THIS TEMPLATE HAS BEEN ADAPTED SPECFICALLY FOR *NAAHLS* FROM:

OIE Validation and Certification of Diagnostic Assays

Validation Pathway for NAAHLS Diagnostic Test Methods*

Dossier Template

* 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.

Advisory Note:

Before embarking on the validation pathway described herein, it is advised that NAAHLS scientific and technical staff read the current introductory chapter found in either web version of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* or the OIE *Manual of Diagnostic Tests for Aquatic Animals*:

1) Principles of validation of diagnostic assays for infectious diseases, Chpt. 1.1.4/5 (*Terrestrial Manual*) or Chpt. 1.1.2 (*Aquatic Manual*)

Either of these chapters may be downloaded from the website (www.oie.int) under the heading *OIE Expertise – Specialist Commissions* and should be used as companion guidelines for this validation pathway.

_____ _____

Approved by: National Laboratory Manager Date

This signature constitutes an approval of this test method by the National Laboratory Manager of the NAAHLS (National Aquatic Animal Health Laboratory System) as being validated and fit for the intended purpose(s).

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Section 1. Guide for applicants

1.1. General information

The first version of the DFO dossier was based on the original template found in the 2006 version of the Standard Operating Procedure (SOP) for OIE Validation and Certification of Diagnostic Assays. The OIE template is currently undergoing revision and this next DFO version incorporates the modifications to the original OIE version which are yet to be unpublished.

A series of specific guidelines to support the new OIE template are also currently under development. Once finalized, they too will be appended to the DFO version as appropriate.

As with the previous DFO version, the new DFO version reflects minor modifications to the new OIE template validation of tests used by the National Aquatic Animal Health Laboratory System (NAAHLS). The original validation principles have not been altered.

1.2. User guide on filling in this form

1. 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).

2. 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.

3. The "Table of Contents" is generated automatically. To update the "Table of Content", place the cursor on the Table of Content area, press F9, select "Update entire table".

Section 2. General information

2.1. Information about the laboratory

2.1.1. Name of the laboratory responsible for the dossier

Laboratory	
Organization	
Address	

2.1.2. Type of organisation

Double click on a check box to switch the option on or off (to answer Yes, select "Checked").

☐ Federal	☐ Provincial	☐ Institutional	☐ Commercial
☐ Other: (specify)			

2.1.3. Name and contact details of the person responsible for this validation dossier

Contact person	
Job title	
Laboratory *(If different from 2.1.1)*	
Organization *(If different from 2.1.1)*	
Address *(If different from 2.1.1)*	
Phone	
Fax	
E-mail	

2.1.4. Accreditation or certification status of laboratory

Double click on a check box to switch the option on or off (to answer Yes, select "Checked").

☐ OIE Quality Standard	☐ ISO/IEC 17025	☐ ISO/IEC 9000 series
☐ Other: (Specify)		

2.2. Name and purpose of the diagnostic test

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

Check the specific purpose(s) of the test from the list below.
***Note**: Specific details on the intended use of the test will be prompted in Section 3.1. below.*

Double click on a check box to switch the option on or off (to answer Yes, select "Checked").

		Purpose or Application
		National Aquatic Animal Health Program (CFIA/DFO)
☐	1	Domestic Movement
☐	2	Export
☐	3	Import
☐	4	Facility Recognition Program
☐	5	Survey
☐	6	Surveillance
☐	7	Response to a Notification
☐	8	Compartmentalization
☐	9	Other: (specify)
		Aquatic Animal Health Science (DFO)
☐	1	Health profile (FHPR)
☐	2	Export
☐	3	Domestic (I&T)
☐	4	Morbidity/mortality investigation
☐	5	Survey
☐	6	Surveillance
☐	7	Laboratory referral (confirmatory)
☐	8	Other: (specify)

2.3. Test description and requirements

2.3.1. Protocol of the test

Reference current version of test method protocol. Include the full title and current version number below.

2.3.2. Disease/analyte target

State infectious agent and analytical targets (e.g. name of viral/bacterial/parasitic pathogen, strains, lineages etc.; whole organism, antibody, antigen, gene targets, etc.)

2.3.3. Species and specimens

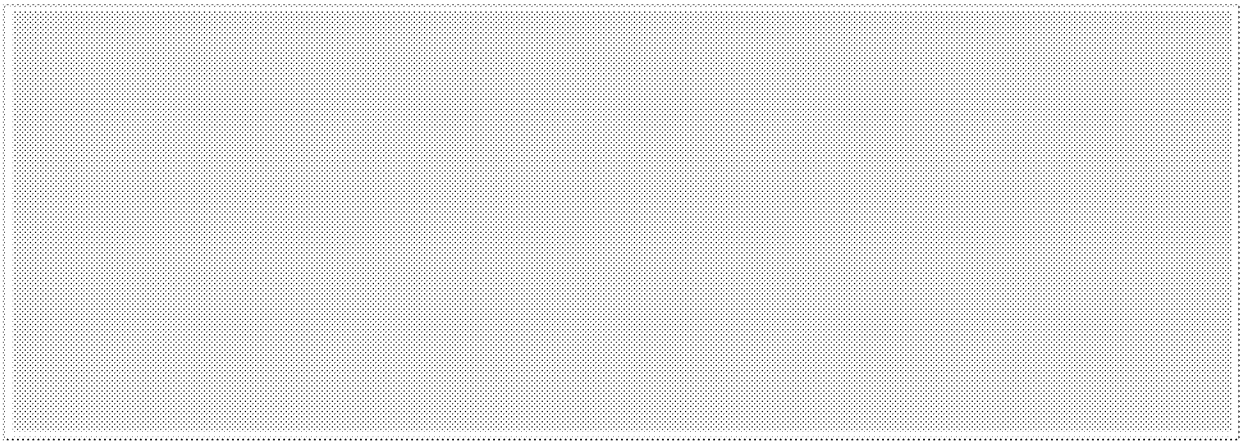
Species and specimens that can be examined (e.g. salmonids, oyster, lobster, etc.; whole animal, kidney, reproductive fluids, gill, etc.). List only those that have been validated sufficiently.

2.3.4. Sampling procedures

Describe briefly the recommended procedures for acquiring, preserving and shipping specimens for the test. The specimen collection protocol may be referenced or appended in Section 5 but include the full title and current version number below.

