Report of the Meeting of the WOAH Biological Standards Commission

Original: English (EN)

6 to 10 February 2023
Paris

Introduction and Member contribution

A meeting of the WOAH Biological Standards Commission (hereafter called 'the Commission') was held from 6 to 10 February 2023 at the WOAH Headquarters in Paris, France. During the meeting, 15 chapters from the WOAH *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) were approved for circulation for second-round Member comment and proposal for adoption at the General Session in May 2023. The Commission wished to thank the following Members for providing comments on draft texts for the *Terrestrial Manual* circulated with the Commission's September 2022 report: Australia, Belgium, Canada, China (People's Republic of), Chinese Taipei, Japan, New Zealand, Switzerland, the United States of America (USA), the United Kingdom (UK) and the 27 Member States of the European Union (EU). The Commission also wished to acknowledge the valuable advice and contributions from numerous experts of the WOAH scientific network.

The Commission reviewed all comments that were submitted on time and were supported by a rationale. Due to the large number of comments, the Commission was not able to provide a detailed explanation of the reasons for accepting or not each of the comments considered, and focused its explanations on significant issues. Where amendments were of an editorial nature, no explanatory text has been provided. The Commission wished to note that not all texts proposed by Members to improve clarity were accepted; in these cases, it considered the text clear as currently written. The Commission made amendments to draft texts in the usual manner by 'double underline' and 'strikethrough'. In relevant chapters, amendments proposed at this meeting are highlighted in yellow to distinguish them from those made previously.

Chapters

The chapters can be downloaded from the following address:

BSC Draft Chapters, March 2023

Deadline to comment

Comments on the draft chapters must reach the Headquarters by 30 April 2023.

Where to send comments

All comments should be sent to the Science Department at: BSC.Secretariat@woah.org

Date of the next meeting

The Commission noted the dates for its next meeting: 4 to 8 September 2023

World Organisation for Animal Health
Founded as OIE

Science Department
BSC.secretariat@woah.org

12, rue de Prony
75017 Paris, France

T. +33 (0)1 44 15 18 88
F. +33 (0)1 42 67 09 87
woah@woah.org
www.woah.org

Table of Contents

List of Annexes

1. Welcome from the directors

1.1. Director General

Dr Monique Eloit, the WOAH Director General, met the Biological Standards Commission on 10 February and thanked its members for their support and commitment to achieving WOAH objectives.

Dr Eloit updated the Commission on the progress of the review of the WOAH Science System and the assessment against other international organisations similar systems. Dr Eliot assured the Specialist Commissions and World Assembly of Delegates that she would keep them informed as the process progresses.

Dr Eloit highlighted the recently published WOAH Observatory annual report and indicated that it will help Members understand how the Observatory programme provides insight into the implementation of WOAH standards. The report contains recommendations that are important for WOAH in supporting Members and for Members in improving their implementation of the standards and national approaches.

The Commission thanked Dr Eloit for these updates.

1.2. Deputy Director General, International Standards and Science

Dr Montserrat Arroyo, WOAH Deputy Director General, International Standards and Science, welcomed members of the Biological Standards Commission and thanked them for their ongoing contributions to the work of WOAH. Dr Arroyo commended the Commission for its ambitious agenda and extended her appreciation to the members' employing institutions and national governments.

Dr Arroyo informed the Commission that the selection process for experts seeking nomination for election to WOAH Specialist Commissions will start with the call for experts in July 2023, and that the elections will take place during the 91th General Session in May 2024. The Performance Management Framework will fit into the process for current members wishing to be re-elected. More information will be provided to the Delegates in due course.

Dr Arroyo briefed the Commission that the 90th General Session will occur in a physical format. She indicated that there will be a forum on current global animal health issues, with a specific focus on avian influenza and that specific sessions throughout the General Session will be webcast for Members. She informed Commission members that a single pre-General Session webinar for each of the three Specialist Commissions involved in the standard-setting process will be organised mid-April with simultaneous interpretation and will be recorded for publication on the WOAH website.

She also informed the Commission that the new WOAH acronym will be applied to the 2023 version of the *Terrestrial Manual*. Dr Arroyo provided an update on the ongoing WOAH initiatives for the revision of the *Basic Texts*, the digitalisation and transparency of comments, including the continuation of work on new digital tools.

Dr Arroyo acknowledged the improved harmonisation between the Specialist Commissions, which has been demonstrated by their presence at Bureau meetings with the Aquatic Animal Health Standards Commission and the increased coordination on harmonised items in the workplan with the Terrestrial Code Commission.

The members of the Commission thanked Dr Arroyo for the excellent support provided by the WOAH Secretariat.

1.3. Updates from the WOAH Headquarters

1.3.1. WOAH Specialist Commission reports

Background

The Secretariats of the WOAH Specialist Commissions are always looking to improve the efficiency of the production and publication of their respective Specialist Commission reports whilst ensuring alignment, as relevant. Dr Arroyo considered the proposals made by the Secretariat and agreed with the following changes to the publication of the Commission reports starting in February 2023:

1. All Commission reports will revert to a single report per Commission. (Note: SCAD has always been produced as a single report);
2. Unofficial reports in English will no longer be published;
3. Commission reports will be published on the Delegates website (in Word format for AAHSC and TAHSC and PDF for BSC and SCAD) and on the public website (all in PDF format) per language (i.e. English,

French and Spanish) once final. A gap between the publication of the English version and the French and Spanish versions is unavoidable because our working language is English. However, we endeavour to keep this period to a minimum.

4. The four Specialist Commission reports will be published in English at least two weeks prior to the pre-GS webinars.

1.3.2. Pre-General Session

1. Pre-General Session information webinars will be held every year for AAHSC, BSC and TAHSC (with SCAD support), in one time-zone only and recorded and uploaded onto General Session website. These webinars will be presented by the President of the respective Commission and will focus on presenting information about new or revised standards that will be proposed for adoption at the General Session. Each webinar will have a duration of a maximum of 2 hours and will be conducted with simultaneous interpretation in English, French and Spanish.

   NOTE: 2023 dates are: Biological Standards Commission – 18 April 2023; Terrestrial Animal Health Standards Commission – 19 April 2023; Aquatic Animal Health Standards Commission – 20 April 2023. All webinars will be held between 12:00 and 2:00 pm CET.

2. WOAH will no longer provide a mechanism for Members to submit pre-General Session positions, as was the case in 2021 and 2022 when General Sessions were held in a virtual or hybrid format. However, if Members wish to unofficially send pre-GS positions to assist the Presidents of the Specialist Commissions prepare their General Session reports, this can be done through email to the relevant Secretariat.

1.3.3. Use of the acronym "WOAH" in the *Terrestrial Manual*

Following the resolution adopted at the 89th General Session in May 2022, the organisation is now the World Organisation for Animal Health (WOAH). The acronym WOAH will be used throughout the *Terrestrial Manual*.

2. Adoption of the agenda

The proposed agenda was presented and adopted. Dr Emmanuel Couacy-Hymann chaired the meeting and the WOAH Secretariat acted as rapporteur. The agenda and the list of participants can be found at Annexes 1 and 2 respectively.

3. Collaboration with other Specialist Commissions

3.1. Scientific Commission for Animal Diseases

3.1.1. Case definitions: infection with Crimean–Congo haemorrhagic fever virus and infection with Nipah virus (Nipah virus encephalitis)

The Biological Standards Commission discussed the case definitions for infection with Crimean–Congo haemorrhagic fever virus (CCHFV) and infection with Nipah virus (Nipah virus encephalitis) and gave its recommendations to the Scientific Commission for Animal Diseases (see agenda items 11.3.2.1 and 11.3.2.2 of the report of the meeting of the Scientific Commission for Animal Diseases, 13–17 February 2023).

In reviewing the Crimean–Congo case definition, the Commission noted the need to amend the ratings of the tests for the purpose *Confirmation of clinical cases in animals* in Table 1 *Diagnostic test formats for Crimean–Congo haemorrhagic fever virus infections in animals*, of *Terrestrial Manual* Chapter 3.1.5. The Reference Laboratory expert reviewed the chapter to remove any conflict between the proposed case definition and the *Terrestrial Manual*. The amended chapter is included in the batch of chapters that will be sent for second-round comment in March 2023 (see agenda item 5.1).

The experts' proposed case definition for Nipah virus states that for the purposes of notification to WOAH, Nipah virus encephalitis is an infection of horses, pigs, dogs, and cats. However, the *Summary* of the current version of *Terrestrial Manual* Chapter 3.1.15. *Nipah and Hendra virus diseases* states that: "Both viruses can infect companion animals, but they do not seem to play a role in the epidemiology of the disease". The Commission noted the significant role of horses in the epidemiology of the disease and the uncertainty about the role of dogs and cats. To address the discrepancy between the case definition and the *Terrestrial Manual*, the Biological Standards Commission agreed to change the statement to read "It is not currently known if the susceptibility of dogs and cats to infection is at a level to have potential for epidemiological significance".

3.2. Terrestrial Animal Health Standards Commission

Matters between the Terrestrial Animal Health Standards Commission and the Biological Standards Commission

3.2.1. Updates from the September 2022 Code Commission meeting

The Biological Standards Commission was updated by the Secretariat of the Code Commission on the current topics under review by the Code Commission to ensure complementarity and alignment of the two Commission's respective work programmes.

3.2.2. Questions on Chapter 12.7 *Infection with* Theileria equi *and* Babesia caballi (*equine piroplasmosis*)

Members commenting on the draft updated *Terrestrial Code* chapter on *Infection with* Theileria equi *and* Babesia caballi (*equine piroplasmosis*) had asked if some corresponding changes would be made to the *Terrestrial Manual* chapter to ensure the alignment of the two publications. The Biological Standards Commission agreed that the following changes would be made to *Terrestrial Manual* Chapter 3.6.8 *Equine piroplasmosis:*

1. In Section A *Introduction*, the following sentence and reference will be added to the first paragraph:

Other genera such as *Ambloyomma* have also been identified as competent vectors (Scoles *et al.,* 2011).

SCOLES G.A., HUTCHESON H.J., SCHLATER J.L., HENNAGER S.G., PELZEL A.M. & KNOWLES D.P. (2011). Equine piroplasmosis associated with *Amblyomma cajennense* Ticks, Texas, USA. *Emerg. Infect. Dis*., **17**, 1903–1905. doi: 10.3201/eid1710.101182.

2. In Section B *Diagnostic techniques*, the following sentence will be added to the end of the first paragraph:

Treatment with antiparasitic drugs may mask infection and give rise to false negative results.

3.2.3. Questions on Chapter 8.8 *Infection with foot and mouth virus*

The Code Commission referred a Member request to define the latent period for FMD[1] virus to the Biological Standards Commission noting that such detail should be in the *Terrestrial Manual* and not the *Terrestrial Code.* The Biological Standards Commission agreed to ask the Reference Laboratory experts who are currently updating the *Terrestrial Manual* chapter to develop a definition of latent period for the *Terrestrial Manual.* The experts would also be asked if they recommend its use in the *Terrestrial Manual* chapter, what its added value would be and what, in their view, would be the impact on the rest of the *Terrestrial Manual* and perhaps the *Terrestrial Code* of including such a definition.

3.2.4. Questions on Chapter 12.6 *Infection with equine influenza virus*

The advice of the Biological Standards Commission was sought regarding draft Chapter 12.6. *Infection with equine influenza virus.* In response to a Member comment, the Code Commission agreed to modify the infective period from 21 days to 10 days, based on the scientific references reviewed, which specified that the incubation period is 1–3 days and that infected horses have been found to shed the virus up to 10 days via nasal discharge.

The Biological Standards Commission advised against the change: the 10-day infective period is based on virus isolation in embryonated eggs. The Biological Standards Commission recommended keeping the infective period at 21 days as a measure to allow for the incubation period and the fact that virus isolation is not very sensitive.

Regarding the proposal to add information on the infective period to *Terrestrial Manual* Chapter 3.6.7 *Equine influenza* (*infection with equine influenza virus*), the Biological Standards Commission believed that information on the infective period based on virus isolation in eggs may not be helpful. The Commission could add such information from experimental infection studies if requested.

[1] FMD: foot and mouth disease

3.2.5. Comments on Chapter 12.2 *Infection with* Taylorella equigenitalis (*contagious equine metritis*)

A Member had noted a discrepancy between Chapter 3.6.2 *Contagious equine metritis* of the *Terrestrial Manual* and Chapter 12.2. *Infection with* Taylorella equigenitalis (*contagious equine metritis*) of the *Terrestrial Code*. The *Terrestrial Code* indicates that sampling cannot be conducted for at least 21 days after treatment with antibiotics whereas the *Terrestrial Manual* indicates that swabbing for *T. equigenitalis* should not recommence until at least 7 days (systemic treatment) or 21 days (local treatment) following treatment.

The Biological Standards Commission's advice was to amend Article 12.2.4. *Recommendations for importation of stallions or mares*, Point 2b) ii of the *Terrestrial Code* so that it reads:

Horses must not have ~~not~~ been treated with local antibiotics nor subjected to antiseptic washing of genital mucous membranes for at least 21 days prior to sampling. ~~and~~ They must not have been treated with systemic antibiotics for at least 7 days prior to sampling. They must not have been mated after sampling.

3.2.6. Use of terms: 'bovid', 'bovidae', 'bovine' and 'cattle'; 'enzootic', 'endemic', 'epizootic' and 'epidemic'

The Biological Standards Commission noted that the Code Commission would replace the English language word "cattle" with "ruminants", "bovids" or "bovine" depending on the context throughout the *Terrestrial Code*. The Code would also use the terms, "endemic" and "epidemic" rather than "enzootic" and "epizootic" except in the names of diseases. The Biological Standards Commission agreed to adopt the same terminology.

