



World Organisation
for Animal Health
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Application Form for Registration of Diagnostic Kits*

****Application Form for the Certification of Diagnostic Kits validated as fit for specific purposes (Application Form)***

Note: When completed, this document contains confidential business information that is intended solely for the information of the WOAH Secretariat for Registration of Diagnostic Kits (WOAH SRDK) in support of an application for registration. The information in this application must, therefore, must not be disclosed, distributed, or otherwise communicated beyond authorised WOAH SRDK staff and collaborating reviewers without express written approval of the applicant.

Use this form to submit an application to the WOAH Procedure for registration of a diagnostic kit.

Before completing this form and submitting an application, applicants should read “Standard Operating Procedure (SOP) for WOAH Registration of Diagnostic Kits: Guide and Administrative Forms” at <https://www.woah.org/en/what-we-offer/veterinary-products/diagnostic-kits/procedure-for-submission/>.

For official use	
Reference No.	
Date of first submission	
Date of last amendment	
Date accepted	
Note	

Note on Terminology: The nouns ‘Test’, ‘Assay’, and ‘Test Method’ are used synonymously in this document. Subtle or preferred distinctions between these terms are not implied nor should they be assumed. These terms refer to the principles, systematic procedures, and processes required for detection of an analyte.

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Section 1. Guide for Applicants

1.1. Information to fill out this application form

Before filling in this form and submitting an application, applicants should read “Standard Operating Procedure (SOP) for WOAHP Registration of Diagnostic Kits: Guide and Administrative Forms”, available at: <https://www.woah.org/en/what-we-offer/veterinary-products/diagnostic-kits/procedure-for-submission/>.

The WOAHP *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* and the WOAHP *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)* should be also consulted (available on the WOAHP website). [Chapter 1.1.6 Principles and methods of validation of diagnostic assays for infectious diseases](#) of the *Terrestrial Manual* and the corresponding [Chapter 1.1.2](#) of the *Aquatic Manual* deal with principles of validation and therefore should be consulted when filling in this dossier. Supporting chapters [2.2.1](#), [2.2.2](#), and [2.2.3](#) of the *Terrestrial Manual* provide information for validation of fundamentally different assays such as for the detection of [antibodies](#), [antigens](#) and [nucleic acid](#). Chapters [2.2.4](#), [2.2.5](#), [2.2.6](#), [2.2.7](#), and [2.2.8](#) provide an overview and introduction to statistical approaches and validation of tests for wildlife. Summaries of validation dossiers for WOAHP registered assays are provided in *Validation Studies Abstracts* that are posted online at <https://www.woah.org/en/what-we-offer/veterinary-products/diagnostic-kits/the-register-of-diagnostic-kits/>.

As shown in Figure 1, from the WOAHP *Terrestrial Manual* [Chapter 1.1.6 Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases](#), the following parameters have to be addressed to enable an objective and transparent assessment: Intended purpose(s), optimisation and standardisation, analytical sensitivity (ASe) and analytical specificity (ASp), repeatability, cut-off, diagnostic sensitivity (DSe) and diagnostic specificity (DSp), reproducibility, and conclusion about fitness for purpose. It is important that validation information supports the specific purpose, e.g. a screening test would need to show high DSe and a confirmatory test high DSp to conclude fitness for purpose.