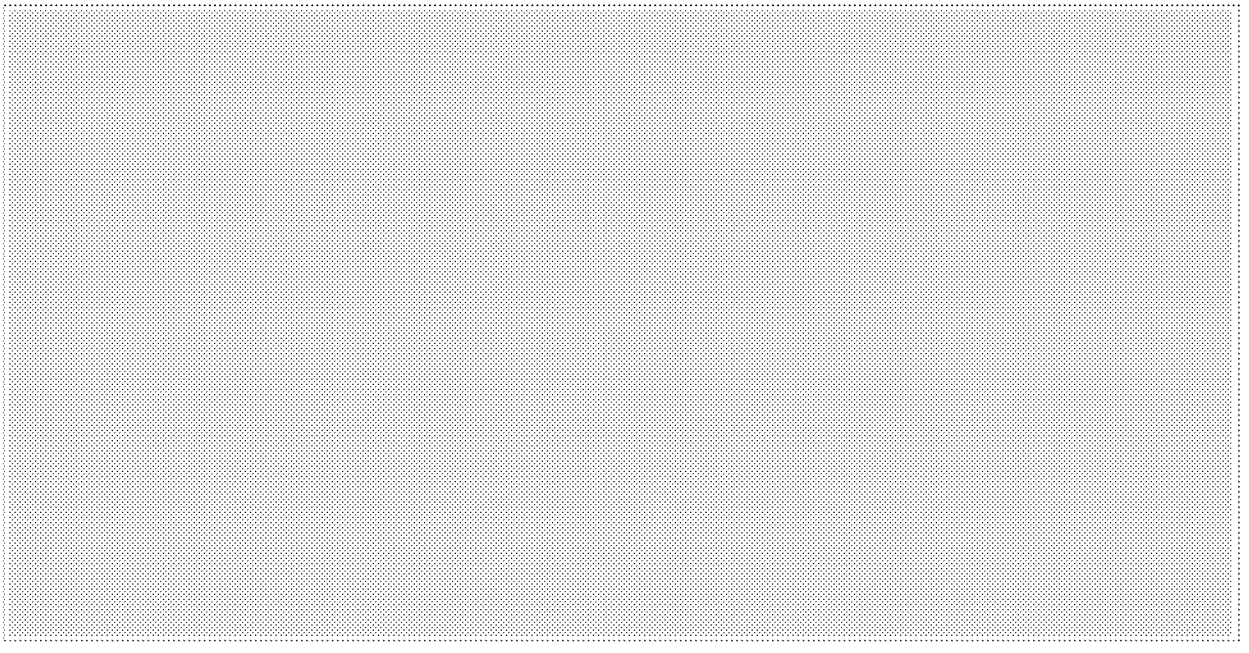
2.3.5. Controls included

If not fully described in the test method protocol, describe positive and negative control materials used in the test, including source, biosafety/biosecurity considerations and test activity.



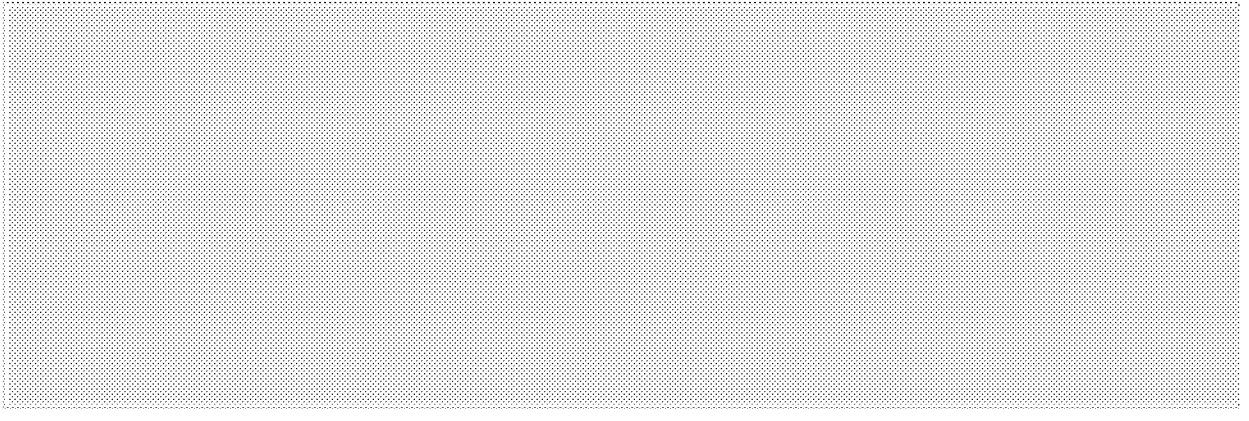
2.3.6. Laboratory requirements

If not specified in the test method protocol, describe minimum laboratory requirements for optimal test performance; include environmental, equipment, chemical and/or biological requisites.



2.3.7. General precautions/ safety aspects/ disposal of reagents

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



Section 3. Development and Validation of the Assay

3.1. Assay Development Pathway

3.1.1 Fitness of assay for its intended purpose

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 Chapter 1.1.4. for an overview of 'reasons for test'. Give a brief description of how you see the test being applied in support of a specific type(s) of testing programmes. Test applications may be relatively broad or highly specific depending on the diagnostic application being targeted.

Note: Design, development, optimization and standardization of the assay

Assay design, development, optimization and standardization, 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. Reviewers, as part of the dossier review process, may ask for documents on, for instance, the statistical methods and conclusions reached in drawing inferences relative to reagent optimization/standardization 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 level of agreement between replicates of a sample both within and between runs of the same test method in a given laboratory.

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.