3.2.7. Use of terms related to diagnosis and diagnostic methods

The advice of the Biological Standards Commission was sought regarding certain terms used throughout the *Terrestrial Code*. The Biological Standards Commission agreed that the following terms were appropriate:

"isolated" for virus and bacteria or other microorganism for which culture is relevant;

"observed" for protozoa, chlamydia or other microorganisms as relevant when referring to the direct visualisation of the agent (i.e. without isolation);

[PATHOGENIC_AGENT] has been isolated "and identified as such" to ensure proper understanding that this point implies the confirmation of the identity of the agent irrespective of the methods required to that end;

if a disease shows pathognomonic clinical signs (i.e. specifically characteristic or indicative of a particular disease), the term "consistent with" will be used to refer to these clinical signs;

"nucleic acid" to refer to nucleic acid-based testing;

antigen/nucleic acid/antibody has been "detected" to refer to nucleic acid-based testing or antigen/antibody detection methods

3.3. Aquatic Animal Health Standards Commission

Meeting of the Bureaus of the Commissions.

3.3.1. Reference Centres: discussion on annual report templates and the use of data collected

In September 2022, the Biological Standards Commission had updated the annual report template used by the Reference Centres with the aim of improving the questions asked so as to receive clearer responses and improve the quality of the data collected. At the bureaus meeting, the Aquatic Animals Commission commented that the templates satisfy their needs, but that there are two principles that need further thought: is there more that can be taken from the report exercise, can we maximise the benefits that we derive from these reports taking into account the effort that experts put into filling in the report template and meeting the deadlines. The Bureaus agreed that there is a need to improve the efficiency of the annual reporting exercise and to determine what outputs could be derived from the data collected; such improvements could satisfy the laboratories that filling in the reports is worth their investment.

Currently all the Reference Centres are supplied with the annual report template around mid-December with a deadline to submit the report by mid- to late January. The Biological Standards Commission asked the

Secretariat to inquire if the online annual report system could be made available to the experts throughout or part of the year so that they could fill in as the activities progress.

The bureau of the Aquatic Animals Commission was informed of the initiative to ask the Reference Laboratories about the usefulness of the annual report and get their feedback on their experience of being a WOAH Reference Laboratory (see agenda item 6.5). The Aquatic Animals Commission supported the purpose of the questionnaire.

3.3.2. Aquatic and Terrestrial Manuals: areas of common interest

3.3.2.1. Aquatic Animals Commission's table on PCR[2] parameters for consideration by the Biological Standards Commission

The Aquatic Animals Commission had developed a table on PCR primer and probe sequences and cycling parameters so that critical information on PCR methods is presented in a uniform way in all the chapters of the *Aquatic Manual*. The Biological Standards Commission commented that presenting PCR parameters in tabular format is extremely useful and agreed to adopt this approach in the *Terrestrial Manual* chapters.

3.3.2.2. Updated *Terrestrial Manual* validation chapter

Terrestrial Manual Chapter 1.1.6 *Principles and methods of validation of diagnostic assays for infectious diseases* had been extensively revised and would be proposed for adoption at the General Session in May 2023. The Aquatic Animals Commission had not reviewed it and commented that it would be crucial to do so because the *Aquatic Manual* includes the same chapter. The Aquatic Animals Commission requested that adoption of the chapter be postponed to 2024 to allow it to review the update to ensure that there are no differences in the horizontal chapters. The Biological Standards Commission agreed to delay the adoption of the chapter until 2024. Following the meeting of the Bureaus, the Aquatic Animals Commission reconsidered its request and agreed that the chapter could be proposed for adoption and inclusion in the *Terrestrial Manual*. The *Aquatic Manual* is concerned with disease notification and determining the immune status of animals, whereas the *Terrestrial Manual* is concerned with disease management: the two Manuals therefore have different validation purposes and thus they will not continue to have a harmonised chapter. The Aquatic Animals Commission has added the update of the *Aquatic Manual's* validation chapter to their work programme; they will take account of the revised *Terrestrial Manual* chapter in their revision. The Presidents of both Commissions will mention this in their presentations at the General Session in May.

3.3.2.3. Addition of a new section to the disease-specific chapters to describe the rationale behind the selection of tests for different purposes given in Table 1 *Test methods available and their purpose* and an explanation for their score

The Biological Standards Commission informed the Aquatic Animals Commission that it is working to add a new section to the disease-specific chapters of the *Terrestrial Manual* to describe the rationale behind the selection of tests for different purposes given in Table 1 *Test methods available and their purpose* and an explanation of their score. This will address queries received from Members and provide justification for different tests. The work is in a pilot stage and the format needs to be finalised to give flexibility for the experts providing the justification.

The Aquatic Animals Commission noted that both Commissions are working to achieve similar outcomes by providing additional information on particular assays. The Aquatic Animals Commission has a different approach in *Aquatic Manual* Table 4.1 *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals,* which includes life stage, validation level and rating against purpose of use. The Aquatic Animals Commission revised the definition of the ratings as readers confused the test rating with the validation level.

The Biological Standards Commission agreed that this effort is good for harmonisation and agreed that it would be valuable to look at *Aquatic Manual* Table 4.1.

[2] PCR: polymerase chain reaction

3.3.2.4. Development of a template for validation reports for tests in the *Terrestrial Manual*

The Biological Standards Commission informed the Aquatic Animals Commission that it had developed a template for the validation data for tests recommended in the *Terrestrial Manual*. Reference Laboratories would be invited to fill in the 'validation report' form, which would be made available in a repository on the website for anyone seeking the validation data available for the test. As a first step in a pilot scheme to test the template's suitability and usability, the document was shared with selected WOAH Reference Laboratories to complete and to provide their feedback.

The Aquatic Animals Commission has received comments from Reference Laboratories about the time it takes for new or changed methods to be included in the *Aquatic Manual* because the methods or validation information must be published in peer-reviewed articles. The Aquatic Animals Commission considered the template developed by Biological Standards Commission has value in urgent scenarios and agreed to review it during their meeting and provide feedback.

3.3.3. Work on the list of WOAH approved reference reagents

The Biological Standards Commission has a list of WOAH-approved International Standard Reagents available online and are planning to expand the list (https://www.woah.org/en/what-we-offer/veterinary-products/#ui-id-4). The Aquatic Animals Commission will consider developing a corresponding list noting that the *Aquatic Manual* relies heavily on PCR methods.

Both the Commissions considered this meeting to be very useful to identify and discuss areas of harmonisation.

4. Work Programme

The updated work programme was agreed and can be found at Annex 3.

5. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*

For this Agenda Item, the Commission was joined by Dr Steven Edwards, Consultant Editor of the WOAH *Terrestrial Manual*.

5.1. Review of Member comments received on draft chapters and their endorsement for circulation for second-round comment and proposal for adoption in May 2022

The Commission reviewed the comments that had been received on the 16 draft chapters that had been sent for first-round Member comment in October 2022. The Commission approved 15 for circulation before presenting them for adoption by the Assembly in May 2023.

Comments had been received from: Australia, Belgium, Canada, China (People's Rep. of), Chinese Taipei, European Union, Japan, New Zealand, Switzerland, and United Kingdom.

The 15 chapters and a summary of the main amendments made in response to Member comments are provided below:

Glossary of terms: in response to a Member comment on Chapter 3.9.7 Influenza A virus of swine, a definition of anthroponosis was added to the glossary.

1.1.6 Principles and methods of validation of diagnostic assays for infectious diseases: made a number of minor editorial amendments throughout to improve the clarity of the text; deleted text from the *Introduction* on the *Aquatic Manual:* a separate validation chapter will be developed for the *Aquatic Manual* in the future; in Section B.1.1.1 *Selectivity*, deleted text on the impact of the use of anticoagulants on the RT-PCR[3] as it was already included in Section A.2.4. *Inhibitory factors in the sample matrix;* reversed the order of Section A.2.2 *Operating range of the assay* and Section A.2.3 *Standardisation and optimisation*; did not specify the exact number of validation purposes in the introduction as the list given there is a list of examples and not limited to those in Table 1; in Section B.1.3 *Analytical sensitivity*, did not change the term "100% or 0%" in the example of a titration response as the meaning is clear in the context of the paragraph; in Section B.2.2 *Samples from animals of unknown status*, clarified that deducing the population's status is more challenging if the infection is subclinical or non-productive and deleted mention of carriers with an active infection.

[3] RT-PCR: reverse transcription PCR

1.1.10. Vaccine banks: in the *Summary* and in Section B *Types of banks*, clarified that it is the pathogenic agent that shows variation, not the disease, and that within a serotype there can be extensive antigenic diversity; in Section B, replaced the word "slaughter" with "stamping out" as the animals referred to will not enter the food chain, clarified that reduced testing "might be acceptable" at the time of release provided that full "finished product" testing has previously been performed on a batch, and reworded the sentences on the main advantages of ready-to-use formulated vaccines for clarity; in Section C *Selection of vaccines for a bank*, added text on the importance of knowledge on the extent of antigenic matching between strains and the virus antigens stored in the vaccine bank; in Section E *Regulatory considerations*, amended the text to include antigen and/or ready-to-use vaccines as mentioned elsewhere in the chapter; for consistency with other chapters in the *Terrestrial Manual*, replaced the word "licencing" with "regulatory approval" and "product licence (authorisation or registration)" with "regulatory approval"; in Section H *Deployment planning*, deleted text referring to international trade and country disease status as it is out of the remit *Terrestrial Manual,* but kept the remaining text as it provides specific advice to those intending to create a vaccine bank that will be used to support a DIVA[4] policy in terms of what needs to be taken into consideration regarding the properties of the vaccine and the accompanying diagnostic test as this advice is not found in the *Terrestrial Code*.

3.1.1. Anthrax: at the request of a number of Members, agreed to reinstate the polychrome methylene blue (M'Fadyean reaction) stain as it is still in use; in the *Introduction*, clarified that blood-feeding insects can mechanically transmit anthrax; still the *Introduction*, stressed the importance of avoiding environmental contamination by closing all natural orifices on carcasses of animals suspected to have died of anthrax, and emphasised that post-mortem examination is prohibited in many countries when anthrax is suspected; in Section A.1 *Zoonotic risk and biosafety requirements*, added a mention of the use of personal protective equipment; changed the rating of the PCR from "+" to "++" for the purpose *Confirmation of clinical cases* in Table 1 *Test methods available for the diagnosis of anthrax and their purpose* as PCR is very suitable for this purpose as the presence of toxin genes can be demonstrated directly; added TSPB[5] agar as an alternative to PLET[6] agar and a brief description of its preparation; in Section B.1.3 *Confirmation of virulence with the polymerase chain reaction,* added information and text references to an evaluation study of 35 PCR-based methods for markers of *Bacillus anthracis*; still in Section B.1.3, added information and text references on molecular typing techniques for *B. anthracis*.

3.1.5. Crimean–Congo haemorrhagic fever: in Table 1. *Diagnostic test formats for Crimean-Congo haemorrhagic fever virus infections in animals*, amended the ratings of the tests for the purpose *Confirmation of clinical cases in animals* to align with the proposed case definition: changed real-time RT-PCR from "+" to "+++", changed IgG ELISA and competitive ELISA from "–" to "+" as these assays have limited use – seroconversion needs to be demonstrated between successive samples, changed IgM ELISA from "–" to "++" because while the presence of IgM antibody confirms active infection in animals, no commercial kits are currently available, and left virus isolation in cell culture as "+" because access to BSL4[7] facilities is necessary for virus isolation thus limiting its use; removed mention of the countries in which human cases have been reported; in the *Introduction*, added a sentence and reference on experimental infections of wild animals and livestock, and on progress in vaccine development; in Section B.1 *Detection and identification of the agent*, added the cell line BSR-T7/5 as it is a highly reproductive and appears to generate the highest titres of virus at this time; in Section B.2 Serological tests, updated the taxonomy

3.1.18 Rabies (infection with rabies virus and other lyssaviruses): ensured that the abbreviation "RABV" for rabies virus is used consistently throughout the chapter; changed the diagnostic method from "PCR" to "RT-PCR" as lyssaviruses are RNA viruses; removed the LFD/RICT[8] antigen detection tests from Table 1. *Test methods available for the diagnosis of rabies and their purpose,* and in Section B.1.3.3 *Rapid immunochromatographic tests* (*lateral flow devices*) stressed the limitations of these tests, including their lack of sensitivity; in Section C.2 *Rabies vaccine for injectable use,* added a statement that for injectable rabies vaccination in animals, inactivated virus (for companion animals and livestock), and recombinant vaccines (for cats) are used, and stressed that as injectable live-attenuated vaccines have been documented to cause vaccine-induced rabies their use should be discontinued. The Commission supported retaining a paragraph in Section C.2 on vaccines in the advanced stages of development and potentially available in the future as it is important for Members to be aware of advances in vaccinology.