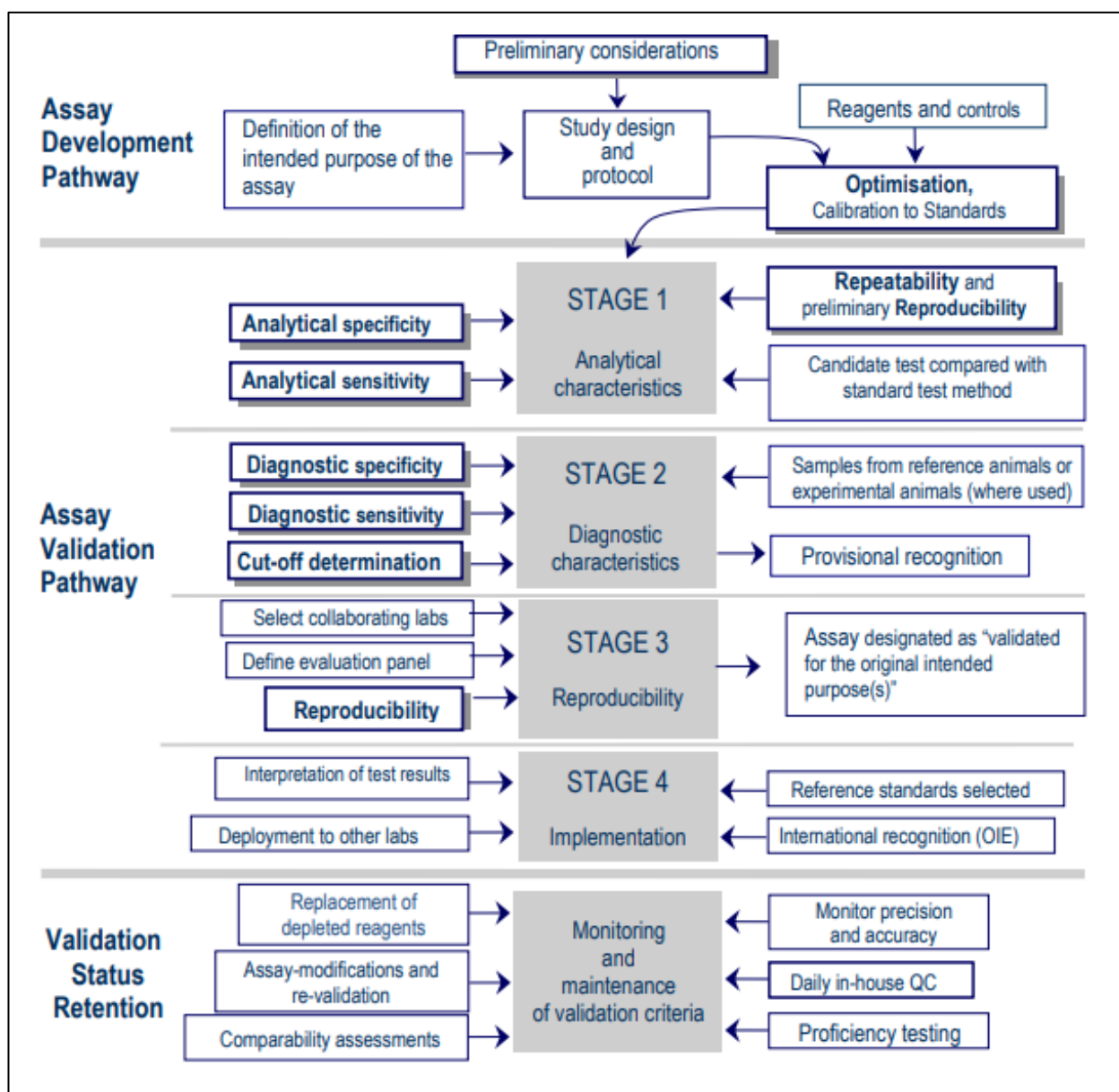


Figure 1: Diagnostic kit assay development and validation pathway.

Source: WOAHP Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 1.1.6 Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases

1.2. Evaluation process by WOAHP

This application will be subjected to formal evaluation by an expert panel appointed by WOAHP, as detailed in the SOP. The WOAHP Secretariat for Registration of Diagnostic Kits (WOAHP SRDK), with support from the experts, will conduct a preliminary screening to check applications for completeness before proceeding with the technical assessment.

Section 2 of this *Application Form* provides general information on the test and its manufacture. Provided assurances are given that the test is manufactured under adequate and acceptable quality control conditions, then Section 2 will not form part of the formal evaluation. As such, the data in the submission is reviewed on the understanding that the data submitted to validate the performance of the test batches is fully representative of the expected performance and batch-to-batch consistency of all subsequently produced batches of the diagnostic kit. Section 3 deals with development and validation with sub-sections for analytical and diagnostic characteristics. Section 4 is a summary of critical data.

The confidential evaluation will be conducted on data submitted in Sections 3 and 4. It is critically important that sub-sections 3.2, 3.3, and 3.4 are fully completed.

1.3. User guide on filling in this form

1. To avoid delays and queries, applicants should fill in all sections of the *Application Form* as completely as possible, and ensure that the data (particularly in Sections 3 and 4) are set out in the specified format. See the WOAH *Terrestrial Manual* [Chapter 1.1.6](#), and Section 2.2 for further guidance.
2. The italic text under each heading is indicative or explanatory of the minimum requirements for the validation template. It should not be considered normative by regulatory bodies using this template for national procedures.
3. In some fields where you are required to select one or more options, double click on a box to switch the option on or off (select “Checked” to answer yes).
4. Type or paste your information inside the yellow box under each field. To keep the yellow background, apply the “Body Text” style for the text (*press Ctrl-Shift-B*). For text consistency, use only “Time New Roman” font, size 10-11 points for normal text.
5. The “Table of Contents” is generated automatically. To update the “Table of Contents”, place the cursor on the Table of Contents area, press F9, select “Update entire table”.

Section 2. General Information

2.1. Information about the applicant

2.1.1. Name of the organisation making this application (*the applicant*)

Organisation	
Address	
Phone	
Fax	
E-mail	

2.1.2. Type of organisation

Double click on a check box to indicate type of organisation. Select 'Checked' to indicate Yes. Select 'Not Checked' to indicate No.