3.2.3. Stage 1. Analytical sensitivity data

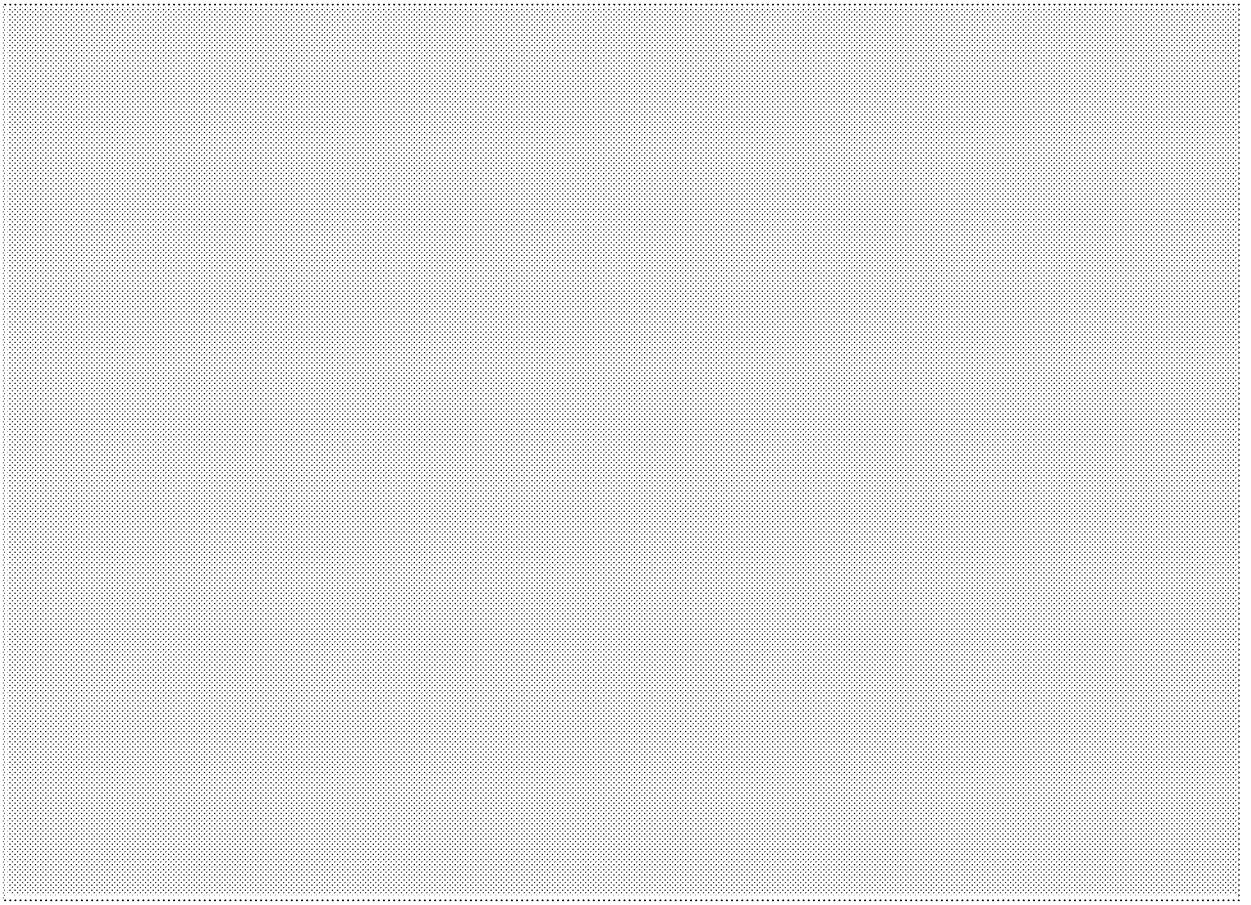
Analytical sensitivity is synonymous with 'Limit of Detection', smallest detectable amount of analyte that can be measured with a defined certainty; analyte may include antibodies, antigens, nucleic acids or live organisms.

3.2.4. Stage 1. Standard(s) of comparison

At a minimum, the standard method(s) of comparison (reference standard) should be run in parallel on small but select group of highly characterized 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. Accuracy of analytical methods

Test methods used solely for the characterization or identification of pathogens are not diagnostic tests per se as they are applied only after the presence of the pathogen has been detected. Never-the-less, these analytical methods need to be verified in terms of their accuracy in correctly characterizing a particular trait or identifying a pathogen type, sub-type, lineage, etc.



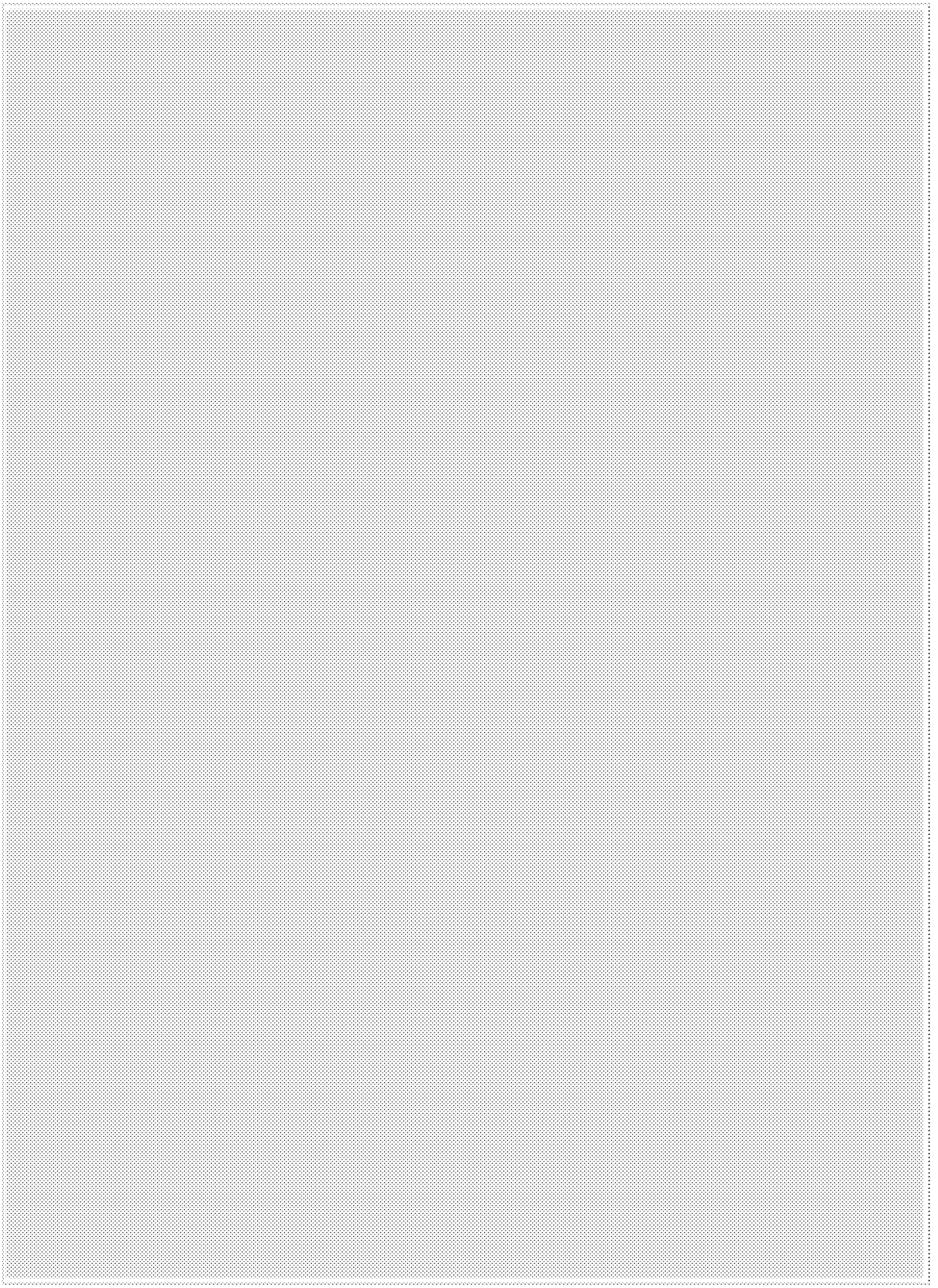
3.3. Stage 2 – Diagnostic characteristics

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.****)*

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.