3.1.19. Rift Valley fever (infection with Rift Valley fever virus): in the *Introduction*, clarified that the disease is mosquito borne, and that clinical presentations have been observed in camels; in Table 1. *Test methods available for diagnosis of Rift Valley fever and their purposes*, amended the ratings of the RT-PCR and

[4] DIVA: detection of infection in vaccinated animals
[5] TSPB: trimethoprim, sulfamethoxazole and polymyxin B
[6] PLET: polymyxin, lysozyme, EDTA (ethylene diamine tetra-acetic acid), thallous acetate
[7] BSL: biosafety level
[8] LFD/RICT: lateral flow devices/rapid immunochromatographic antigen detection tests

antigen detection for the purpose *Individual animal freedom from infection prior to movement* as the experts recommend these test for that purpose*;* in Section B.1.4.1 *Agarose gel-based RT-PCR assay,* stressed that reagent volumes and cycling parameters may need to be adapted according to the manufacturer's recommendations; in Section B.1.4.2 *Real-time RT-PCR assay*, added mention of and references to alternative RT-PCR methods; in Section B.1.5.1 *Antigen ELISA*[9] *procedure*, emphasised that controls and antisera used in the performance of this assay, and samples, should be treated to inactivate any viable Rift Valley fever viral particles, and included the inactivation method for the samples; added a protocol for the virus neutralisation test method (new Section B.2.3) as the test is mentioned as an alternative to the plaque reduction neutralisation test in Table 1; in Section C.1.3 *The MP-12 RVF vaccine*, added text and a reference that the vaccine has also been successfully tested in Camelidae.

3.1.22. Trichinellosis (infection with *Trichinella* spp.): made a number of editorial changes to improve the clarity of the text including changing "flesh-eating" to *"*not-strictly herbivorous"; in Table 1 *Test methods available for detecting* Trichinella *infections in pigs and their purpose,* changed "enzymatic digestion" to "artificial digestion" as this is the term used by the ICT[10], replaced "multiplex PCR" with "PCR" as multiple different formats can be used, amended the rating of the PCR from "+" to "++"for the purpose *Prevalence of infection – surveillance* as species-level information is relevant for surveillance purposes, and edited footnote (b) to clarify that PCR is used as confirmatory test and species-determination; deleted Section B.1.3.2 Trichinoscopy as the method is not recommended. The Commission, on the advice of the Reference Laboratory experts, did not accept to include a new serological test in the chapter as there are insufficient data to recommend this method as fit for purpose at this time: a sentence and a reference to the test was included.

3.2.2. American foulbrood of honey bees (infection of honey bees with *Paenibacillus larvae*): in the *Introduction*, clarified that genotype ERIC V has been identified as a new genotype and included a reference; in Section A.1 *Epizootology and clinical signs*, clarified that larvae infected with ERIC I usually die after brood cell capping, whereas larvae infected with other types usually die before cell caping; shortened Section B.1.3.4.vi) *Microscopy*, to remove details of standard laboratory methods such as Gram staining. The Commission did not agree to replace Figure 1a. *Combs have mottled appearance* and Fig. 1b *A matchstick draws out the brown, semi-fluid larval remains in a ropy thread*, as the proposed photographs did not present a significant improvement.

3.2.3. European foulbrood of honey bees (infection of honey bees with *Melissococcus plutonius*): in the *Summary* clarified that identification of the presence of European foulbrood is only unreliable in the absence of specialist training, and that both disease signs and the presence of *M. plutonius* are required for diagnosis; replaced Figure 1 *Clinical European foulbrood: irregular capping of the brood,* with an improved photograph; in Section A.1 *Epizootiology and clinical signs*, replaced the reference to typing of *M. plutonius* with the original publication; in Table 1. *Test methods available for the diagnosis of European foulbrood and their purpose*, deleted the row for real-time PCR as it was identical to and is covered by the row on PCR; in Section B.1.2.1 *Culture media*, added a statement that *M. plutonius* can be stored by suspension in liquid media containing 10–30% glycerol and kept at –80°C, and mentioned that the current taxonomic position of *Achromobacter* eurydice remains uncertain; in Section B.1.4 *Polymerase chain reaction*, added a new reference to a review article

3.3.10. Fowlpox: added tongue to the list of tissues affected by lesions; added histopathology to Table 1. *Test methods available for diagnosis of fowl pox and their purpose* as histopathological observation of characteristic lesions can confirm fowlpox even in the absence of fresh sample for PCR or isolation; in Section B.1.3 *Molecular methods*, clarified that DNA extracted from paraffin-embedded tissues are suitable for molecular assays, but that prolonged fixation in formalin especially unbuffered formalin, can reduce ability to detect nucleic acid of fowlpox virus and other pathogens.

3.3.13. Marek's disease: made a number of editorial changes to improve the clarity of the text in Section A *Introduction*, and added a statement that atrophy of the bursa of Fabricius and the thymus, and enlargement of the spleen are common; in Table 2. *Test methods available for the diagnosis of Marek's disease and their purpose*, amended the rating of the PCR from "+++" to "++" for the purpose *Confirmation of clinical cases* because PCR cannot directly identify MD tumour formation or tumour cells, and separated PCR and real-time PCR. PCR only reveals the presence or absence of MDV in animals and cannot quantify the virus. PCR is not useful for MD diagnosis because MDV including vaccine strains can cause subclinical persistent infection without lymphoma formation; in Section B.1.3 *Molecular methods – detection of nucleic acids*, added a sentence and reference to Marek's disease virus gene-deleted vaccines that can distinguish the tumorigenic strain from the vaccine strain by PCR because of the specific gene deletion fragment; deleted

[9] ELISA: enzyme-linked immunosorbent assay
[10] ICT: International Commission on Trichinellosis

Section C.2.3.4 *Vaccines permitting a DIVA strategy* (*detection of infection in vaccinated animals*), as the information was not relevant for an international standard.

3.4.12. Lumpy skin disease (diagnostic techniques section only): the chapter received few Member comments – made editorial changes to improve the clarity of the text.

3.7.2. Rabbit haemorrhagic disease: did not agree to add two references to the *Introduction* – the Commission emphasised that the *Terrestrial Manual* is not intended to provide comprehensive reviews of the literature, but rather to provide key, up-to-date references as an entry point to the literature for those who wish to study further and that the number of references is limited to 30 per chapter; in Table 1 *Test methods available for the diagnosis of rabbit haemorrhagic disease and their purpose*, did not agree to change the rating of the haemagglutination test from "+" to "++" for the purpose *Confirmation of clinical cases* because the test is performed using human red blood cells, which is a limitation, and also has low sensitivity and specificity; did not accept to change the rating of the isotype ELISA from "+" to "–" for the purpose *Confirmation of clinical cases* because this rating is aligned with the case definition (see item 12.3.2.2. Infection with pathogenic rabbit lagoviruses [rabbit haemorrhagic disease] of the report of the meeting of the Scientific Commission for Animal Diseases, September 2022); reworded the first paragraph of Section C.3 *Vaccines based on biotechnology*, to improve the clarity of the text.

3.9.7. Influenza A virus of swine: reinstated the abbreviation IAV-S (influenza A viruses of swine) throughout the chapter; reorganised the order of the tests in the *Introduction* Section on *Detection and identification of the agent,* and changed real-time PCR to real-time RT-PCR to align with Section B *Diagnostic tests*; in Table 1. *Test methods available for diagnosis of IAV-S and their purpose* amended the rating of the ELISA from "+++" to "+" for the purpose *Individual animal freedom from infection prior to movement*, because the presence or absence of antibodies is not useful – the animal may have maternal antibodies, may be vaccinated of may have had a prior infection and recovered; in Section B.1.6 *Reverse-transcription polymerase chain reaction*, clarified that sequencing is often more precise than real-time RT-PCR for discriminating between subtypes and lineages within a subtype due to the high diversity of swine HA[11] and NA[12] gene sequences; still in Section B.1.6, added a statement and a reference on using high throughput sequencing to obtain genomic information on the isolate or directly from field samples to speed up characterisation of the influenza virus. In reply to a comment on the use of the term "anthroponosis", the Commission proposed a definition to be added to the glossary of terms.

3.10.1. Bunyaviral diseases of animals (excluding RVF fever and Crimean–Congo haemorrhagic fever): the chapter received few Member comments – made editorial changes to improve the clarity of the text; replaced PCR with RT-PCR in the *Introduction* and Table 1 to align with the method described in the text.

NB: All amendments made in response to Member comments are highlighted in yellow in the chapters.

To recap, below is a list of the 15 chapters that are proposed for adoption at the 90th General Session in May 2023. The chapters can be downloaded from the following address:

BSC Draft Chapters, March 2023

The chapters are also available on the Delegates website and on the website of the Biological Standards Commission.

		Glossary of terms
1.	1.1.6.	Principles and methods of validation of diagnostic assays for infectious diseases
2.	1.1.10.	Vaccine banks
3.	3.1.1.	Anthrax
4.	3.1.5.	Crimean–Congo haemorrhagic fever
5.	3.1.18.	Rabies (infection with rabies virus and other lyssaviruses)
6.	3.1.19.	Rift Valley fever (infection with Rift Valley fever virus)
7.	3.1.22.	Trichinellosis (infection with *Trichinella* spp.)
8.	3.2.2.	American foulbrood of honey bees (infection of honey bees with *Paenibacillus larvae*)
9.	3.2.3.	European foulbrood of honey bees (infection of honey bees with *Melissococcus plutonius*)

[11] HA: hemagglutinin
[12] NA: neuraminidase

10.	3.3.10.	Fowlpox
11.	3.3.13.	Marek's disease
12.	3.4.12.	Lumpy skin disease
13.	3.7.2.	Rabbit haemorrhagic disease
14.	3.9.7.	Influenza A virus of swine
15.	3.10.1.	Bunyaviral diseases of animals (excluding RVF fever and Crimean–Congo haemorrhagic fever)

5.2. Chapter 3.1.15 *Nipah and Hendra virus diseases:* modifying the susceptible species to align with the case definition

See agenda item 3.1.1.

5.3. Follow-up from September 2021: conclusions and recommendations from the WOAH *Scientific and Technical Review* issue on diagnostic test validation science

5.3.1. Progress on development of a validation report form for tests recommended in the *Terrestrial Manual*

At the meeting in September 2022, the Commission agreed that the template originally developed for the validation data that would be requested of applicants wishing to add their test to a future online list of WOAH validated tests could be better used as a 'validation report' form for tests recommended in the *Terrestrial Manual*. With this new purpose in mind, the Commission addressed the comments submitted by experts participating in a pilot scheme to test the template's suitability and usability, and simplified and streamlined the form, as suggested. The new version of the document will be shared again with those WOAH Reference Laboratories in the pilot scheme for further feedback on its utility.

The main purpose of the validation report is to allow experts contributing to *Terrestrial Manual* chapters to post their validation data online so that users have access to it, can compare the data with their own platform's performance or simply to know who to ask should they have a question about a test method. Reference Laboratories would be invited to fill in the 'validation report' form, which would be made available in a repository on the website for anyone seeking the validation data available for the test. The Commission believes that this is an important advance, particularly for new technologies.

As stated in the September report, the template will also be used for experts requesting to add tests to the *Terrestrial Manual*.

5.3.2. Progress on development of a template for a new *Terrestrial Manual* section on the rationale behind the selection of tests included in Table 1. *Test methods available and their purpose*

At the meeting in September 2021, the Commission agreed to include test validation data in the *Terrestrial Manual* disease chapters and to justify the selection of tests considered to be fit for purpose in Table 1, along with their rating, based on expert opinion. It was felt that this information would help the reader to find relevant information for the selection of tests while making sure that the selection process is evidence-based and transparent. With the aim of developing an example to aid contributors to the *Terrestrial Manual*, the Commission gave the draft template to a group of experts currently updating a chapter with the request that they use it to provide the rationale for the selection and scoring of tests in the updated chapter. It was hoped that the feedback could be used to make the template useful and less time consuming if necessary. Unfortunately the experts were unable to complete the task.

At this meeting, the Commission decided to go ahead and to give the template to all experts in the next review cycle when they are invited to update or draft a *Terrestrial Manual* chapter. The experts will be asked to use the template or to choose another format: the experts' opinion on how best to collect and present this information in the *Terrestrial Manual* chapters will be reviewed at the next Commission meeting.

5.4. Instructions to authors: inclusion of text on point of care tests

The Commission reviewed and agreed on amendments to the instructions to authors to include information on POCTs [13].

5.5. Amendment to Chapter 3.10.7 *Salmonellosis*

WOAH had received a query regarding the following instruction in the rapid slide agglutination test in Chapter 3.10.07 *Salmonellosis*: "If nonspecific false-positive reactions are suspected, positive/suspicious sera may be retested after heat-inactivation at 56°C for 30 minutes."

The Member noted that heat inactivation will degrade antibodies (especially IgM) to a titre below detection in the RSA testing method so is not advised as it could cause false negatives. The instruction could have the potential in possibly leading to incorrect negative *Salmonella* serovar Gallinarum biovar gallinarum/pullorum results worldwide in poultry should there be an outbreak.

In consultation with the WOAH Reference Laboratories, the Commission agreed to amend the text as follows:

To replace "after heat-inactivation at 56°C for 30 minutes with "with the ELISA", so the sentence reads: "If non-specific false-positive reactions are suspected, positive/suspicious sera may be retested with the ELISA."

5.6. Inclusion of videos on diagnostic techniques on the WOAH website disease portals: development of a process, roles and responsibilities

The Commission had received some requests from Reference Laboratory experts updating *Terrestrial Manual* chapters to include links to videos illustrating the diagnostic techniques in question. The Commission welcomes this initiative but is aware that the video must meet a certain standard, be free from components such as trade names and be approved by consensus among Reference Laboratories. Rather than adding a direct link to videos, the Commission agreed that a link to the Reference Laboratory website could be added at the end of disease chapters with the note: "Videos of diagnostic methods available from the WOAH Reference Laboratories". To advance this initiative, the Secretariat were asked to contact the Reference Laboratories to ask if they have videos of diagnostic techniques they would like added to their chapter. At its next meeting in September, the Commission would review any videos submitted before approving the link to the site where they can be found to the *Terrestrial Manual* .