- Commercial
 Institutional
 Governmental
 State/Province
 Federal
 Other: (specify): _____

2.1.3. Name and contact details of the person who will be authorised to represent the applicant regarding this application for registration, and name and contact details of the authorised contact person who would serve as the applicant's post-registration representative for responding to technical and regulatory questions and post-registration surveillance, if it is different.

Contact person	
Job title	
Organisation	<i>(If different from that in 2.1.1)</i>
Address	<i>(If different from that in 2.1.1)</i>
Phone	
Fax	
E-mail	

2.1.4. Name and contact details of manufacturer/producer of the test or test components (*if different from 2.1.3*)

Organisation	
Address	
Phone	
Fax	
Contact person	
Job title	
E-mail	

2.1.5. Name and contact details of post-registration representative (if different from 2.1.3)

Organisation	
Address	
Phone	
Fax	
Contact person	
Job title	
E-mail	

2.1.6. Accreditation or certification status of applicant (where relevant to test performance)

Double click on a check box to indicate accreditation or certification status of applicant. Select 'Checked' to indicate Yes. Select 'Not Checked' to indicate No.

- ISO/IEC 17025 ISO/IEC 9000 series
 GLP/GMP Other: (Specify): _____

2.1.7. Accreditation or certification status of producer(s) (if applicable)

Double click on a check box to indicate accreditation or certification status of producer. Select 'Checked' to indicate Yes. Select 'Not Checked' to indicate No.

- ISO/IEC 17025 ISO/IEC 9000 series
 GLP/GMP Other: (Specify): _____

2.1.8. Declarations and signature

Insert name of responsible person below, to confirm agreement on behalf of the applicant:

We,

hereby declare that we have read and will adhere to the [Standard Operating Procedure for the Validation and Certification of Diagnostic Tests](#) and that we are aware of all of its terms and conditions.

On behalf of the applicant, we hereby declare that a) the data presented in this *Application Form* are an accurate representation of the formulation and performance of the diagnostic kit, such that commercially manufactured batches would be expected to exhibit the same performance characteristics, b) the manufacturer employs a quality assurance system and post-registration monitoring to ensure that all batches meet these standards, and c) only those batches that conform to the established manufacturing and testing standards and 'fitness for purpose' parameters' will be released for distribution and use under the attestations that this test conforms to WOH standards and WOH SRDK validation procedures.

We hereby declare that all the information contained in this application form and all documentation submitted further in support of the application form are true and complete in all respects.

We acknowledge that incomplete *Application Forms* will be returned to the applicant and that the review of the application by a panel of experts will only commence once the application is complete and all required information is provided.

APPLICATION FORM SOP

We understand and agree that any misrepresentation of the information furnished in this *Application Form* will result in the automatic end of the procedure or revocation of the potential certification obtained.

Signature: _____

Name, Position: _____

[Company representative of applicant of the concerned diagnostic test]:

Date: _____

2.2. Name and purpose of the diagnostic kit

2.2.1. Type of method

Indirect or competitive ELISA, conventional or real-time PCR, etc.

2.2.2. Commercial name (if applicable)

2.2.3. Intended purpose(s) of the test

Where possible, please select the specific purpose(s) of the test from the list of intended purposes provided below. If none of these purposes are suitable, please select "Other", and describe the purpose using similar terminology to that of the listed options below. Specific details on the intended use of the test will be prompted in Section 3.1. below. Suitable data need to be provided to substantiate fitness for each selected purpose in the application.

Double click on a check box to indicate the purpose of the test. Select 'Checked' to indicate Yes. Select 'Not Checked' to indicate No.

Intended Fitness for Purpose

- 1 Demonstrate freedom from infection in a defined population (country/zone/compartments/herd)
 - 1a "Free" with vaccination
 - 1b Historical freedom
 - 1c Re-establishment of freedom after outbreaks
- 2 Certify freedom from infection or agent in individual animals or products for trade/movement purposes
- 3 Eradication of infection from defined populations
- 4 Confirmatory diagnosis of clinical cases (includes confirmation of suspect cases and a positive screening test)
- 5 Estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control)
- 6 Determine immune status in individual animals or populations (post-vaccination)
- 7 Other [please specify]*: _____

* Detection of antigen, antibody, or nucleic acid associated with current infection or an immune response to previous exposure or vaccination in an individual animal, group of animals, or a defined population. For use in conjunction with other tests or diagnostic procedures, as an aid in diagnosis or other clinical or epidemiological assessments.

Note: Interpretation of test results may vary according to history, clinical signs, pathological lesions, epidemiological context, and results of other diagnostic tests.