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.).



3.3.2. Stage 2. Negative reference animals/samples

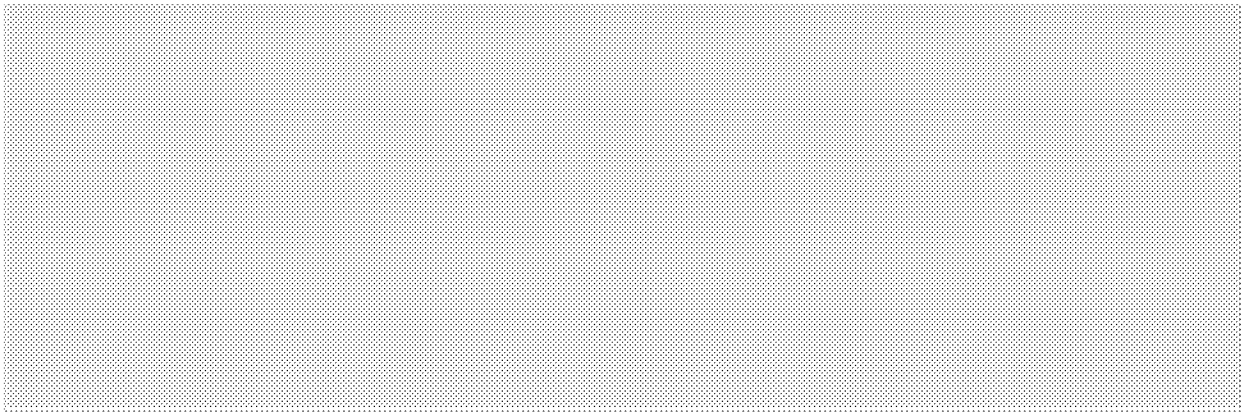
(Note: Negative refers to lack of exposure to, or infection with, the agent in question).

Complete description: age, sex, breed, etc. Relatedness to intended target population. 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).

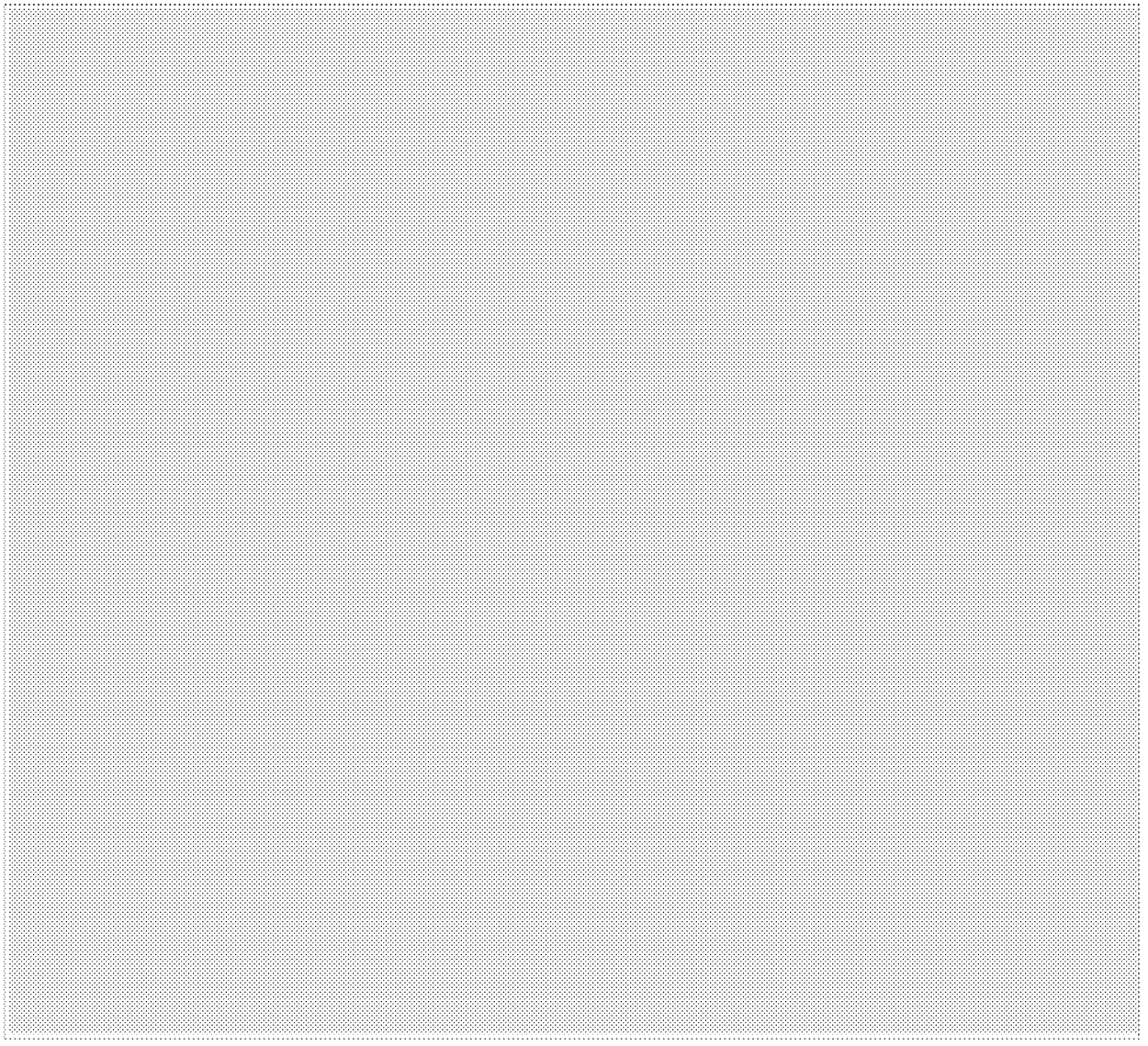
Complete description: age, sex, breed, etc. Relatedness to intended target population. Selection criteria including historical, epidemiological and/or clinical data. Pathognomonic and/or surrogate tests used to define status of animals or prevalence within population. Sampling plan and procedures.