5.7. Request to further update the vaccine section of the Chapter 3.9.3. *Classical swine fever*

The Commission was informed that the recently adopted Chapter 3.9.3. *CSF* [14] still lacks some important information in the vaccine section, such as on minimum requirements for live recombinant vaccines and the potency test for batch release of the modified live marker vaccines. The gaps are causing confusion among Regulatory Authorities. The WOAH Reference Laboratories also agreed that the vaccine section needs to be updated to incorporate new generation vaccines. The chapter has thus been added to the 2023/2024 review cycle.

5.8. Review of advice submitted by experts on seven *Terrestrial Manual* chapters updated and circulated in October 2022 on whether the update had an impact on the corresponding chapter in the *Terrestrial Code*

At the September 2022 meeting of the Bureaus of the Code and Biological Standards Commissions it was agreed that the experts who reviewed a *Terrestrial Manual* chapter be requested to advise the Biological Standards Commission as to whether the proposed revision could have an impact on the corresponding *Terrestrial Code* chapter. The Secretariat of the Code Commission had identified seven *Terrestrial Manual* chapters in the current review cycle where the update may impact the *Terrestrial Code.* The Biological Standards Commission reviewed the advice received from experts who had undertaken the updates and agreed to submit the following recommendations to the Code Commission:

[13] POCTs: point-of-care tests
[14] CSF: classical swine fever

Code chapter	Recommendations from Biological Standards Commission to the Code Commission
Chapter 8.1. Anthrax	The Commission agrees the *Terrestrial Manual* update has no impact on the *Terrestrial Code* chapter
Chapter 8.14. Infection with rabies virus	The Commission agrees the *Terrestrial Manual* update has no impact on the *Terrestrial Code* chapter
Chapter 8.15. Infection with Rift Valley fever	The *Terrestrial Code* chapter only mentions quarantine as a control measure for animal movement. The Code Commission should consider including the recommended tests that are suitable for certifying animals for international movement
Chapter 8.17. Infection with *Trichinella* spp.	The Commission agrees that the *Terrestrial Code* chapter needs to update the number of taxa to include the new taxa
Chapter 9.2. Infection with honey bees with *Paenibacillus larvae* (American foulbrood)	The Commission agrees the *Terrestrial Manual* update has no impact on the *Terrestrial Code* chapter
Chapter 9.3. Infection with honey bees with *Melissococcus plutonius* (European foulbrood)	The Commission agrees the *Terrestrial Manual* update has no impact on the *Terrestrial Code* chapter
Chapter 11.9. Infection with lumpy skin disease	The Commission agrees the *Terrestrial Manual* update has no impact on the *Terrestrial Code* chapter, but that the *Terrestrial Manual* should add a sentence in the introduction that some wildlife species are susceptible to lumpy skin disease

5.9. Update on the development of guidelines on the manufacture of safe vaccines for African swine fever

The Commission was provided with an early draft of the guidelines for the development and manufacture of pure, potent, safe and efficacious. vaccines for ASF[15]. In the elaboration of the guidelines, a number of meetings have been held and the main WOAH ASF experts have been consulted. As the safety of ASF vaccines is a crucial issue, the Commission proposed that Regulatory Authorities be invited to participate in any future meetings.

5.10. *Terrestrial Manual* status: update on chapters selected for the 2023/2024 review cycle

The Commission examined the status of chapters that had previously been identified for update in the 2022/2023 review cycle but had not been received. As there are 34 chapters on the list, the Commission did not add the remaining chapters last adopted in 2018. The Commission encouraged those Reference Laboratories with outstanding chapters to deliver by the deadline. The following chapters have been identified for update in 2023/2024 (year last adopted in brackets after the title).

1.1.2. Collection, submission and storage of diagnostic specimens (2013)

1.1.4. Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities (2015)

1.1.5. Quality management in veterinary testing laboratories (2017)

1.1.7. Standards for high throughput sequencing, bioinformatics and computational genomics (2016)

1.1.9. Tests for sterility and freedom from contamination of biological materials intended for veterinary use (2017)

2.1.3. Managing biorisk: examples of aligning risk management strategies with assessed biorisks (2014)

2.2.1 Development and optimisation of antibody detection assays (2014)

2.2.2 Development and optimisation of antigen detection assays (2014)

[15] ASF: African swine fever

2.2.3 Development and optimisation of nucleic acid detection assays (2014)

2.2.4 Measurement uncertainty (2014)

2.2.5 Statistical approaches to validation (2014)

2.2.6 Selection and use of reference samples and panels (2014)

2.2.7 Principles and methods for the validation of diagnostic tests for infectious diseases applicable to wildlife (2014)

2.2.8. Comparability of assays after minor changes in a validated test method (2016)

2.3.1. The application of biotechnology to the development of veterinary vaccines (2010)

2.3.3. Minimum requirements for the organisation and management of a vaccine manufacturing facility (2016)

2.3.5. Minimum requirements for aseptic production in vaccine manufacture (2016)

3.1.8. Foot and mouth disease (infection with foot and mouth disease virus) (2021)

3.2.4. Nosemosis of honey bees (2013)

3.3.4. Avian influenza (including infection with high pathogenicity avian influenza viruses) (2021)

3.3.6 Avian tuberculosis (2014)

3.3.8. Duck virus hepatitis (2017)

3.3.12. Infectious bursal disease (Gumboro disease) (2016)

3.4.1. Bovine anaplasmosis (2015)

3.4.7. Bovine viral diarrhoea (2015)

3.4.11. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (2017)

3.4.12. Lumpy skin disease (vaccine section) (2021)

3.4.15. Theileriosis in cattle (infection with *Theileria annulata, T. orientalis* and *T. parva*) (2018)

3.6.10. Equine viral arteritis (2013)

3.6.9. Equine rhinopneumonitis (infection with equid herpesvirus-1 and -4) (2017)

3.8.1. Border disease (2017)

3.8.2. Caprine arthritis/encephalitis and Maedi-visna (2017)

3.8.12. Sheep pox and goat pox (2017)

3.9.3. Classical swine fever (infection with classical swine fever virus) (2022: diagnostic tests section)

3.9.9. Teschovirus encephalomyelitis (2017)

3.9.10. Transmissible gastroenteritis (2008)

3.10.4. Infection with *Campylobacter jejuni* and *C. coli* (2017)

3.10.8. Toxoplasmosis (2017)

3.10.9. Verocytotoxogenic *Escherichia coli* (2008)

6. WOAH Reference Centres

6.1. Annual reports of Reference Centre activities in 2022

A new electronic system had been launched in December 2022 to collect the annual reports of WOAH Reference Centre activities in 2022. Unfortunately a number of Reference Centres experienced problems filling in and submitting their reports. In view of these problems, the deadlines for submission of the reports were cancelled and those Reference Centres experiencing problems were invited to contact the WOAH IT Department. WOAH would like express its gratitude to the highly valued network for its collaboration and understanding.

At the last meeting in September 2022, the Commission reviewed the template and identified critical activities that all Reference Laboratories should report on. For the next meeting in September 2023, the Commission will take a closer look at the reports of those laboratories that appear to be underperforming in these areas, along with the reports of

the newly designated Reference Centres. The laboratories would be given feedback on the outcomes of the Commission's review.

The Commission expressed its appreciation for the continued support and expert advice given to WOAH by the Reference Centres. In accordance with the SOPs, those Reference Centres that were not complying with the performance criteria will be asked to provide an explanation of their situation; the Delegate will be in copy of all correspondence.

6.2. Applications for WOAH Reference Centre status

The Commission recommended acceptance of the following applications for WOAH Reference Centre status:

WOAH *Reference Laboratory for American foulbrood* (*infection of honey bees with* Paenibacillus larvae)
Animal Health Laboratory, Diagnostic and Surveillance Services, Biosecurity New Zealand, Ministry for Primary Industries, 66 Ward Street, Wallaceville, Upper Hutt 5018, NEW ZEALAND
Tel.: (+64-4) 894.56.00
E-mail: info@mpi.govt.nz / Richard.Hall@mpi.govt.nz
Website: https://www.mpi.govt.nz/science/laboratories/national-animal-health-laboratory/
Designated expert: Dr Richard Hall

WOAH *Reference Laboratory for varroosis of honey bees*
Animal Health Laboratory, Diagnostic and Surveillance Services, Biosecurity New Zealand, Ministry for Primary Industries, 66 Ward Street, Wallaceville, Upper Hutt 5018, NEW ZEALAND
Tel.: (+64-4) 894.56.00
E-mail: info@mpi.govt.nz / Richard.Hall@mpi.govt.nz
Website: https://www.mpi.govt.nz/science/laboratories/national-animal-health-laboratory/
Designated expert: Dr Richard Hall

WOAH *Collaborating Centre for Economics of Animal Health in the Americas Region*
Department of Agricultural Economics, Kansas State University, UNITED STATES OF AMERICA
Tel.: (+1-785 532.35.25)
E-mail: dpendell@ksu.edu
Website: www.ksu.edu
Contact Point: Dustin L. Pendell.

This multi-national WOAH Collaborating Centre will include participation from the following institutions:

Department of Economics, Business and Sociology (ESALQ/USP), University of São Paulo, BRAZIL
and
Faculty of Agronomy and Veterinary Medicine, University of Brasília, BRAZIL
Tel: (+55-19) 34.29.44.44 and (+55-61) 992.09.06.66
E-mail: shgdmira@usp.br / vitorspg@unb.br
Web site: www.esalq.usp.br / www.unb.br
Designated Contact Points: Dr Silvia Helena Galvão de Miranda / Prof. Vitor Salvador Picão Gonçalves.

Department of Business, Economics and Rural Development, Faculty of Veterinary Medicine and Husbandry, Universidad Nacional Autonoma De México, MEXICO
Tel: (+1-52) 56.22.59.05
E-mail: jldf@fmvz.unam.mx
Web site: www.fmvz.unam.mx
Designated Contact Point: Prof. José Luis Dávalos Flores.

School of Economic Sciences, Paul G. Allen School for Global Health, Washington State University, UNITED STATES OF AMERICA
Tel: (+1-509) 335.85.97
E-mail: tl_marsh@wsu.edu
Web site: www.wsu.edu
Designated Contact Point: Prof. Thomas L. Marsh.

WOAH *Reference Laboratory for lumpy skin disease*
Exotic and vector-borne diseases (EXOVEC), Department of infectious diseases in animals, Sciensano, Groeselenberg 99, 1180 Uccle, BELGIUM

Tel.: (+32-2) 379.06.27
E-mail: nick.deregge@sciensano.be
Websites: https://www.sciensano.be/en https://www.eurl-capripox.be/homepage
Designated expert: Dr Nick de Regge.

WOAH *Reference Laboratory for mammalian tuberculosis*
Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense de Madrid, Avenida Puerta de Hierro s/n 28040 Madrid, SPAIN
Tel.: (+34-394) 4033 /3992
E-mail: visavet@visavet.ucm.es mycobacteria@visavet.ucm.es
Websites: www.visavet.es / www.bovinetuberculosis.eu
Designated expert: Dr Beatriz Romero Martínez.

An application had been received for a Collaborating Centre for Genomic Outbreak Monitoring of Swine Diseases. This Commission was fully satisfied with the quality and scientific excellence of the applicant institution. However, the Commission found that the scope of the application was too narrow for a Collaborating Centre focusing on one disease only. The Commission encouraged the applicant to reapply emphasising their strengths and expertise in a broader range of services, such as vaccinology, and extending the application to include more experts in the proposed Centre and other swine diseases.

Finally an application had been received for a Collaborating Centre for Impacts of Global Changes on Infectious Animal Diseases. The applicant had chosen wildlife health and biodiversity as the main focus area with climate change and biodiversity, and drivers for emerging disease risks as the two specialties. The Commission acknowledged that it is important to have WOAH Collaborating Centres in these areas, especially for climate change and biodiversity. However, although the applicant has a large amount of expertise and work in these areas, the focus is national rather than international with details of what the applicant institutions do but not what the Collaborating Centre will do. It was noted that there are no collaborations with institutes in places where climate change will and is having its greatest impact, such as in Africa and South America. The applicant will be asked to re-draft the application so that it is more focused and precise, includes evidence of international collaborations, information on what training will be provided, of how they plan to interact with other Collaborating Centres, and information on the projected output of evidence-based veterinary research, and what will be delivered to WOAH.

The Commission noted that there are no longer WOAH Reference Laboratories for West Nile fever, equine encephalomyelitis (Eastern and Western) or Venezuelan equine encephalomyelitis in the Americas though they are important diseases in this region. The Commission also noted that there are no WOAH Reference Laboratories for glanders in Asia or the Americas, only two WOAH Reference Laboratories for lumpy skin disease and only one Reference Laboratory for Marek's disease. The Commission would welcome applications from suitable candidates for these diseases.

6.3. Changes of experts at WOAH Reference Centres

The Delegates of the Members concerned had submitted to WOAH the following nominations for changes of expert at WOAH Reference Laboratories. The Commission recommended their acceptance:

Brucellosis (Brucella abortus and B. melitensis): Dr Svetlana Berdenstein to replace Dr Menachem Banai at the Kimron Veterinary Institute, Beit Dagan, ISRAEL

Classical swine fever: Dr Yu-Liang Huang to replace Dr Chia-yi Chang at the Animal Health Research Institute, New Taipei City, CHINESE TAIPEI

The Commission noted that the expertise of the designated expert should not be limited to laboratory techniques but that in addition to being a leading and active researcher, they must be capable of providing advice on the control of the disease for which the Reference Laboratory is responsible.

6.4. Review of new and pending applications for laboratory twinning

As of February 2023, 73 projects have been completed and 29 projects are underway, and three projects are on hold. Of the completed projects, 11 Reference Laboratories and four Collaborating Centres have achieved WOAH designation status.

