**
Bacterium	9	5	9	0	4	2	1	4	3	0	1	0	4
Fungus	1	0	0	0	0	0	0	0	0	0	0	0	0
Mycoplasma	2	2	1	0	0	0	0	0	2	0	0	2	0
Parasite	11	2	0	0	0	7	3	1	1	0	0	0	2
Protozoan	1	1	0	0	0	0	1	0	0	0	0	0	0
Virus	36	34	19	22	9	0	4	1	0	5	4	2	4
Total	60	44	29	22	13	9	9	6	6	5	5	4	10
%	27%	20%	13%	10%	6%	4%	4%	3%	3%	2%	2%	2%	5%

a) Miscellaneous includes immunolabelling, haemagglutination, loop-mediated isothermal amplification, rodent inoculation, skin tests, micro-arrays and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: Virus neutralisation test/plaque reduction neutralisation test

Table V

Tests that contribute to eradication policies in different species

Test	Ab ELISA	PCR	Culture	Ag ELISA	VN/PRNT	Visualisation	Immunolabelling	Agglutination	Immunolabelling	Precipitation	CFT	Immunoblot	Haemagglutination	Miscellaneous [a]
Species	**Immune**	**Agent**	**Agent**	**Agent**	**Immune**	**Agent**	**Immune**	**Immune**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Both**
Aves	7	9	2	3	3	0	0	2	0	2	0	1	1	1
Bovinae	9	6	2	3	3	4	2	0	2	1	1	1	0	2
Caprinae	6	5	2	3	2	0	0	0	1	2	2	0	0	2
Equidae	6	4	2	1	0	0	2	0	0	1	1	2	1	1
Leporidae	3	2	0	1	0	0	0	0	0	0	0	0	1	0
Multiple species	17	9	3	2	8	2	1	5	2	0	1	0	1	2
Other diseases	1	1	2	1	0	1	0	1	0	0	0	0	0	0
Suidae	6	8	3	1	1	0	4	0	1	0	0	1	0	0
Total	55	50	18	17	17	11	9	8	6	6	5	5	4	8

%	25%	23%	8%	8%	8%	5%	4%	4%	3%	3%	2%	2%	2%	4%

a) Miscellaneous includes immunoblot, agglutination, loop-mediated isothermal amplification, rodent inoculation, micro-arrays and skin tests

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: Virus neutralisation test/plaque reduction neutralisation test

Table VI

Tests for different types of infectious agents, which contribute to eradication policies

Test	Ab ELISA	PCR	Culture	VN/PRNT	Ag ELISA	Visualisation	Immunolabelling	Agglutination	Precipitation	Immunolabelling	CFT	Immunoblot	Haemagglutination	Miscellaneous [a]
Type	Immune	Agent	Agent	Immune	Agent	Agent	Immune	Immune	Immune	Agent	Immune	Immune	Immune	Both
Bacterium	9	10	8	0	5	2	1	7	1	1	3	2	0	0
Fungus	1	1	1	0	0	0	1	0	0	0	0	0	1	1
Mycoplasma	2	0	1	0	0	0	0	0	0	0	2	1	0	0
Parasite	4	6	1	0	1	9	2	0	0	0	0	0	0	1
Prion	0	0	0	0	1	0	0	0	0	2	0	0	0	2
Protozoan	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Virus	38	32	7	17	10	0	4	1	5	3	0	2	3	4
Total	55	50	18	17	17	11	9	8	6	6	5	5	4	8

%	25%	23%	8%	8%	8%	5%	4%	4%	3%	3%	2%	2%	2%	4%

a) Miscellaneous includes immunoblot, agglutination, loop-mediated isothermal amplification, rodent inoculation, micro-arrays and skin tests