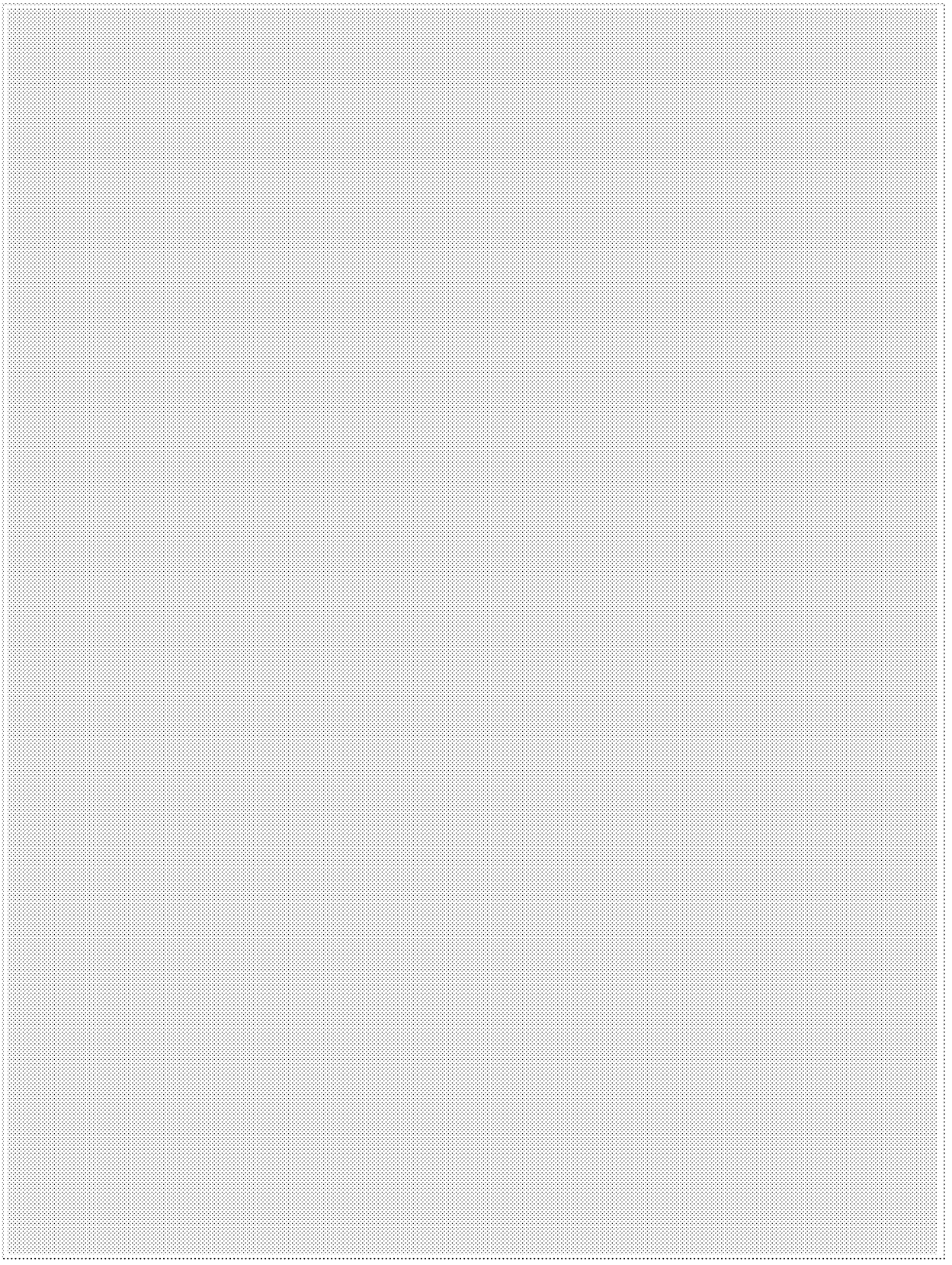


3.3.4. Stage 2. Experimental animals (where used)

(Note; Experimental animals maybe be used when it is not possible to define or obtain sufficient positive reference animals from the field.).

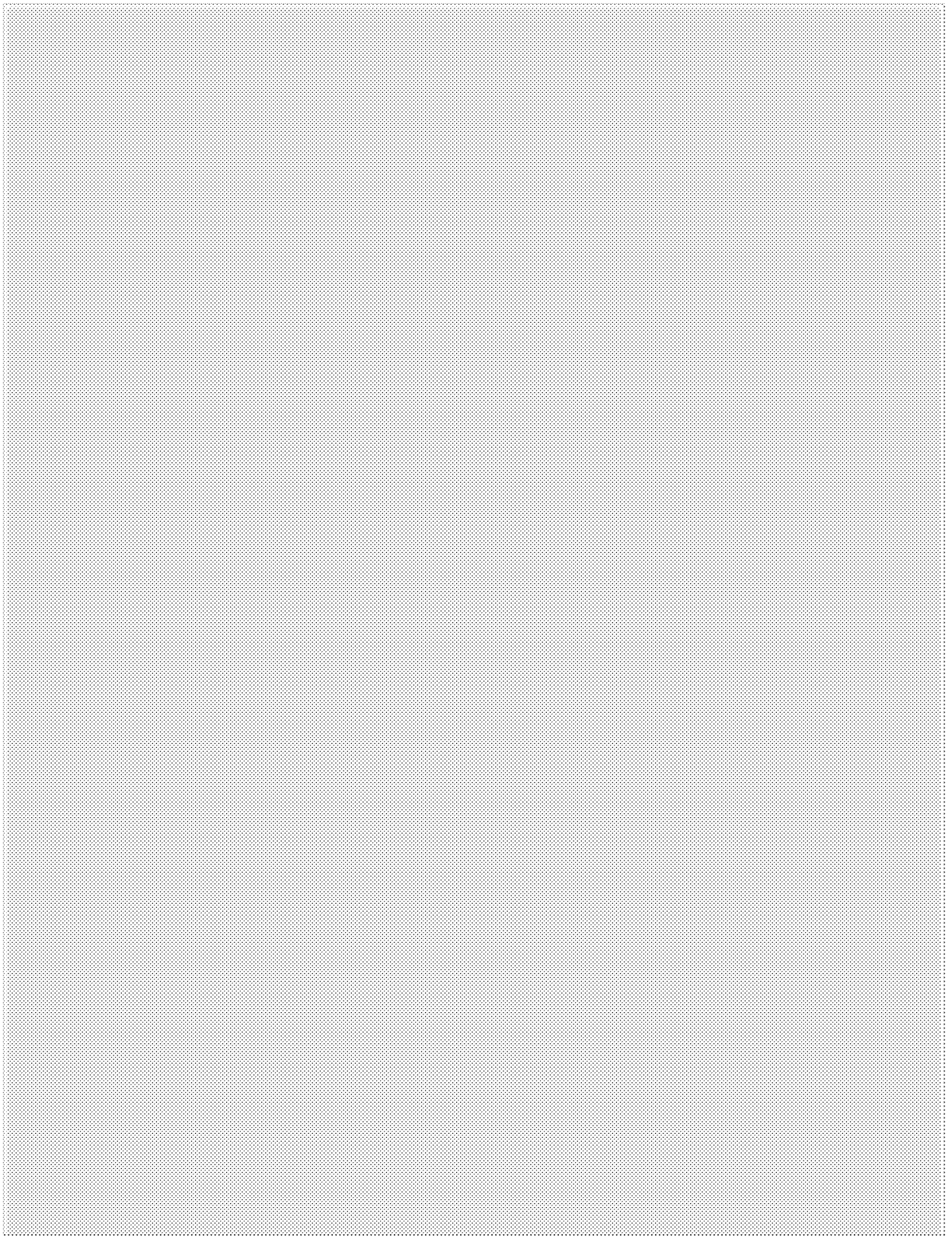
Complete description: age, sex, breed, etc. Immunological status, if applicable. Relatedness to intended target population. Challenge material, source, dose, etc. Type of exposure – inoculation, aerosol, contact, etc. Sampling plan and procedures.