2.3. Test description and requirements

2.3.1. Protocol of the test

Include your working protocol here as would be given to laboratory analysts. If applicable, provide instructions that would be included with the commercial test. Please include information on the protocol for interpretation of test results, i.e. colour changes or lines to indicate positive, negative, or indeterminate results.

2.3.2. Disease target/analyte target

State targets in analytical terms (e.g. antibody isotype and specificity, gene sequence and association, etc.)

2.3.3. Target species and specimens

Species and specimens that can be examined (e.g. swine serum, bull semen, fish kidney). List only those for which you have sufficient validation data. Describe briefly the recommended procedures for acquiring, preserving and shipping specimens for the test.

2.3.4. Controls included

Describe the positive and negative control materials in the test, including source and test activity.

2.3.5. End-User Requirements (laboratory or field use)

Describe minimum laboratory requirements for optimal test performance; include laboratory biocontainment/biosafety requirements, environmental conditions (temperature and humidity ranges), equipment, chemical and/or biological requirements (not specified in the protocol or included in the test).

2.3.6. Computational requirements (if applicable)

Describe hardware and software requirements for assay operation and data processing. Indicate what is supplied and what is not.

2.3.7. Test kit format (if applicable)

For commercial tests, outline the number of samples that can be tested with one kit. Describe any flexibility in kit formats that would accommodate various test throughput volumes (e.g. multi-well plate vs. strip formats).

2.3.8. General precautions/ safety aspects/disposal of reagents

List potential health hazards and safety precautions, refer to Material or Biological Safety Data Sheets if necessary.

2.4. Technical information about the diagnostic test

2.4.1. Chemical reagents

List all chemical reagents specified in the test protocol or supplied with test, indicate their use, and briefly describe composition and characteristics in chemical terms (e.g. buffer formula, molarity, pH, etc.).

Reagent	Use in assay	Description

2.4.2. Equipment and consumables included (if applicable)

List all pieces of equipment supplied with the test, indicate their use, and briefly describe operating characteristics (e.g. accuracy, precision, etc.).

Equipment name	Use in assay	Description

2.4.3. Biological components used in test

List each biological component, its function in the test, and briefly describe its origin, composition, and presentation in biological terms (e.g. affinity purified, rabbit anti-bovine IgG, etc.). Biological agents should be described as live or killed. Note any other applicable guidance for safe handling of diagnostic reagents.

Component	Use in assay	Description

2.4.4. Control of the final product of the test (if applicable)

Briefly describe the method used to document and approve a serial or batch release of the test, include description of (reference) reagents/panels used to assess test performance including the number of serials or batches tested to assure quality and batch-to-batch consistency.

2.4.5. Shipping requirements

List conditions and precautions required for shipping, include critical factors that may adversely affect test performance e.g., how does time, humidity, and temperature impact kit stability?

2.4.6. Troubleshooting and technical support (if applicable)

Indicate scope, format, and availability of technical support.

Section 3. Development and Validation of the Assay

Please read the text in Section 1 before completing the form.

3.1. Assay Development Pathway

3.1.1 Intended purpose(s) of use

The design of the test must be consistent with its intended purpose and the population for which it is intended. Please refer to the discussion of this topic in the WOAAH Terrestrial Manual [Chapter 1.1.6 Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases](#) and the corresponding [Chapter 1.1.2](#) of the WOAAH Aquatic Manual for an overview of 'reasons for test'. These chapters provide a list of the most common purposes, and a flow chart that summarises the key steps in the assay development validation pathway for diagnostic kits. Give a clear summary description of the specific purposes of use that this kit has been validated for based on answers to question 2.2.3.

3.1.2. Design, development, optimisation and standardisation of the assay

Assay design, development, optimisation and standardisation, as well as validation, must be based on sound scientific principles and carried out using best practices, leading to a validated assay that is publishable in peer reviewed journals. For guidance, refer to Chapters [2.2.1 Development and optimisation of antibody detection assays](#), [2.2.2 Development and optimisation of antigen detection assays](#), and [2.2.3 Development and optimisation of nucleic acid detection assays](#) of the WOAAH Terrestrial Manual relevant to the type of test being certified. The Review Panel, as part of the review process, may ask for documents on, for instance, the statistical methods and conclusions reached in drawing inferences relative to reagent optimisation/standardisation or result interpretations (any factors which may affect data acceptance and interpretation of the test result) – or any other data deemed essential to drawing conclusions about the validity of the test.