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table VII

Tests for the confirmation of clinical cases in different species

Test	PCR	Culture	Ab ELISA	Ag ELISA	Visualisation	Immunolabelling	VN/PRNT	CFT	Agglutination	Haemagglutination	Immunolabelling	Histopathology	Precipitation	Miscellaneous [a]
Species	**Agent**	**Agent**	**Immune**	**Agent**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Agent**	**Immune**	**Both**
Apinae	6	2	0	2	4	0	0	0	0	0	0	0	0	0
Aves	14	11	1	6	1	3	1	0	0	1	0	2	0	2
Bovinae	14	5	4	2	8	3	1	1	0	0	0	0	1	3
Caprinae	12	7	4	3	0	2	3	3	1	0	0	1	2	3
Equidae	10	7	6	2	2	0	3	4	0	3	2	0	1	3
Leporidae	2	1	2	1	2	1	0	0	0	0	0	0	0	0
Multiple species	28	20	11	5	5	4	4	3	5	1	3	2	0	5
Other diseases	5	4	1	1	3	2	0	0	1	0	1	0	0	0

Suidae	10	7	3	3	0	2	2	1	0	1	0	0	0	1
Total	101	64	32	25	25	17	14	12	7	6	6	5	4	17
%	30%	19%	10%	7%	7%	5%	4%	4%	2%	2%	2%	1%	1%	5%

a) Miscellaneous includes immunoblot, skin tests, agglutination, lateral flow, complement fixation test, micro-array, *in situ* hybridisation, mass spectrometry, rodent inoculation, immunochromatographic assay, native hapten and cytosol protein-based tests and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table VIII

Tests for different types of infectious agents to confirm clinical cases

Test	PCR	Culture	Ab ELISA	Ag ELISA	Visualisation	Immunolabelling	VN/PRNT	CFT	Agglutination	Haemagglutination	Immunolabelling	Histopathology	Precipitation	Miscellaneous [(a)]
Type	**Agent**	**Agent**	**Immune**	**Agent**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Agent**	**Immune**	**Both**
Bacterium	23	19	7	5	4	3	0	3	4	0	2	1	1	3
Fungus	1	1	1	0	0	0	0	0	0	1	1	0	0	1
Mycoplasma	4	3	3	0	0	0	0	3	1	0	0	0	0	2
Parasite	14	3	1	1	15	0	0	1	1	0	1	0	0	2
Prion	0	0	0	0	0	2	0	0	0	0	0	0	0	2
Protozoan	3	0	2	1	2	1	0	0	1	0	2	0	0	0
Virus	56	38	18	18	4	11	14	5	0	5	0	4	3	7
Total	101	64	32	25	25	17	14	12	7	6	6	5	4	17

%	30%	19%	10%	7%	7%	5%	4%	4%	2%	2%	2%	1%	1%	5%

a) Miscellaneous includes immunoblot, skin tests, agglutination, lateral flow, complement fixation test, micro-array, *in situ* hybridisation, mass spectrometry, rodent inoculation, immunochromatographic assay, native hapten and cytosol protein-based tests and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table IX

Tests to determine prevalence of infection during surveillance, in different species

Test	Ab ELISA	PCR	VN/PRNT	Culture	Immunolabelling	Ag ELISA	Visualisation	Agglutination	CFT	Haemagglutination	Immunolabelling	Precipitation	Miscellaneous [(a)]
Species	**Immune**	**Agent**	**Immune**	**Agent**	**Immune**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Agent**	**Immune**	**Both**
Apinae	0	4	0	2	0	2	4	0	0	0	0	0	0
Aves	5	8	2	4	0	3	0	1	0	1	0	1	3
Bovinae	9	5	2	1	4	3	3	0	1	0	2	1	2
Caprinae	7	9	3	1	0	1	0	0	3	0	1	2	1
Equidae	8	5	3	2	3	1	1	0	2	3	0	1	2
Leporidae	3	0	0	0	1	0	0	0	0	1	0	0	0
Multiple species	24	14	10	3	3	1	2	7	1	1	2	1	3

Other diseases	4	2	1	2	1	2	2	1	0	0	1	0	0
Suidae	6	8	2	3	4	2	0	0	0	1	0	0	1
Total	66	55	23	18	16	15	12	9	7	7	6	6	12
%	26%	22%	9%	7%	6%	6%	5%	4%	3%	3%	2%	2%	5%

a) Miscellaneous includes immunoblot, skin tests, haemagglutination, loop-mediated isothermal amplification, micro-arrays and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table X