3.3.5. Stage 2. Threshold determination

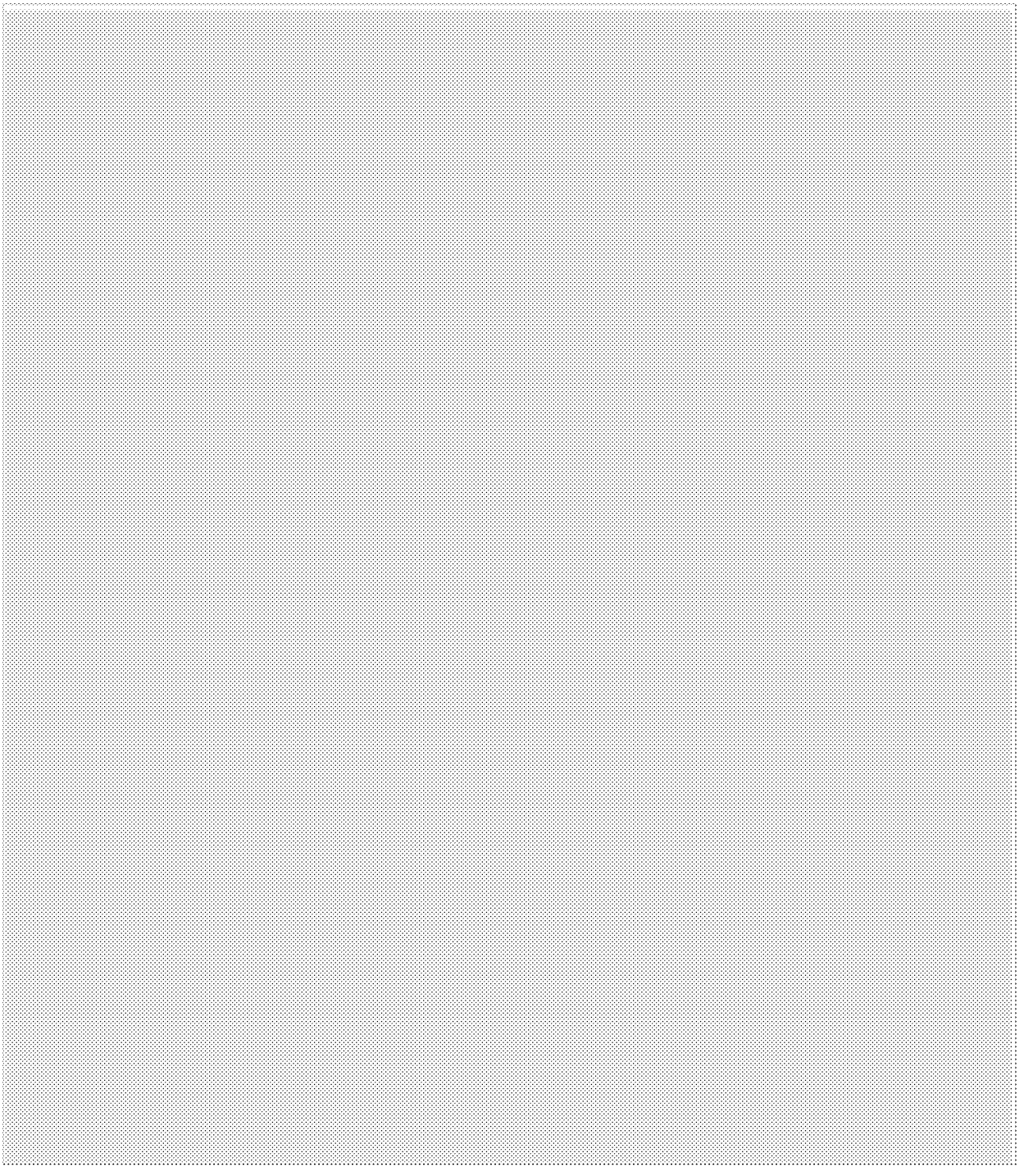
Complete description of method used to determine thresholds (cut-off(s)) used to classify animals as test positive, negative or indeterminate (if relevant). Include statistical calculations, frequency distributions, etc., as applicable.



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

Complete either 3.3.6 or 3.3.7 as appropriate.