3.2. Validation Pathway Stage 1 - Analytical characteristics

3.2.1. Stage 1. Repeatability data

Repeatability is the level of agreement between replicates of a sample both within (intra-assay) and between (inter-assay) runs of the same test method in a single laboratory. Repeatability is estimated by evaluating variation in results of replicates. The number of replicates should preferably be determined in consultation with a statistician with a suggested minimum of three different samples representing analyte activity within the operating range of the assay. Within or intra-assay variation can be assessed using three or more replicates of each sample in one run (one operator). Intra-assay and inter-assay variation can be assessed by testing the panel of samples over several days, using two or more operators, e.g. for a total of 10-20 runs.

The data/detail provided must be clear, including:

- i) the number of different isolates use, ideally minimum of three covering analytical range of test (strong/moderate/weak)*
- ii) the number of replicates per sample for intra-assay and inter-assay analysis*
- iii) the number of different operators used at a single site*

For repeatability of PCR, all replicates are treated like individual diagnostic samples subject to individual extraction and testing. Therefore, for PCR the WOAAH Terrestrial Manual recommends using virus and including independent extraction step in each measure of repeatability, so that repeatability assesses both extraction and PCR (WOAH Terrestrial Manual [Chapter 2.2.3](#)).

For serology, all replicates are treated like individual diagnostic samples including preparation of any working dilutions. For serology, a minimum of three different sera, covering analytical range should be assessed (WOAH Terrestrial Manual [Chapter 2.2.1](#)).

Implicit to the testing of repeatability is that the samples used are homogeneous and stable for the period of testing, so that any variation in repeated testing reflects variation of the assay and not heterogeneity and/or stability of the samples.

3.2.2. Stage 1. Analytical specificity data (as appropriate for the test type and disease)

Analytical specificity is the degree to which the assay distinguishes between the target analyte and other components in the sample matrix; the higher the analytical specificity, the lower the level of false positives. The assessment of analytical specificity is qualitative, and the choice and sources of sample types, organisms and sequences for the assessment should reflect test purpose and assay type. Analytical specificity is further characterised by determining:

- a) selectivity, which is the extent to which a method can accurately quantify the targeted analyte in the presence of interferents, for example, a) of matrix components such as inhibitors of enzymes in the reaction mix, b) degradants (toxic factors), c) nonspecific binding of reactants to a solid phase, e.g. conjugate of an ELISA absorbed to well of microtiter plate, and d) antibodies to vaccination that may be confused with antibodies to active infection,*
- b) exclusivity, which is the capacity of the assay to detect an analyte or genomic sequence that is unique to a targeted organism, and excludes all other known organisms that are potentially cross-reactive. This would also define a confirmatory assay, and*
- c) inclusivity, which is the capacity of an assay to detect several strains or serovars of a species, several species of a genus, or a similar grouping of closely related organisms or antibodies thereto. It characterises the scope of action for a screening assay.*

3.2.3. Stage 1. Analytical sensitivity data

Analytical sensitivity is synonymous with 'Limit of Detection', the smallest detectable amount of analyte in a specified matrix that would produce a positive result with a defined certainty. An analyte may include antibodies, antigens, nucleic acids, or live organisms. The WOAHS Terrestrial Manual [Chapter 2.2.1](#) suggests each dilution in the series should be tested in 10 replicates, however, three to five replicates are acceptable. The dilution series must extend to at least one dilution past end-point (negative/not detectable). Criteria for end-point dilution must be established, e.g. the end-point is the last dilution for which all replicates are positive. A precise estimate of ASe is often not available for assays for infectious diseases, except for PCR where it is possible to calculate the threshold number of copies of a target nucleic acid sequence that can be detected by the assay. Alternatively, it is possible to compare the limit of detection between the candidate and reference test to obtain a relative estimate for ASe.

3.2.4. Stage 1. Standard of comparison

For a preliminary evaluation, the standard method(s) of comparison (reference standard) should be run in parallel on a small but select group of highly characterised test samples representing the linear operating range of the new method(s). Identify and cite the reference method(s) and protocol(s) used in the study.

3.2.5. Stage 1. Preliminary evaluation of reproducibility

Reproducibility is the ability of a test method to provide consistent results when applied to aliquots of the same sample tested by the same method in different laboratories. Where possible, the reproducibility assessment should include data from tests conducted at a WOAHP Reference Laboratory or national laboratory. For provisional recognition where it is not possible to complete Stages 2 or 3, a preliminary evaluation of reproducibility must be presented. This will be a scaled down version of Stage 3 and may utilise the test samples used in 3.2.4. above. The panel should contain at least 20 samples and at least three laboratories should participate in the reproducibility testing. Further information is available in the WOAHP Terrestrial Manual [Chapter 2.2.6 Selection and use of reference samples and panels](#). Laboratories participating in this type of study should be known to the test developer and may be in close geographic proximity.