Two Laboratory Twinning project proposals were presented for the Commission's review:

1. ***Germany – Kyrgyzstan*** for brucellosis: the Commission supported the technical contents of this project proposal.
2. ***United Kingdom – China (People's Rep. of)*** for bovine tuberculosis: the Commission supported the technical contents of this project proposal.

6.5. Review of the draft questionnaire for Reference Laboratories

In the September 2022 meeting, the Commission decided to ask the Reference Laboratory experts feedback on their experience of being a WOAH Reference Laboratory. With this in mind, a member of the Commission developed a questionnaire for review by the Commission. The questionnaire is focused on the Reference Laboratory system, and includes topics that are not already covered in the annual report template, with a particular emphasis on the point of contact between Reference Laboratories and WOAH and the Commission. The survey will present an opportunity for experts to make suggestions for changes and improvements to the system. The Commission approved the questionnaire, which will be sent to all WOAH Reference Laboratories. The replies will be analysed at the September 2023 meeting. The questionnaire can be filled in anonymously, but respondents can include their contact details if they want to be approached.

6.6. Reference laboratories – implementation of the SOPs

6.6.1. Follow-up February 2022 meeting: further feedback from the laboratory that are not complying with the key ToR according to their 2018 annual report

The Commission reviewed the feedback received from the Reference Laboratory that was not complying with the key performance criteria according to its 2018 annual report. At the last meeting in September 2022, the Reference Laboratory was asked to detail the efforts taken to improve the laboratory's performance. The laboratory provided a description of the measures undertaken to improve sample submission, information on diagnostic and research activities, proficiency testing schemes and training programmes for the region. The Commission accepted the explanation submitted.

6.6.2. Follow-up from September 2022 meeting: feedback from the Laboratories that are not complying with the key ToR according to 2021 annual report

The Commission reviewed the feedback received from 15 Reference Laboratories that were not complying with key performance criteria according to their 2021 annual reports.

Many of the laboratories cited the impact of the COVID-19 pandemic as one of the reasons for the lack or limited number of international activities. Apart from the challenges caused by travel restrictions and shipment of samples, these laboratories provided lists of the activities where they remained active along with future plans to improve the performance. The Commission accepted the explanation.

Three laboratories reported that they did not receive any requests for diagnostic testing because they are located in regions free from the disease or because of improved testing capacity in national laboratories. However, these laboratories remained active producing or distributing reference reagents, organising inter-laboratory comparison tests, and providing scientific guidance. The Commission accepted the explanations and agreed that there is a need to maintain these Reference Laboratory facilities, and the accompanying training provided for certain diseases, irrespective of the low demand for diagnostic testing. The Commission will consider how to evaluate those laboratories in situations where the disease is well controlled or not widely distributed.

Of those laboratories that were requested to clarify their accreditation status to ISO 17025 or equivalent quality management system, three submitted the necessary certificate. The Commission accepted to grant two other Reference Laboratories an extension of 2 years to submit their accreditation certificate. A laboratory that had wrongly indicated that it did not maintain a biorisk management system clarified that it was an error and confirmed the ability to meet biosafety and biosecurity standards.

The Commission accepted the proposals provided by three laboratories for improvement in performance and placed them on a watch list for follow-up review in the next annual report review cycle.

6.7. Collaborating Centres – implementation of the SOPs

6.7.1. Develop a plan of how to evaluate the Collaborating Centres' activities in the past 5 years against their submitted 5-year work plans

Since implementation of the procedures for approval and maintenance of Collaborating Centre status [16] (SOPs) began, the term for a WOAH Collaborating Centre is for 5 years after which the designation will be reviewed by the relevant WOAH Specialist Commission. The 5-year period for the first batch of Collaborating Centre designations is 2020 to 2024, thus the first reviews are due to be communicated to the Centres in early 2025. In view of this, the Commission discussed the procedures to be followed to evaluate the summary of achievements of the Centres over the 5-year designation period.

The Commission developed a template for the Centres to submit evidence of their achievements against each of the activities listed in their submitted 5-year work plan. The Centres will be asked how its specific speciality supported the WOAH and its Members, the value provided, the achievements and its impact over the 5-year designation period. In September 2024, those Centres concerned will be provided with the evaluation template and asked to return it completed in early 2025. At the meeting in February 2025, the Commission will discuss the performances of those Centres: Centres with a positive evaluation will be asked to submit a new 5-year work plan for renewal of their designation for the years 2025 to 2029.

6.8. Reference Centre networks

6.8.1. Update on the three Reference Laboratory networks (rabies, peste des petits ruminants and African swine fever)

The WOAH ASF Reference Laboratory network held regular virtual meetings to exchange scientific and technical expertise, including recent developments on ASF vaccines, and discussed activities in developing training programmes to assist at-risk countries, including the organisation of proficiency tests. In November 2022, a regional laboratory expert meeting for ASF for the Asia-Pacific region was held in Geelong, Australia, attended by key ASF laboratories in the region, to share updates on diagnostic tools and their applications, information on the current situation, surveillance activities, research updates on ASF virus and vaccine development in the region. The network continues to work on a laboratory manual, including diagnostic algorithms to detect low virulent and novel emergent variants, and to explore user requirements on an open access information sharing platform for ASF virus genome sequence data. There are also plans to launch a website for the network.

The WOAH PPR [17] Reference Laboratory network organised its second workshop on 1 December 2022 (https://www.ppr-labs-oie-network.org/activities/meetings-and-workshops/2022-annual-workshop) with the participation of international and national laboratories from different regions. Laboratories from Austria, Bangladesh, Cameroon, China (People's Rep. of), France, India, Kenya, Nigeria, Pakistan, Russia, Senegal, South Africa, Tanzania, United Arab Emirates and the United Kingdom participated in this workshop. The aim of this workshop was to discuss the purpose of the network, its planned activities and how the network can be improved to benefit all members. The participants exchanged updates on PPR from different regions, validation assays, sequencing efforts, comparison of available serological assays for detecting PPR virus antibodies in wildlife and other diagnostic assays. A survey showed that the PPR network website (https://www.ppr-labs-oie-network.org/) is quite useful for the members to find validated protocols, information on training, various PPR activities, and contact details of other members. The participants made suggestions to improve the network activities. In addition, the PPR network will produce annual newsletters on its activities to be posted on its dedicated website. The first newsletter was disseminated in September 2022.

The WOAH Rabies Reference Laboratory Network (RABLAB) held quarterly virtual meetings to exchange scientific and technical expertise, including recent developments in rabies diagnostics, kits, training programmes and support to assist endemic countries. The network supports the FAO [18]/IAEA [19] Animal Production and Health Laboratory, Austria to produce anti-rabies reference serum. In December 2022, the WOAH RABLAB and WHO [20] Collaborating Centre for rabies had the first joint in-person meeting. This was also the first in-person meeting of the RABLAB network. At the meeting the experts exchanged information on the state of play of rabies biologicals, laboratory capacities, surveillance, and policy and finally identify the needs of both networks. The RABLAB network continues to participate in several twinning programmes to

16 https://www.woah.org/en/what-we-offer/expertise-network/collaborating-centres/#ui-id-2
17 PPR: peste de petits ruminants
18 FAO: Food and Agriculture Organization of the United Nations
19 IAEA: International Atomic Energy Agency (of the United Nations)
20 WHO: World Health Organization

build laboratory capacity, with network experts also supporting countries in the development and implementation of national control programmes for rabies. The Commission recommended providing RABLAB more visibility in the public domain and suggested developing a website dedicated to the network as was done for other disease networks. For the next meeting, the Commission asked RABLAB to give an update on the use of lateral flow devices as a tool for the diagnosis of rabies.

The Commission appreciated the efforts of all the three network Reference Laboratories in establishing scientific collaboration and exchange of technical information to contribute to the global eradication efforts.

6.8.2. Review of the current list of main focus areas and specialties

The Commission reviewed and updated the current list of Main Focus Area and Specialities for WOAH Collaborating Centres. The main edits include elaboration or addition of new specialities in each Main Focus Area, and changing the title of focus area 'Wildlife health and biodiversity' to 'Environment and climate change', which is broader and more relevant in the current context. The list will be reviewed by the Aquatic Animals Commission and the Working Group on Wildlife at their next meetings. The updated document with the changes shown is available at Annex 4.

6.8.3. Clarify the role of the Contact Point in providing advice and services to WOAH Members

Each WOAH Collaborating Centre has one designated Contact Point to supervise the Centre's activities and act as the liaison between WOAH, the Commission, and WOAH Members. The Contact Point is often the Director of the institute that hosts the Centre, though in reality, other members of the Centre's personnel often take responsibility for responding to administrative issues or requests for assistance from Members on behalf of the official Contact Point. The official Contact Point will be asked if they are willing to continue to receive and re-direct requests or if they would prefer to nominate another staff member to be the first point of contact for their Centre. This member will not replace the official Contact Point of the Centre but will facilitate all contact between the Centre and the Commission or WOAH.

7. *Ad hoc* Groups: Update on activities of past *ad hoc* Groups

7.1. *Ad hoc* Group on Replacement of the International Standard Bovine Tuberculin (ISBT) and Avian Tuberculin (ISAT)

The Commission was updated on the progress with the development of the replacement ISBT. The UK Health Security Agency (UK HSA) has completed the first round of trials and is currently analysing the results and preparing the report to present to the *ad hoc* Group. The Commission commended the Group's efforts to date and look forward to the results of the UK HSA trials that are expected to be completed in May 2023.

The Group is also working on the replacement ISAT. The National Institute for Biological Standards and Control (NIBSC), the main custodian of the international standards for purified protein derivatives of bovine and avian tuberculin, is currently declining all requests for ISAT and has removed it from their catalogue. WOAH initiated the call for replacement candidates for avian tuberculin during the Third Partnership Review meeting held in Paris in February 2023. A call for tender is also currently under preparation.

A member of the Commission was identified to join the Group as an observer.

8. International Standardisation/Harmonisation

8.1. WOAH Register of diagnostic kits – update and review of new or renewed applications

The Secretariat for the Registration of Diagnostic Kits (SRDK) informed the Commission that at present, there are 14 registered kits; one new application (2023), which was endorsed during the meeting, with two diagnostic kits renewals, and two other products extending claims.

8.1.1. Endorsement of VDRG® FMDV 3Diff/PAN Ag Rapid kit

The Commission was informed that the evaluation of the dossier on "VDRG® FMDV 3Diff/PAN Ag Rapid kit (MEDIAN Diagnostics Inc.) has been completed. Based on the final report from the Expert Review Panel, the Commission endorsed the Panel's recommendation to approve the kit's 'fitness for purpose' as described in the Validation Studies Abstract and User's Manual (Instructions for Users).

The VDRG® FMDV 3Diff/PAN Ag Rapid kit is a lateral flow test intended for the universal detection of FMD virus (FMDV) of serotypes A, O and Asia-1 in tissue samples (epithelium) or fluid from blisters or ruptured lesions of suspected swine or cattle. The test is designed to be used for the rapid diagnosis of FMDV infection in samples from swine or cattle.

The Validation Studies Abstract drafted by the manufacturer and approved by the Expert Review Panel was endorsed by the Commission (see Annex 5).

8.1.2. Addition of new claim (milk) for the Enferplex Bovine TB antibody test

The Commission was further informed that Enferplex Bovine TB antibody test's (Enfer Scientific ULC) application for extension of the claim has been completed. The Commission endorsed the Panel's recommendation to approve the supplementary validation data to support the new claim for milk: *for the detection of IgG anti-*Mycobacterium bovis *in cattle milk samples.* The test is designed to be used for the diagnosis of bovine tuberculosis infection and evaluation of antibody response to *M. bovis.*

In 2019 this test was provisionally approved for testing milk samples from cattle as a herd screening test, or as a supplemental confirmatory test for use in individual animals, when used in conjunction with other methods for diagnosing and managing *M. bovis* infection (https://www.woah.org/app/uploads/2021/03/a-r31-diagnostic-kits.pdf).

The Validation Studies Abstract drafted by the manufacturer and approved by the Expert Review Panel was endorsed by the Commission (see Annex 6).

8.1.3. Extension of claim (additional species: water buffalo) of BOVIGAM® – *Mycobacterium bovis* Gamma interferon test kit for cattle

The Commission was also informed that the evaluation of the dossier on "BOVIGAM® – *Mycobacterium bovis* Gamma interferon test for cattle (registered at WOAH originally in 2015, renewed 2020 for the Marketing Authorisation holder: Thermo Fisher Scientific Prionics AG, approval number: 20150110) application for extension has been completed; the application was submitted by Thermo Fisher Scientific Prionics Lelystad B.V. legal dossier holder. Based on the final report from the Expert Review Panel, the Commission endorsed the Panel's recommendation to approve the supplementary validation data for this previously approved diagnostic kit and approve the application for extension of the claim to use in water buffalos (*Bubalus bubalis*).

The BOVIGAM® – *Mycobacterium bovis* Gamma interferon test kit is an indirect assay intended for the detection of interferon-gamma (IFNγ) response elicited to specific stimulation *by M. bovis specific* peptides or proteins, in plasma obtained from stimulated blood samples of suspected water buffalos (*Bubalus bubalis*)

The Validation Studies Abstract – Supplementary Data, drafted by the manufacturer and approved by the Expert Review Panel, was endorsed by the Commission (see Annex 7).

8.1.4. Renewal of Rapid MERS-CoV Ag Test (BioNote Inc.)

The Commission endorsed the recommendation for the 5-year renewal of the BioNote® Rapid MERS-CoV[21] Ag Test (BioNote, Inc.) based on the conclusions and recommendations of the Review Panel Final Report on the supplementary data and Validation Studies Abstract. The kit was originally registered in 2016.