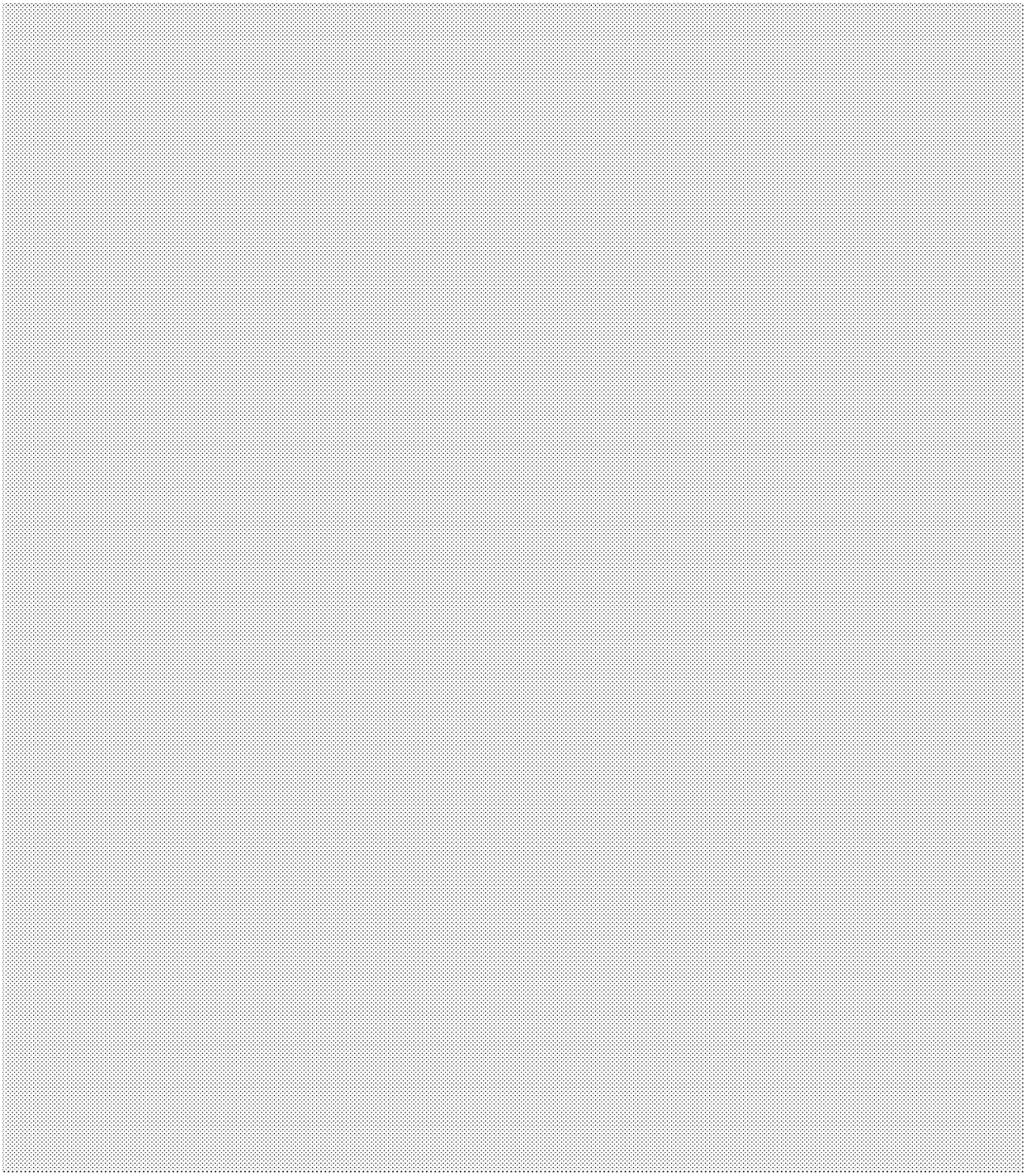
Diagnostic sensitivity is the proportion of known infected reference animals that test positive in the assay; infected animals that test negative are considered to have false-negative results. Diagnostic specificity is the proportion of known uninfected reference animals that test negative in the assay; uninfected reference animals that test positive are considered to have false-positive results.



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

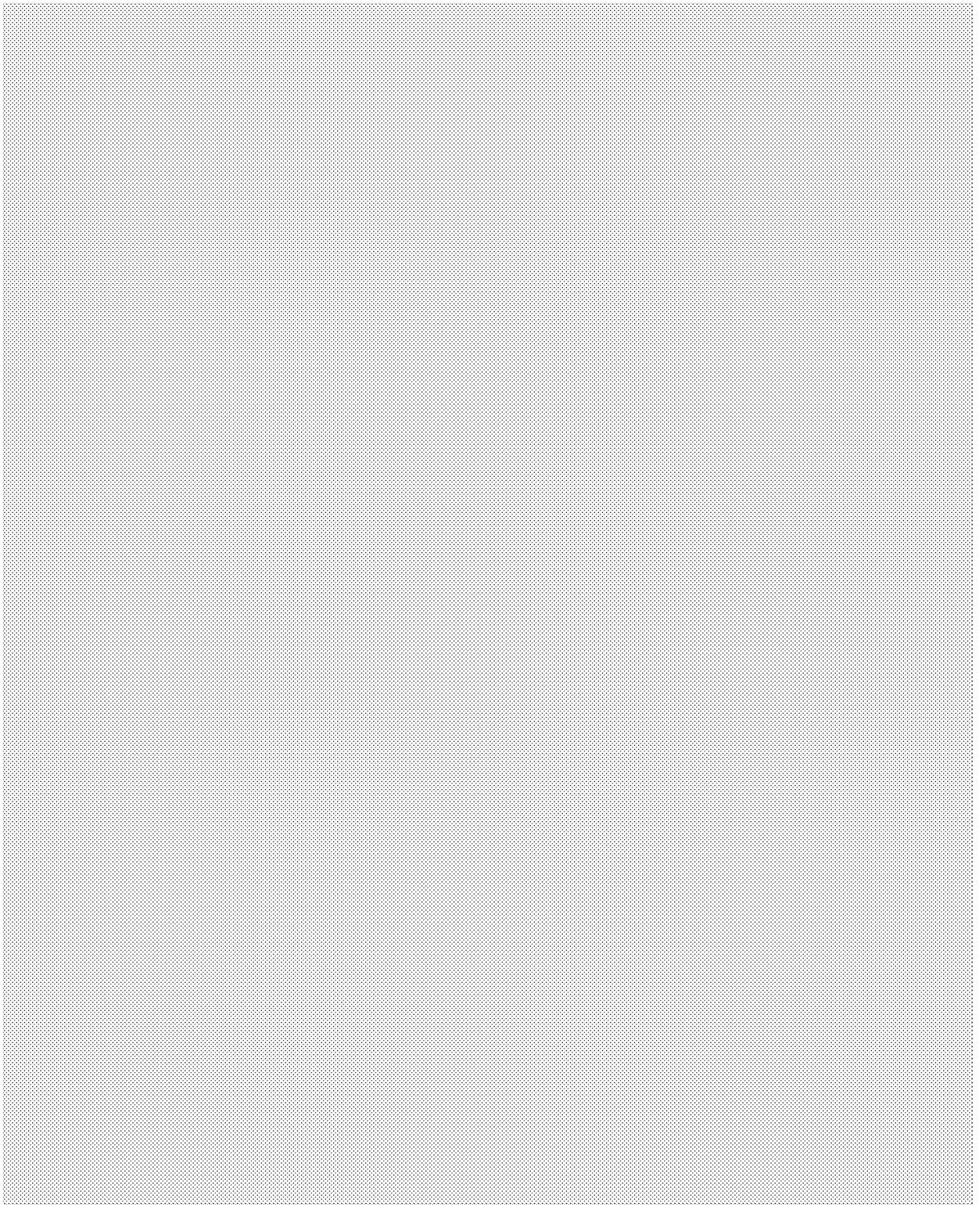
Complete either 3.3.6 or 3.3.7 as appropriate.