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3.3. Stage 2 – Diagnostic characteristics

3.3.1. Study design(s)

(Note: Several approaches may be taken in the determination of diagnostic sensitivity and specificity estimates. The most suitable and/or feasible approach for any given disease agent and host should be considered. The availability of reference animals or reference populations will have the greatest impact on the approach. **Therefore, once decided, only those applicable sections below need be completed.**) A clear case definition needs to be developed, e.g., what constitutes an infected and what constitutes a non-infected animal and applied consistently throughout the application, e.g. for 3.3.2 and 3.3.3 below.)

Ideally, study design(s) should be done with the assistance of a statistician and a disease expert to ensure that the sample size and the experimental approach are valid.

Ideally, estimates of diagnostic characteristics should be derived from testing a panel of samples from reference animals of known history and infection status relative to the disease/infection in question (source population) and relevant to the country or region in which the test is to be used (target population), and for the purpose of use. If more than one purpose was selected it is necessary to provide supporting data for each purpose. Reference samples may be obtained from the field or from experimentally infected animals as appropriate to the nature of the disease. Their key characteristic is that their true status (positive/negative etc) should be independently verified by a different technique.

The “source/population of samples used” should be indicated by selecting the appropriate tick box responses noted below:

- a) reference animal populations
- b) animals of unknown status (in which case a reference test or latent class analysis would be used)
- c) experimentally infected or vaccinated reference animals
- d) other [please specify]: _____

Give an overview of the chosen approach used for determination of diagnostic specificity and sensitivity estimates. Include rationale for statistical design, choice of populations, animals or animal models, numbers of animals used to generate confidence intervals for sensitivity and specificity etc. See Table 1. For example, an assay with an expected DSe of 90%, would need to test 864 infected animals to be 95% confident about the result (DSe 90%) and with a 2% error margin. A higher error margin, e.g. 5%, decreases the number of infected animals to be tested, e.g. $n=138$. Numbers of non-infected animals to estimate DSp are calculated in the same way.

Table 1: Theoretical number of samples from animals of known infection status required for establishing diagnostic sensitivity (DSe) and specificity (DSp) estimates depending on likely value of DSe or DSp and desired error margin and confidence.

Estimated DSe or DSp	2% error allowed in estimate of DSe and DSp			5% error allowed in estimate of DSe and DSp		
	Confidence			Confidence		
	90%	95%	99%	90%	95%	99%
90%	610	864	1493	98	138	239
92%	466	707	1221	75	113	195
94%	382	542	935	61	87	150
95%	372	456	788	60	73	126
96%	260	369	637	42	59	102
97%	197	279	483	32	45	77
98%	133	188	325	21	30	52
99%	67	95	164	11	15	26

Source: WOAHP Terrestrial Manual Chapter 1.1.6 Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases.

3.3.2. Stage 2. Negative reference animals/samples

(Note: Negative refers to lack of exposure to, or infection with, the agent in question). Complete description: age, sex, breed, etc. Representativeness of intended target populations consistent with the proposed purpose(s) of use. Selection criteria including historical, epidemiological and/or clinical data. Pathognomonic and/or surrogate tests used to define status of animals or prevalence within population. Sampling plan and procedures.

3.3.3. Stage 2. Positive reference animals/samples

(Note: Positive refers to known exposure to, or infection with, the agent in question). Complete description: age, sex, breed, clinical signs if evident, etc. Representativeness of intended target population consistent with the proposed purpose(s) of use. Selection criteria including historical, epidemiological and/or clinical data. Pathognomonic and/or surrogate tests used to define status of animals or prevalence within population. Sampling plan and procedures.

3.3.4. Stage 2. Experimental animals (where used)

(Note: Experimental animals maybe be used when it is not possible to define or obtain sufficient positive reference animals from the field.) Representativeness of intended target population. Complete description: age, sex, breed, etc. Immunological status. Type of exposure, inoculation, source, aerosol, contact, sampling plan and procedures, etc.

3.3.5. Stage 2. Threshold determination

Complete description of method used to determine thresholds (cut-off(s)) used to classify animals as test positive, negative, or indeterminate (if relevant). Include statistical calculations, frequency distributions, receiver operating characteristic (ROC) analysis, etc. as applicable. See WOAHS Terrestrial Manual [Chapter 1.1.6](#) and [Chapter 2.2.5](#).

3.3.6. Stage 2. Diagnostic sensitivity and specificity estimates – with defined reference animals

Complete either 3.3.6 if defined reference animals were used, or 3.3.7 if a latent class model was used.

Diagnostic sensitivity is the proportion of known infected reference animals that test positive in the assay; infected animals that test negative are considered to have false-negative results. Diagnostic specificity is the proportion of known uninfected reference animals that test negative in the assay; uninfected reference animals that test positive are considered to have false-positive results. Please include a 2x2 table and confidence intervals for estimates of these parameters.