The Rapid MERS-CoV Ag Test (BioNote Inc.) for the qualitative detection of MERS-CoV antigens from nasal swabs in dromedary camels for the following purposes:

- Detection of MERS CoV infected herds (herd test) with acutely infected animals with high virus loads;
- When used as a supplemental test, to estimate prevalence of infection to facilitate risk analysis, e.g. surveys, herd health schemes and disease control programmes.

The Validation Studies Abstract – Supplementary Data, drafted by the manufacturer and approved by the Expert Review Panel, was endorsed by the Commission (see Annex 8).

[21] MERS-CoV: Middle East Respiratory Syndrome Coronavirus

8.1.5. Third Renewal of IDEXX *M. bovis* Antibody Test Kit

The Commission endorsed the additional 5 year renewal of *Mycobacterium bovis* Antibody Test Kit (IDEXX Laboratories) based on the consolidated recommendation of three WOAH Reference Laboratories. The kit was originally registered in 2012 and renewed 2017.

Mycobacterium bovis Antibody Test Kit is intended for the detection of antibody to *M. bovis* in cattle serum and plasma samples. The test is designed to be used as a supplemental test, in conjunction with other methods, for diagnosing and managing tuberculosis infection. The test also has utility when performing serosurveys to understand prevalence and risk at a herd management level.

There is no Validation Studies Abstract for this kit, as this is a renewal without any additional data evaluation or changes.

8.1.6. Additional information on kits

The Commission was informed that the Aquatic Animal Health Commission (AAHC) endorsed the Experts Panel's recommendation to approve Innocreate Bioscience WSSV RP Rapid test to be added to WOAH's Register of Diagnostic kits, validated as fit for purpose (qualitative detection kit for *Whispovirus, White Spot Syndrome Virus* (WSSV) infection in shrimps.

Two diagnostic kits are scheduled to be renewed for the next 5 years in May 2024, Avian Influenza Disease Antibody Test Kit, Newcastle Disease Virus antibody detection ELISA (BioCheck UK Ltd) and one new application, which is under assessment.

8.1.7. The Future Secretariat for Registration of Diagnostics Kits – New Concept Note for the Registration of Diagnostic Kits

The Commission was informed on the work to review the registration process to increase the value of what WOAH provides to Members in the field of diagnostics kits. After 20 years of existence and with only 14 kits included in WOAH's registry. A consultation was carried out with key stakeholders in the field, yielding three leads worth exploring:

a) Mechanisms that could be implemented for facilitating regulatory harmonisation of diagnostic kits;

b) The value of setting minimum criteria needed for reliable registration of diagnostic kits, facilitating accessibility to Members regardless of their regulatory capacity;

c) Streamlining kit recognition procedures and aligning WOAH Reference Centres with SRDK Diagnostic kit activities.

This exercise could take around 24 months and will lead to a totally renewed and restructured SRDK. While the leads are explored, SRDK will stop reviewing and validating new dossiers. Only those currently under evaluation or potential renewals will be processed, as well as exceptional cases linked to an emergency animal health situation.

8.2. Standardisation programme

8.2.1. Association française de normalisation: questions for the Commission

On behalf of AFNOR[22] (CEN/TC 469), a technical committee established in the summer of 2021 at the level of the European Committee for Standardization (CEN), the following questions were submitted to the Commission for feedback.

1) How changes to WOAH manuals could be proposed by CEN/TC 469 and considered by WOAH

The Commission responded that AFNOR could submit their proposals for changes to the WOAH manuals through the European Union representative.

[22] AFNOR: Association française de normalisation

2) How CEN/TC 469 could be more directly involved in the work and discussions of the WOAH Biological Standards Commission

The Commission responded that CEN could not be involved directly in the work and discussions of Commission. Members of the Commission are elected by the Assembly as independent neutral experts in a given area. CEN can contribute to the work through the submission of comments on the Commission's work programme within the normal procedure of the standard-setting process.

3) In Chapter 1.1.6 *Principles and methods of validation of diagnostic assays for infectious diseases*, it is mentioned that "at least three laboratories" should be involved in the assessment of inter-laboratory reproducibility during the validation of a diagnostic tool. However, several European experts consider, on a statistical basis, that five to eight laboratories should be involved in such assessment for the results to be interpreted appropriately

To evaluate this request, the Commission asked for the data on the statistical analysis mentioned by CEN so that it could be submitted to the experts revising the chapter.

8.2.2. Project to extend the list of WOAH-approved reference reagents: review of guidelines

At the September 2022 meeting, the Commission discussed the project to extend the list of WOAH-approved reference reagents. The Commission asked the disease-specific networks, namely ASF, rabies and PPR, to review the guidelines for antibody standards[23], antigen standards[24] and PCR assays[25], and also to consider submitting candidate reagents.

One of the networks commented that the requirements set out in the guidelines are of a too high standard for Reference Laboratories wishing to have their reagents approved and added to the list. The Commission pointed out that the guidelines set out the ideal situation, but that the inclusion of the phrase "where possible" allows the user to not meet all of the provisions. The Commission will discuss this topic at the next meeting when more feedback has been received. In the meantime the Commission asked the Secretariat to make the template for the data to be submitted with a request to add an antibody assay available online.

8.2.3. Guidance document for production of an in-house positive control serum for rabies serology

Rabies serological tests are undertaken worldwide in laboratories working on rabies activities. The *Terrestrial Manual* recommends the use of the WOAH anti-rabies positive reference serum of dog origin to titrate serum samples in international units (IU)/ml for use in rabies serological tests. This reagent has been produced by the WOAH Reference Laboratory in France since 1991. As it is running out, the Reference Laboratory is currently working with the WOAH Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis (Austria) to produce a new batch as part of the RABLAB network activities (see agenda item 6.8.1). The laboratory, with the support of RABLAB, has developed a guidance document to ensure the appropriate use of the WOAH international rabies-positive serum and support national laboratories in the production and calibration of their in-house positive control serum. This document can be used for producing rabies control serum in dogs and could also be potentially used to produce rabies control serum from different animal species. The Commission approved the guidance document, which will be published on the WOAH website.

9. Resolutions for the General Session

The Commission noted that the following resolutions would be proposed for adoption at the General Session in May 2023:

A resolution proposing the adoption of 15 draft chapters and the glossary of terms for the *Terrestrial Manual*;

A resolution proposing the new WOAH Collaborating Centres.

The following resolutions would be proposed for adoption by the alternative procedure before the General Session in May 2023:

A resolution proposing the new WOAH Reference Laboratories for terrestrial animal diseases;

A resolution on the WOAH Register of Diagnostic Kits.

[23] https://www.woah.org/app/uploads/2021/03/a-guideline-antibody-standards.pdf
[24] https://www.woah.org/app/uploads/2021/03/a-guideline-antiegen-standards.pdf
[25] https://www.woah.org/app/uploads/2021/03/a-guideline-pcr-standards.pdf

10. Conferences, Workshops, Meetings

10.1. Update on the WAVLD seminar in Lyon, France in 2023

WOAH plans to hold its customary 1-day seminar at the biennial International Symposium of the World Association of Veterinary Laboratory Diagnosticians (ISWAVLD) in Lyon, France, 29 June–1 July 2023. WOAH is a member of the Scientific Committee of ISWAVLD 2023. The Theme of the conference is “Towards the Veterinary Diagnostics of the Future”.

The One Health concept will be a major theme of the symposium. In light of the COVID-19 pandemic and the resulting spotlight on laboratory diagnosis along with the significant work at WOAH related to laboratory sustainability, pandemic preparedness, and resilience, the topics of the WOAH seminar will highlight how the lessons learnt from the COVID-19 pandemic and the involvement of the veterinary laboratory sector have maintained WOAH’s seat at the policy-making table to inform more holistic and efficient disease prevention from a One Health perspective.

11. Matters of interest for consideration or information

11.1. Update on OFFLU

The Commission was briefed on OFFLU[26]’s contribution to the WHO Consultation on the Composition of Influenza Virus Vaccines on avian influenza and swine influenza for the period February to September 2022.

During the reporting period, the avian influenza epidemic continued with high numbers of detections reported globally in poultry and non-poultry including wild birds mainly in the Europe and Americas Regions. The disease also spread to several new countries in Central and South America. The predominant subtype circulating in the current epidemic period is H5N1, and there was unusual persistence of the virus in wild birds during the summer months. A rising number of H5N1 avian influenza cases has been reported in several mammalian animals both terrestrial and aquatic, causing morbidity and mortality. In response to these outbreaks, OFFLU network experts participated in teleconferences to share epidemiological and molecular data on currently circulating viruses and released situation updates and statements needed to inform surveillance and control policies. There were regular communication exchanges with WHO to share public health and animal health data so that risk assessments are updated on issues related to the animal–human interface, including pandemic preparedness.

Data for 588 HPAI H5, 60 LPAI H7 and 89 H9 avian influenza genetic sequences were contributed by animal health laboratories in countries representing Africa, the Americas, Asia-Pacific and, Europe. Additionally, data for 345 swine H1 sequences from 18 different clades and 116 swine H3 sequences from eight different clades were analysed and submitted. Antigenic characterisations were undertaken by OFFLU contributing laboratories and subsequently there were updates to the WHO recommendations for the development of new candidate vaccine viruses for pandemic preparedness purposes. Based on this contribution, two new candidate vaccine viruses for an H5 clade 2.3.4.4b-like virus and an H3N8-like human, avian lineage virus were proposed against avian origin viruses.

OFFLU embarked on a project called avian influenza matching (AIM) for characterisation of circulating avian influenza viruses in different regions to support poultry vaccination. Annual reports will be published to provide up-to date information to the animal health sector, governments, and poultry vaccine manufacturers on antigenic characteristics of circulating avian influenza viruses including comparisons with vaccine antigens. This information will facilitate selection of appropriate vaccines for poultry and updating of poultry vaccine antigens in places where vaccines are being used.

In view of the significant changes observed in the epidemiology of HPAI viruses in the recent years, WOAH collaborated with FAO through the GF-TADs[27] mechanism and established the HPAI task force to initiate revision of the global strategy for prevention and control of HPAI which was last updated in October 2008. Experts from the OFFLU network will be engaged to support the revision of the strategy.

[26] OFFLU: Joint WOAH-FAO Network of Expertise on Animal Influenza
[27] GF-TADs: Global Framework for the Progressive Control of Transboundary Animal Diseases

11.2. Update on rinderpest

The Commission was informed that there were five countries holding RVCM[28] outside of FAO-WOAH designated RHFs[29]. Regarding the RHFs, five of the seven facilities were inspected in 2022 by an independent expert team coordinated by FAO and WOAH. The inspectors recommended that the mandate of the inspected RHFs be extended for another 3-year period. The procedure for inspecting and designating RHFs, nominating inspectors, and the policy on destruction and sequestration will be discussed and reviewed during a workshop with analogous teams from WHO and FAO later in 2023. The 18th meeting of the FAO-WOAH Joint Advisory Committee (JAC) for Rinderpest took place in Paris from 10 to 11 December 2022. In addition to discussing the extension of the RHFs, for which JAC's recommendation was in line with that of the inspectors, the JAC also recommended a 3-year extension of the mandate of the two RHFs that were not inspected in 2022, conditional to their agreement to be inspected in 2024. With regards to research, JAC recommended the approval of two 'sequence and destroy' projects in two different RHFs. The size, membership, and scope of the JAC will be reviewed throughout 2023 to reflect the evolving challenges of the second phase of the post-eradication era.

11.3. Update on Global Burden of Animal Diseases Programme

The Commission was updated on key milestones met by the GBADs[30] programme team in developing, refining and testing GBADs methodologies and informatics. The key milestones include deriving initial animal disease and health burden estimates within the Ethiopia country case study (the programme's proof-of-concept cases study), the first Ethiopia case study stakeholder workshop to present work done and prioritise possible follow-up work of value to national stakeholders, and obtaining initial user feedback for the various dashboards developed thus far presenting various estimates. In the coming months, the work plan will focus on: i) completing the scientific validation process of the GBADs approach, ii) demonstrating the utility of GBADs in Ethiopia, and iii) updating the knowledge engine prototype built to align with overall progress to move GBADs out of the proof-of-concept phase.

11.4. Update on VICH[31] activities

The Commission was updated on the 41st VICH Steering Committee, 15th VICH Outreach Forum (VOF), which took place from 14 to 17 November 2022 in Washington (USA).

The VOF Members received an overview of the approaches taken by the VICH full Members (European Union, Japan and Untied States) to address the stability testing of vaccines for veterinary use. The training material is available on the VICH website (https://www.vichsec.org/en/training.html)

The VICH Steering Committee agreed a structural reorganisation: as a result the VOF will become the VICH Forum to better address the needs. New member of the VOF include the Southern African Development Community (SADC), Eastern African Community (EAC) and Botswana showing that significant progress has been made on harmonisation of the authorisation of veterinary medicinal products and implementation of different VICH Guidelines.

The Commission noted that the 7th VICH Public Conference will take place in Amsterdam, the Netherlands in November 2024. The programme will be shared when it is available.

11.5. Update on the Grand Challenge for sustainable laboratories

WOAH, in partnership with Global Affairs Canada, other G7 Global Partnership countries, Grand Challenges Canada, and the Pirbright Institute, was exploring possibilities to launch a grand challenge to seek solutions to improve the sustainability of laboratories. The partnership was undertaking a feasibility study to assess the likely success of a grand challenge. As part of the feasibility study the partnership would test the water (to see if potential innovative solutions exist) with a request for expressions of interest that would be launched on 24 February 2023. The outcome of the feasibility study would inform the decision on whether to hold a full grand challenge, which would involve the mobilisation of considerable resources. The Commission was supportive and would like to be kept informed.