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 ± 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. Provide statistical measures of agreement between the reference methods and the new test being validated and suggest explanations for results <u>not</u> 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 utilization, part of fitness studies and reproducibility assessments (e.g. shipping conditions, technology transfer, reagents batches, equipment, testing platforms and/or environments).

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 OIE Reference Laboratories where they exist.

3.4.2. Stage 3. Evaluation panel

Description of test panel used for independent reproducibility study (interlaboratory comparisons).

3.4.3. Stage 3. Analysis of reproducibility

Description of reproducibility study and interpretation of results.

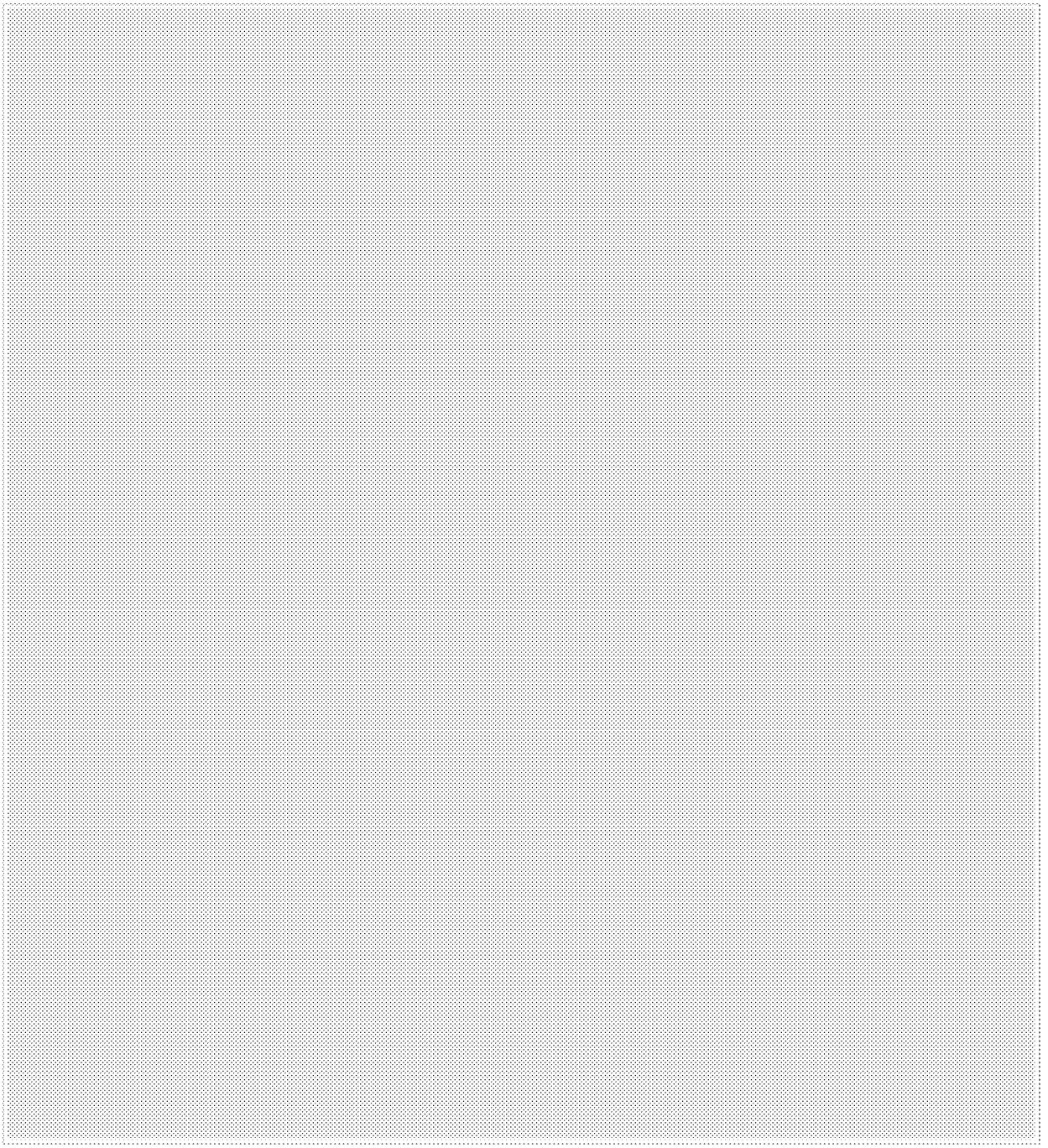
3.5. Stage 4 - Applications

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 the OIE evaluation. Please complete where the information is available.

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. Listed international standards containing this test method. Listed international programmes employing this test method.

Section 4. Performance summary

4.1. Summary of validation data

SECTION	ELEMENT	SUMMARY DATA
2.2 General	**Type & purpose**	
2.2.1	Type of test	
2.2.2	Intended purpose	

2.3 General	**Test description**	
2.3.2	Disease/analyte	
2.3.3	Species/specimen	
3.2 Validation	**Stage 1 - Analytical Characteristics**	
3.2.1	Repeatability	
3.2.2	Analytical specificity	
3.2.3	Analytical sensitivity	
3.2.4	Std of comparison (analytical correlation)	
3.2.5	Reproducibility (national)	
3.3 Validation	**Stage 2 - Diagnostic characteristics** **(Note: complete only 3.3.6 or 3.3.7)**	
3.3.5	Threshold (diagnostic)	

3.3.6 (i)	Diagnostic specificity	
3.3.6 (ii)	Diagnostic sensitivity	
3.3.7 (i)	Diagnostic specificity	
3.3.7 (ii)	Diagnostic sensitivity	
3.3.8 (i)	Std of comparison (diagnostic specificity)	
3.3.8 (ii)	Std of comparison (diagnostic sensitivity)	
3.4 Validation	**Stage 3 - Reproducibility**	
3.4.3	International (if applicable)	
3.5 Validation	**Stage 4 - Applications**	
3.5.1	Diagnostic integration	
3.5.2	Laboratories (in scope)	

Section 5. 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 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			
3			
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Section 6. 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.