Tests to determine prevalence of infection with different types of infectious agents

Test	Ab ELISA	PCR	VN/PRNT	Culture	Immunolabelling	Ag ELISA	Visualisation	Agglutination	CFT	Haemagglutination	Immunolabelling	Precipitation	Miscellaneous [(a)]
Type	**Immune**	**Agent**	**Immune**	**Agent**	**Immune**	**Agent**	**Agent**	**Immune**	**Immune**	**Immune**	**Agent**	**Immune**	**Both**
Bacterium	14	16	0	9	2	5	2	6	3	0	1	2	4
Fungus	1	1	0	1	1	0	1	0	0	1	0	0	0
Mycoplasma	3	2	0	0	0	0	0	0	3	0	0	0	1
Parasite	6	5	0	2	6	1	8	1	1	0	0	0	1
Prion	1	0	0	0	0	1	0	0	0	0	2	0	2
Protozoan	3	2	0	0	2	1	1	1	0	0	1	0	0
Virus	38	29	23	6	5	7	0	1	0	6	2	4	4

Total	66	55	23	18	16	15	12	9	7	7	6	6	12
%	26%	22%	9%	7%	6%	6%	5%	4%	3%	3%	2%	2%	5%

a) Miscellaneous includes immunoblot, skin tests, haemagglutination, loop-mediated isothermal amplification, micro-arrays and single radial haemolysis

Ab ELISA: antibody enzyme-linked immunosorbent assay
Ag ELISA: antigen enzyme-linked immunosorbent assay
CFT: complement fixation test
PCR: polymerase chain reaction
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table XI

Tests to determine immune status in individual animals or populations post vaccination, in different species

Test	Ab ELISA	VN/PRNT	Haemagglutination	Immunolabelling	Precipitation	Agglutination	CFT	Skin test	Immunoblot	Micro-arrays	SRH	IFN-γ release assay
Species	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**	**Immune**
Aves	10	4	2	0	2	2	0	0	1	0	0	0
Bovinae	5	3	0	2	0	0	0	0	0	0	0	0
Caprinae	6	4	0	0	1	0	2	0	1	0	0	0
Equidae	1	4	2	0	0	0	0	1	0	0	1	0
Leporidae	3	0	1	1	1	0	0	0	0	0	0	0
Multiple species	16	12	1	1	1	1	1	1	0	1	0	1
Other diseases	2	1	0	0	0	1	0	0	0	0	0	0

Suidae	4	2	1	2	0	0	0	0	0	0	0	0
Total	47	30	7	6	5	4	3	2	2	1	1	1
%	43%	28%	6%	6%	5%	4%	3%	2%	2%	1%	1%	1%

Ab ELISA: antibody enzyme-linked immunosorbent assay
CFT: complement fixation test
IFN-γ: interferon gamma test
SRH: single radial haemolysis
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test

Table XII

Tests for different types of infectious agents, to monitor immune status in individual animals or populations post vaccination

Test	Ab ELISA	VN/PRNT	Haemagglutination	Immunolabelling	Precipitation	Agglutination	CFT	Skin test	Immunoblot	Immunoblot	Micro-arrays	SRH	IFN-γ release assay
Type	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune	Immune
Bacterium	11	0	0	1	2	3	2	1	1	0	0	0	1
Fungus	0	0	0	0	0	0	0	1	0	0	0	0	0
Mycoplasma	2	0	0	0	0	0	1	0	0	1	0	0	0
Parasite	2	0	0	2	0	0	0	0	0	0	0	0	0
Virus	32	30	7	3	3	1	0	0	0	0	1	1	0
Total	47	30	7	6	5	4	3	2	1	1	1	1	1
%	43%	28%	6%	6%	5%	4%	3%	2%	1%	1%	1%	1%	1%

Ab ELISA: antibody enzyme-linked immunosorbent assay

CFT: complement fixation test
IFN-γ: interferon gamma test
SRH: single radial haemolysis
VN/PRNT: virus neutralisation test/plaque reduction neutralisation test