11.6. Biosafety Research Roadmap

The Biosafety Research Roadmap (BRM) had two main outcomes: 1) a review of laboratory acquired infections and escapes (due to be published in peer-reviewed journals) and 2) a series of papers assessing the current evidence to inform commonly used biosafety and biosecurity measures for selected pathogens (due to be published in *Applied*

[28] RVCM: Rinderpest virus-containing materials
[29] Rinderpest holding facilities
[30] GBADs: Global Burden of Animal Diseases Programme
[31] VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

Biosafety in spring 2023). A member of the Commission had been representing the Commission on the BRM technical working group. The next step, in March 2023, would be to draft a policy paper (in partnership with WHO and Chatham House) around the need for a greater evidence base to inform laboratory biological risk management.

11.7. Dual use research of concern

In recent times the topic of Dual Use Research of Concern (DURC) stimulated WOAH's interest with the publication of studies by Fouchier[32] and Kawaoka[33] on aerosol transmission of H5N1 between mammalian hosts. There have been numerous other instances over the past decade, and interest was reignited with speculation around the origin of COVID-19 and laboratory experiments with CoV viruses. Although the risk from DURC has always been there, it may be increasing owing to advances in technology, and the widening availability and decreased cost of synthetic biology. The perception of what is DURC may be varied and wide, and the debate also risks creating barriers to important research (particularly basic research) and dissemination of scientific findings. Within the context of WOAH's work on Biothreat Reduction, WOAH convened a working group to develop some 'Guidelines for responsible conduct in veterinary research', which were published in 2019. https://www.woah.org/app/uploads/2021/03/a-guidelines-veterinary-research.pdf

The Commission noted that WHO has issued the *Global guidance framework for the responsible use of the life sciences: mitigating biorisks and governing dual-use research*. The guidance calls on WHO Member States and other stakeholders to mitigate biorisks and safely govern dual-use research, which has a clear benefit but can be misused to harm humans, other animals, agriculture and the environment.

The framework underlines that there is no one-size-fits-all approach to prevent and mitigate biorisks and adopts an integrated approach of biorisk management, which relies on three core pillars: biosafety, laboratory biosecurity and the oversight of dual-use research. It raises awareness about the importance of undertaking biorisk management within the context of the One Health approach to optimize the health of people, animals and ecosystems. A first regional workshop was organised on 24–25 January 2023, in collaboration with Africa CDC[34], in Nairobi, Kenya to start the operationalisation of the framework in the African region. Partners from WOAH, UNEP[35] and FAO attended this first workshop in the region.

WOAH proposes to collaborate with WHO by jointly holding some informal discussions which follow a format previously used by WHO ('DURC dialogues') to explore awareness, attitudes and risks in the animal health sector.

The Commission agreed that it would be a good idea for WOAH to partner with WHO on a framework to manage risks around Dual Use Research of Concern. The Commission said it would be important to define the term and to ensure animal, plant and environment sector interests were represented in such a framework. WHO and WOAH would host a DURC discussion on 14 March 2023. A member of the Commission was identified to represent the Commission in this initiative.

11.8. WOAH Terrestrial Standards Coordination

The Commission was informed of a new mechanism established within the WOAH Secretariat, and chaired by the Deputy Director General, International Standards and Science aimed at achieving more efficient and integrated management of the process of developing new or revised standards for terrestrial animals. The mechanism involves integrating the planning of activities of WOAH teams providing technical support, coordination, and input to WOAH Standard-setting work, as well as coordinating the work plans of the Specialist Commissions involved in the development of WOAH standards for terrestrial animals. The Commission was informed that this mechanism was supported by a process agreed upon by the Commissions' Presidents on the steps and specific Commissions intervention and interaction in standard setting.

The Biological Standards Commission supported the initiative and noted that their regular review cycle for the revision of the *Terrestrial Manual* chapters is established and fitted well with the proposed approach. The Commission highlighted the new process implemented at this meeting to provide early advice to the Code Commission on the potential need to update the *Terrestrial Code* as a consequence of updates being proposed for adoption in the *Terrestrial Manual* (see agenda item 5.8), and noted that it was a critical contribution to this coordination, to ensure consistency between these two complementary sets of standards and continuity between the work programmes of the two Commissions.

[32] https://www.science.org/doi/10.1126/science.1213362?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
[33] https://www.nature.com/articles/nature10831
[34] CDC: Centers for Disease Control and Prevention
[35] UNEP: United Nations Environment Programme

11.9. Guidelines for the national procurement of veterinary vaccines

The Commission was informed about the development of Practical Guidelines for National Procurement of Veterinary Vaccines. This initiative resulted from the acknowledgement of the challenges faced by WOAH Members in the national procurement of quality veterinary vaccines. Drawing from its transversal experience (vaccine banks, PVS Pathway, including PVS Veterinary Legislation Support Programme, Procurement, etc.), WOAH drafted short and practical guidelines with checklist and templates, with the support of a consultant and the collaboration of a group of experts (composed of WOAH Collaborating Centres, public and private partners). At the time of the meeting the guidelines were being piloted by a few countries.

The Commission highlighted the importance for these guidelines to ensure that the quality of the vaccines would be carefully considered in the procurement process and asked WOAH to share these guidelines for their information once finalised.

_____

…Annexes/

Annex 1. Adopted Agenda

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

1. Welcome

1.1. Director General
1.2. Deputy Director General, International Standards and Science
1.3. Updates from the WOAH Headquarters

2. Adoption of Agenda

3. Collaboration with other Commissions

3.1. Scientific Commission for Animal Diseases

3.1.1. Case definitions: infection with Crimean–Congo haemorrhagic fever virus and infection with Nipah virus (Nipah virus encephalitis)

3.2. Terrestrial Animal Health Standards Commission

3.2.1. Updates from the September 2022 Code Commission meeting
3.2.2. Questions on Chapter 12.7 *Infection with* Theileria equi *and* Babesia caballi (*equine piroplasmosis*)
3.2.3. Questions on Chapter 8.8 *Infection with foot and mouth virus*
3.2.4. Questions on Chapter 12.6 *Infection with equine influenza virus*
3.2.5. Comments on Chapter 12.2 *Infection with* Taylorella equigenitalis (*contagious equine metritis*)
3.2.6. Use of terms: 'bovid', 'bovidae', 'bovine' and 'cattle'; 'enzootic', 'endemic', 'epizootic' and 'epidemic'
3.2.7. Use of terms related to diagnosis and diagnostic methods

3.3. Aquatic Animal Health Standards Commission

3.3.1. Meeting of the Bureaus of the Commissions

4. Work Programme

5. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

5.1. Review of Member comments received on draft chapters and their endorsement for circulation for second-round comment and proposal for adoption in May 2022
5.2. Chapter 3.1.15 *Nipah and Hendra virus diseases:* modifying the susceptible species to align with the case definition
5.3. Follow-up from September 2021: conclusions and recommendations from the WOAH *Scientific and Technical Review* issue on diagnostic test validation science

5.3.1. Progress on development of a validation report form for tests recommended in the *Terrestrial Manual*
5.3.2. Progress on development of a template for a new *Terrestrial Manual* section on the rationale behind the selection of tests included in Table 1. *Test methods available and their purpose*

5.4. Instructions for authors: inclusion of text on point of care tests
5.5. Amendment to Chapter 3.10.7 *Salmonellosis*
5.6. Inclusion of videos on diagnostic techniques on the WOAH website disease portals: development of a process, roles and responsibilities
5.7. Request to further update the vaccine section of the Chapter 3.9.3. *Classical swine fever*
5.8. Review of advice submitted by experts on seven *Terrestrial Manual* chapters updated and circulated in October 2022 on whether the update had an impact on the corresponding chapter in the *Terrestrial Code*
5.9. Update on the development of guidelines on the manufacture of safe vaccines for African swine fever.
5.10. *Terrestrial Manual* status: update on chapters selected for the 2023/2024 review cycle

6. WOAH Reference Centres

6.1. Annual reports of Reference Centre activities in 2022

6.2. Applications for WOAH Reference Centre status

6.3. Changes of experts at WOAH Reference Centres

6.4. Review of new and pending applications for laboratory twinning

6.5. Review of the draft questionnaire for Reference Laboratories

Reference Laboratories – Implementation of the SOPs

6.6. Follow-up February 2022 meeting: further feedback from the laboratory that are not complying with the key ToR according to their 2018 annual report

6.7. Follow-up September 2022: feedback from the Laboratories that are not complying with the key ToR according to 2021 annual report

Collaborating Centres – Implementation of the SOPs

6.8. Develop a plan of how to evaluate the Collaborating Centres' activities in the past 5 years against their submitted 5-year work plans

Reference Centre networks

6.9. Update on the three Reference Laboratory networks (rabies, peste des petits ruminants and African swine fever)

6.10. Review of the current list of main focus areas and specialties

6.11. Clarify the role of the Contact Point in providing advice and services to WOAH Members

7. *Ad hoc* Groups

Update on activities of past *ad hoc* Groups

7.1. *Ad hoc* Group on Replacement of the International Standard Bovine Tuberculin (ISBT) and Avian Tuberculin (ISAT)

8. International Standardisation/Harmonisation

8.1. WOAH Register of diagnostic kits: Update and review of new or renewed applications

8.1.1. Endorsement of VDRG® FMDV 3Diff/PAN Ag Rapid kit

8.1.2. Addition of new claim (milk) for the Enferplex Bovine TB antibody test

8.1.3. Extension (additional species: water buffalo) of BOVIGAM® - *Mycobacterium bovis* Gamma interferon test kit for cattle

8.1.4. Renewal of Rapid MERS-CoV Ag Test (BioNote Inc.)

8.1.5. Third Renewal of IDEXX *M. bovis* Antibody Test Kit

8.1.6. Additional information on kits

8.1.7. The Future Secretariat for Registration of Diagnostics Kits – New Concept Note for the Registration of Diagnostic Kits

8.2. Standardisation programme

8.2.1. Association française de normalisation: questions for the Commission

8.2.2. Project to extend the list of WOAH approved reference reagents: review of guidelines

8.2.3. Guidance document for production of an in-house positive control serum for rabies serology

9. Resolutions for the General Session

10. Conferences, Workshops, Meetings

Future Conferences, Workshops, Meetings

10.1. Update on the WAVLD seminar in Lyon, France in 2023

11. Matters of interest for consideration or information

11.1. Update on OFFLU

11.2. Update on rinderpest

11.3. Update on Global Burden of Animal Diseases programme

11.4. Update on VICH activities
11.5. Update on the Grand Challenge for sustainable laboratories
11.6. Biosafety Research Roadmap
11.7. Dual use research of concern
11.8 WOAH Terrestrial Standards Coordination
11.9. Guidelines for the national procurement of veterinary vaccines

Annex 2. List of Participants

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

MEMBERS OF THE COMMISSION

Prof. Emmanuel Couacy-Hymann
(President)
Professor of Virology,
CNRA/LIRED,
Abidjan,
CÔTE D'IVOIRE

Prof. Ann Cullinane
(Vice-President)
Head of Virology Unit,
Irish Equine Centre,
Naas,
IRELAND

Dr John Pasick
(Vice-President)
Formerly National Centre for Foreign Animal Disease,
Winnipeg,
CANADA

Dr Joseph S. O'Keefe
(Member)
Head of Animal Health Laboratory,
Ministry for Primary Industries,
Upper Hutt,
NEW ZEALAND

Dr Satoko Kawaji
(Member)
Principal Scientist
National Institute of Animal Health,
Naro,
JAPAN

Prof. Chris Oura
(Member)
Professor of Veterinary Virology,
The University of the West Indies,
St-Augustine,
TRINIDAD AND TOBAGO

CONSULTANT EDITOR OF THE *TERRESTRIAL MANUAL*

Dr Steven Edwards
c/o WOAH, Paris, FRANCE

WOAH HEADQUARTERS

Dr Gregorio Torres
Head
Science Department

Ms Sara Linnane
Scientific Officer
Science Department

Dr Gounalan Pavade
Scientific Coordinator
Science Department

Annex 3. Work Programme for the WOAH Biological Standards Commission

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

Subject	Issue	Status and Action
Updating the *Terrestrial Manual*	1) Circulate the chapters approved by the BSC to Member Countries for second-round comment	March 2023
	2) Remind authors of the chapters identified previously for update but not yet received and invite authors of chapters newly identified for update	On-going
	3) Create a database of validation reports to be published on the WOAH Website for tests recommended in the *Terrestrial Manual*	On-going
	a) Send the template for the validation data for tests recommended in the *Terrestrial Manual* to the experts who submitted the original comments	March 2023
	4) Add a new section to the disease-specific chapters to describe the rationale behind the selection of tests for different purposes given in Table 1 *Test methods available and their purpose* and an explanation for their score. Subsequently, add links to the validation reports (point 3 above)	On-going
	a) Send the template for this new section to the experts updating Manual chapters asking they use it or provide a justification in an alternative format of their choice	On-going
	5) Ask Reference Centres to provide links to suitable instructional videos to be added to the end of the disease-specific chapters. Videos to be reviewed by the BSC when the chapter is up for review	On-going
Collaborating Centres	1) Implementation of the adopted SOPs:	
	a) Ask the Collaborating Centres to submit a report of their assessment of their performance in the past 5-years to be compared with the 5-year work plan	December 2024
	2) Review the designations of those Centres that completed 5 years	September 2025
	3) Ask the Contact Point to nominate a first point of contact to address administrative queries, enquires, etc. on behalf of the Centre	April/May 2023
Reference Laboratories	1) Put under-performing labs on watch list	On going
	2) Update document detailing past history of annual report reviews	For September 2023
	3) Send a questionnaire to Reference Laboratories to get feedback on their experiences as a WOAH Ref. Lab.	March/April 2023
	4) Analyse the answers to the questionnaire	September 2023

Subject	Issue	Status and Action
	5) Explore enhancements to the annual report process: the possibility of filling in the annual report template throughout the year	For September 2023
Reference Centre Networks	1) Follow up with the three newly launched Reference Laboratory networks (ASF, PPR and rabies)	On-going
Standardisation/ Harmonisation	1) Project to extend the list of OIE approved reference reagents	On-going
	2) Ask the networks to review the three guidelines for standard reagents and consider submitting candidate reagents	For September 2023
	3) Project to develop Replacement International Standard Bovine and Avian Tuberculin: finalise report and propose for adoption	On-going
***Ad hoc* Groups**	1) *Ad hoc* Group on Sustainable Laboratories	On-going
Projects	1) Veterinary Biobanking (project)	On-going
Conferences, Workshops and Meetings with participation by BSC Members	1) Biosafety research roadmap	On-going
	2) ISWAVLD OIE seminar: theme and programme and speakers	June 2023
Performance	1) Engage with the ongoing processes around performance issues with Reference Labs	On-going
Develop laboratory standards for emerging diseases	1) Discuss the *Terrestrial Code* chapter once adopted with the aim of introducing a corresponding chapter for the *Terrestrial Manual*	After May 2023
Case definitions	1) Follow up the implementation of the SOPs for case definitions	On-going

Annex 4. List of Main Focus Area and Specialties for WOAH Collaborating Centres

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

The role of WOAH Collaborating Centres is anchored to the WOAH's founding mandate and to the Seventh Strategic Plan (2021–2025)[36].