For quantitative diagnostic tests, a useful adjunct to estimates of diagnostic sensitivity and specificity is an estimate of the area under the receiver operating characteristic (ROC) curve. Please include this information where relevant.

3.3.7. Stage 2. Diagnostic sensitivity and specificity estimates – without defined reference animals

Complete either 3.3.6 if defined reference animals were used, or 3.3.7 if a latent class model was used.

Complete description of latent class model used (Bayesian or maximum likelihood). Describe rationale for use of this approach, and sources of priors (e.g. experts and published papers) for Bayesian models providing relevant, supporting data. Population selection criteria should be presented, including prevalence estimates. Other test methods evaluated should also include the standard method of comparison. The source data tables with cross-classified test results should be presented for each test population. Using best available priors, choose test populations with appropriate prevalences and select animals in sufficient numbers to generate estimates of sensitivity and specificity with an allowable error of $\pm 5\%$ at a level of 95% confidence. If multiple laboratories are involved in the study design, data on reproducibility should be presented in Section 3.4.3.

3.3.8. Stage 2. Comparison of performance between tests

For standard method(s) of comparison (reference methods) used in full field studies, indicate diagnostic sensitivity and specificity estimates as determined in either Section 3.3.6 or 3.3.7. The reference method could also be used to calculate relative DSe and DS_p of the candidate test. Provide statistical measures of agreement between the reference method(s) and the new test being validated and suggest explanations for results not in agreement.

3.4. Stage 3 - Reproducibility

Reproducibility is the ability of a test method to provide consistent results when applied to aliquots of the same sample tested by the same method in different laboratories. This is the same definition found in Section 3.2.5; however, Stage 3 is more international in scope and is a better indicator of the ruggedness of the test method. Ruggedness is a measure of an assay’s capacity to remain unaffected by substantial changes or substitutions in test conditions anticipated in multi-laboratory utilisation, part of fitness studies and reproducibility assessments (e.g. shipping conditions, technology transfer, reagents batches, equipment, testing platforms, and/or environments). To assess the reproducibility of an assay, each of at least three laboratories should test the same panel of samples (blinded) containing a suggested minimum of 20 samples, with identical aliquots going to each laboratory (see [Chapter 2.2.6](#) of the WOAH *Terrestrial Manual*).

3.4.1. Stage 3. Laboratory identification

Selection criteria for laboratories involved in the reproducibility study. Location, i.e. country. Status, i.e. regional, national, provincial/state. Level of expertise, familiarity with technology. Accreditation status. State the number of laboratories included (minimum of three) which should also include WOAH Reference Laboratories or Collaborating Centres, or national laboratories where they exist.

3.4.2. Stage 3. Evaluation panel

Description of test panel used for independent reproducibility study (interlaboratory comparisons), nature and number of samples and assessment of homogeneity and stability.

3.4.3. Stage 3. Analysis of reproducibility

Description of reproducibility study, analysis, and interpretation of results.

3.5. Stage 4 - Applications

Stage 4 validation is recognised as an ongoing process that continues for the lifetime of the assay. Although this section gives important information regarding the validation of the diagnostic test, it is not a compulsory requirement for WOAH evaluation. Please complete where the information is available. If information is not available or if not applicable or not done, please indicate this in sections 3.5.1 to 3.5.5.

3.5.1. Stage 4. Test applications

(Note: This Section applies to tests that have been incorporated into routine diagnostic regimens.) Describe functional test applications (i.e. screening, confirmatory, supplemental applications) and integration with other tests into diagnostic regimen. Include flowcharts and decision trees where applicable.

3.5.2. Stage 4. Laboratories

List laboratories where this test method is in current use. Location, i.e. Country. Status, i.e. Regional, national, provincial/state. Accreditation status. For each laboratory, indicate purpose of test, integration with other tests and status of test, i.e. official test, supplementary, etc.

3.5.3. Stage 4. International reference standards

List type and availability of international reference reagents. Source. Negative, weak/strong positive reference reagents. Other key biologicals, e.g. antigens, antibodies, etc.

3.5.4. Stage 4. Inter-laboratory testing programmes

Describe programmes involving inter-laboratory comparisons using this test method. National, international. Describe eligibility and number of laboratories participating.

3.5.5. Stage 4. International recognition

List internationally recognised reference laboratory responsible for this test method and/or biologicals. List international standards containing this test method. List international programmes employing this test method.

Section 4. Performance Summary

4.1. Summary of validation data (*Validation Studies Abstract*)

Provide a concise summary of the validation results using the template below. Please ensure that the information provided below is consistent with the information included elsewhere in the application. This information will be documented in a *Validation Studies Abstract* that serves as a publicly available summary of the validation data for registered kits. The *Validation Studies Abstracts* are referenced when proposing new kits for registration, and once a kit has met the registration requirements, the *Validation Studies Abstract* and a copy of the *User's Manual* are posted on the *WOAH Register of diagnostic kits certified by WOAHA as validated as fit for purpose* at <https://www.woah.org/en/what-we-offer/veterinary-products/diagnostic-kits/the-register-of-diagnostic-kits/>. If the application is successful, the information in Section 4.1 will be placed in the public domain on the WOAHA website.

4.1.1. Summary information on the test

Please provide the following information:

- Name of the diagnostic test:

- Manufacturer:

- Disease:

- Pathogen Agent:

- Type of Assay:

- Purpose of Assay:

- Species and Specimens:

- Provide e-mail address and/or web site where prospective customers may make enquiries and/or view information about the test.

4.1.2. Summary of validation studies

STAGE 1 Validation

Provide a succinct summary of Section 3.2; include statistical data where applicable, e.g. coefficients of variation or upper and lower ranges.

- Repeatability:

- Analytical specificity:

- Analytical sensitivity:

STAGE 2 Validation

Provide a succinct summary of Section 3.3; indicate approach taken in study design for the determination of diagnostic sensitivity and specificity estimates.

- Threshold Determination:

- Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Using tabular format below, indicate diagnostic sensitivity and specificity estimates as determined in either Section 3.3.6 or 3.3.7. If the kit is intended for use in multiple species or specimens, please enter each species/specimen in a separate table.

Test method under evaluation: _____		Target Species/Specimen: _____
Diagnostic sensitivity	N	<i>(Number of animals tested)</i>
	DSe	<i>(DSe estimate)</i>
	CI	<i>(95% confidence interval)</i>
Diagnostic specificity	N	<i>(Number of animals tested)</i>
	DSp	<i>(DSp estimate)</i>
	CI	<i>(95% confidence interval)</i>

- Comparative performance:

Using tabular format below, indicate comparative performance data on diagnostic sensitivity and specificity estimates as determined in either Section 3.3.6 or 3.3.7. If the kit is intended for use in multiple species or specimens, please enter each species/specimen in a separate table.

Test method under evaluation:		Target Species/Specimen:
Diagnostic sensitivity	N DSe CI	(Number of animals tested) (DSe estimate) (95% confidence interval)
Diagnostic specificity	N DSp CI	(Number of animals tested) (DSp estimate) (95% confidence interval)

- Agreement and discrepancies:

Indicate level of agreement and suggest explanations for discrepant results.

STAGE 3 Validation

- Reproducibility:

Briefly describe results of inter-laboratory comparisons, including number of laboratories involved and statistical data for a range of positive and negative samples as outlined in Section 3.4.

STAGE 4 Validation

Validation is recognised as an ongoing process that continues for the lifetime of the test. Ultimately, confidence in test performance builds with successful application in diagnostic laboratories and programmes.

- Applications:

If possible, briefly describe where this test method has been integrated in diagnostic regimens and programmes as outlined in Section 3.5.

4.1.3 References:

Include a short list of references, if available, relevant to the assay performance characteristics stated above. Preferably, these should include relevant recently published manuscripts with estimates of diagnostic sensitivity, diagnostic specificity, and analytical characteristics.

Section 5. User's Manual

The applicant must provide, as part of its application, a copy of the draft labelling and instructions for use (i.e., kit insert or *User's Manual*) and a 'mock-up' or specimen of the diagnostic kit 'ready-to-use' packaging and labelling. A 'mock-up' is a copy of the flat artwork design (computer generated), providing a two-dimensional replica of both the outer and inner packages with labelling. At this stage, the 'mock-up' may be in black and white and in English only. Once the registration requirements have been fulfilled, the final English, French and Spanish versions of the *User's Manual* must be provided for approval and posting on the WOAHA website.

Section 6. Additional Data

Tables of raw data or other supporting information can be provided at the discretion of the applicant. Such material can be helpful to the expert panel in completing their evaluation. If you choose not to provide such information, reviewers may request it in order to clarify their decisions.

The raw data can be provided as separate password protected Microsoft Excel files. You may also provide other documents as PDF files. Please specify the name and purpose for each file and use a meaningful title. If a file is providing data for more than one claim, then indicate each link to the headings in submission form in "what does the file show?" column.

No.	File Name	Links to submission numbers	What does file show? Describe very briefly the intention of the data in the file.
1			
2			
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Add rows as needed.

Section 7. References Cited in the Dossier

List the scientific literature related to the diagnostic test described in this application and cited in this dossier. Use a consistent reference style throughout.



Section 8. Current Registration Status

Please list the WOAHA Members where the submitted application has previously been registered and note any special conditions or restrictions, including registration status in the country where the diagnostic kit is manufactured.