1. Laboratory expertise

This topic covers issues related to management and operation of veterinary diagnostic laboratories. It corresponds essentially to provisions of Chapters 1.1.1 to 1.1.7 of the *Terrestrial Manual*, as well as Chapter 2.1.2, and to Chapters 1.1.1 and 1.1.2 of the *Aquatic Manual*. Beyond WOAH standards, the topic is expected to assist WOAH and its Members to follow the recommendations of the first two International Conferences on Biological Threat Reduction, as well as to contribute to the Seventh WOAH Strategic Plan and commitment to modern technology.

- Biorisk management
- Quality management systems
- Biobanking and reference collections
- Genomics and bioinformatics
- Laboratory information systems technology
- Validation ~~procedures for diagnostic tests~~ of laboratory methods

2. Training and education

It is part of the WOAH's founding mandate to improve the legal framework, competency and resources of national Veterinary Services, and particularly their global public good components. This topic covers the scientific and technical veterinary knowledge and skills needed for veterinarians, animal health professionals and veterinary para-professionals to implement WOAH Standards. The topic primarily, but not exclusively, corresponds to provisions of the Section 3 of the *Terrestrial* and *Aquatic Codes*. The topic is also expected to assist the WOAH and its Members to follow-up on the recommendations of ~~the first two~~ International Conferences of Veterinary Education.

- Veterinary undergraduate education
- Veterinary post-graduate training and education (scientific and technical) ~~and capacity building~~
- Veterinary specialisation and laboratory expertise in infectious diseases
- ~~Capacities~~ Capacity building of Veterinary Services

3. Animal health management

WOAH has the responsibility to collect, analyse and disseminate relevant scientific information, especially on disease control methods, and to provide expertise in the control of animal diseases including zoonotic diseases, as well as at the animal–human–ecosystems interface, while taking into account the "One Health" concept whenever possible. This topic covers issues primarily, but not exclusively, related to Sections 2 and 4 of the *Terrestrial* and *Aquatic Codes* and to Part~~s~~ 3 of the *Terrestrial Manual* and Part 2 of ~~both~~ the ~~*Terrestrial* and~~ *Aquatic Manuals,* respectively. The topic is expected to assist WOAH and its Members to fulfil the core missions of the organisation.

- Disease ~~control~~ prevention, risk assessment and preparedness
- Species related (e.g. molluscs, bees, camelids)
- Preventing animal disease along the value-chain ~~biosecurity~~
- Emerging animal diseases (early detection and response)
- Animal health emergencies
- Zoonotic diseases
- Epidemiology, modelling, surveillance
- Social and economic implications of animal diseases
- Biothreat reduction

36 https://www.woah.org/en/document/seventh-strategic-plan/

4. Animal production

WOAH's founding mandate has evolved and has been adapted to Members' needs, it now includes improving the safety of food of animal origin from hazards originating in animal production, and establishing standards and guidelines for animal welfare through a science-based approach and promote their application. This topic corresponds to this mandate and more specifically to Section 7 of the *Terrestrial* and *Aquatic Codes* on animal welfare, and the relevant provisions on food and feed safety in ~~the chapters in~~ Section 6 on Veterinary Public Health of the *Terrestrial Code* ~~(Chapters 6.1, 6.2, 6.3, 6.5, 6.12, 6.13)~~ and Chapter ~~4.8~~ 4.9 of the *Aquatic Code*.

- Animal welfare
- Animal production food safety
- Sustainable animal production
- Safety of animal feed

5. Veterinary products

This topic corresponds to Chapters 1.1.8 to 1.1.10, and most of the specific recommendations included in the Part 2 of the *Terrestrial Manual*. Progress made on vaccines, diagnostics and the development of new drugs is believed to contribute to the global efforts against antimicrobial resistance. As for antimicrobial resistance, the topic also corresponds to Chapters 6.1 to 6.4 of the *Aquatic Code*, Chapters 6.6 to 6.10 of the *Terrestrial Code*, and Chapter 2.1.1 of the *Terrestrial Manual*.

- Vaccines, diagnostics ~~(kits)~~, and drugs
- Antimicrobial ~~agents~~ resistance
- New technologies

6. ~~Wildlife health and biodiversity~~ Environment and climate change

WOAH provides expertise to Members in understanding and managing the effects of environmental and climate changes on animal health and welfare. Climate change is likely to increase pressure on animal production, and provide newly suitable conditions for invasive pests and pathogens. The risk of emergence of new pathogens has increased as a consequence of global changes in the way food is produced, moved and consumed. This topic is expected to address animal health issues, including aquatic animals, connected to wildlife, biodiversity, climate change, and emerging risks.

- Threats to livestock or wildlife health
- Climate change and biodiversity
- Disease related (including vector-borne)
- Drivers for emerging risks

Annex 5. WOAH Procedure for Registration of Diagnostic Kits
Validation Studies Abstract

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

Name of the diagnostic kit: VDRG® FMDV 3Diff/PAN Ag Rapid kit

Manufacturer: MEDIAN Diagnostics Inc.

Procedure /Approval number: WOAH 022029

Date of Registration: May 2023

Disease: Foot and Mouth Disease (FMD) in swine and cattle.

Pathogen Agent: Foot and Mouth Disease virus (FMDV)

Type of Assay: Lateral flow test or pen-side test

Purpose of Assay: The VDRG® FMDV 3Diff/PAN Ag Rapid kit is a lateral flow test or pen-side test intended for the universal detection of foot-and-mouth disease virus (FMDV) and differentiation of serotypes A, O and Asia-1 in tissue samples (epithelium) or fluid from blisters or ruptured lesions of suspected swine or cattle. The test is designed to be used for the rapid diagnosis of foot-and-mouth disease virus infection in samples from swine or cattle.

Species and Specimens

Tissue samples (epithelium) or fluid from blisters or ruptured lesions of suspected swine or cattle

1. **Information on the kit**
   Please refer to the kit insert available on the WOAH Registry web page or contact the manufacturer at MEDIAN Diagnostics Inc.
2. **Summary of validation studies**

Analytical specificity

***Conclusion*:** The kit did not respond to other viruses causing vesicular lesions like the clinical symptoms of FMDV, namely Vesicular stomatitis virus, Swine vesicular disease virus and Seneca valley virus). In addition, there was no cross-reaction for other serotypes in each line.

No.	Virus name	Cross-reaction
1	Vesicular stomatitis virus	No
2	Swine vesicular disease virus	No
3	Seneca Valley virus	No

Analytical sensitivity

***Conclusion*:** The detection limit was measured by serially diluting the virus culture solution 10-fold using the negative samples. The virus culture solution was previously titrated with $TCID_{50}$/ml. And it was compared with Ag ELISA (FMDV ANTIGEN DETECTION and SEROTYPING ELISA (FMDV O, A, C, Asia1, SAT1-2, Pirbright, UK) and RT-PCR (AccupPower FMDV Real-Time RT-PCR MasterMix kit, BIONEER).

Although there is a slight difference from strain to strain, this Ag Rapid kit could detect up to $1.12x10^4TCID_{50}$/ml for type O, up to $1.12x10^4TCID_{50}$/ml for type A, and $8.43x10^4TCID_{50}$/ml for type Asia1. The limit of detection (LoD) of this Ag rapid kit

for Type SAT1 was 8.43x10^{5}TCID$_{50}$/ml; Type SAT2 was 1.5x10^{5}TCID$_{50}$/ml, and Type SAT3 was up to 7.38x10^{4}TCID$_{50}$/ml when using the spiked viral culture solution in saliva.

Type O was detectable by 5.01x10^{4}TCID$_{50}$/ml, Type A was 3.16x10^{4}TCID$_{50}$/ml, Type Asia1 was 3.2x10^{4}TCID$_{50}$/ml, Type SAT1 was 2x10^{5}TCID$_{50}$/ml, Type SAT2 was 7.9x10^{4}TCID$_{50}$/ml, Type SAT3 was detectable up to 5.01x10^{4}TCID$_{50}$/ml when using the spiked viral culture solution in 20% homogenate of tissue.

Limit of detection (Saliva spiking)

Serotype	Strain	Topotype	TCID$_{50}$/㎖	Rapid Cut-off		
				VDRG FMDV 3Difff/PAN(TCID$_{50}$/ml)		RT-PCT
				Strip 3Diff	Strip PAN	Ct value
O	Jin-cheon	SEA/Mya-98	1.5x10^6	1.5x10^4	1.5x10^4	24.94
O	O/Hapcheon/KOR/2014	SEA/Mya-98	1.12x10^6	1.12x10^4	1.12x10^4	26.91
O	Gim-je	SEA/Mya-98	1.5x10^6	1.5x10^4	1.5x10^4	25.82
O	Bo-eun	ME-SA/ind-2001d	1.42x10^7	1.42x10^5	1.42x10^5	18.41
O	Jeong-eup	ME-SA/Ind-2001d	3.56x10^6	3.56x10^4	3.56x10^4	19.18
O	O1manisa	ME-SA	1.12x10^6	1.12x10^4	1.12x10^4	20.2
A	Po-cheon	Asia/Sea-97	4.74x10^6	4.74x10^5	4.74x10^5	16
A	Yeon-cheon	Asia/Sea-97	1.50x10^6	1.5x10^5	1.5x10^5	16.02
A	Malaysia97	Asia/Sea-97	2.0x10^6	2.0x10^4	2.0x10^4	22.27
A	P1A-189	FMDV A/SAU/2/2015	4.74x10^5	4.74x10^4	4.74x10^4	16.38
A	Iran05	Asia/Iran-05	6.32x10^5	6.32x10^4	6.32x10^4	17.06
A	A22 Iraq	Asia/G-IV	1.12x10^6	1.12x10^5	1.12x10^4	19.6
Asia1	MOG/05	G-V	1.50x10^7	1.5x10^5	1.5x10^5	17.08
Asia1	CAM/9/80		8.43x10^6	8.43x10^4	8.43x10^4	17.56
Asia1	Shamir		1.12x10^7	1.12x10^5	1.12x10^5	20.61
SAT1	SAT1/BOT/1/68	WZ(Ⅲ)	8.43x10^6	-	8.43x10^5	15.33
SAT2	SAT2/ZIM/5/81	WZ(Ⅱ)	1.50x10^6	-	1.5x10^5	15.84
SAT3	SAT3/ZIM/4/81		7.38x10^6	-	7.38x10^4	19.05

Limit of detection (Tissue spiking)

Serotype	Strain	TCID$_{50}$/㎖	Rapid Cut-off		
			VDRG FMDV 3Difff/PAN(TCID$_{50}$/ml)		RT-PCT
			Strip 3Diff	Strip PAN	Ct value
O	O1manisa	5.01x10^7	5.01x10^4	5.01x10^4	24.94
A	A22 Iraq	3.16x10^6	3.16x10^4	3.16x10^4	24.85
Asia1	Shamir	3.2x10^5	3.2x10^4	3.2x10^4	24.76
SAT1	SAT1/BOT/1/68	2x10^6	-	2x10^5	19.33
SAT2	SAT2/ZIM/5/81	7.9x10^5	-	7.9x10^4	22.01
SAT3	SAT3/ZIM/4/81	5.01x10^6	-	5.01x10^4	24.96

Repeatability

***Conclusion*:** Using a series of three-lot products, three independent operators tested the standard substance (O, A, Asia1 each strong, medium, weak positive sample, negative 4 samples, total 13 samples) twice a day for 10 days per lot. The within-run, between-run, between-day and within-laboratory precision test results were all determined to be consistent.

Three experimenters tested repeatability with three lots of products and found that 100% of the results were consistent.

Standard No.	#1		#2		Rate of matching
	Strip 3Diff	Strip PAN	Strip 3Diff	Strip PAN	
FMDVO-001	3+	3+	3+	3+	100%
FMDVO-002	2+	2+	2+	2+	100%
FMDVO-003	1+	1+	1+	1+	100%
FMDVA-001	3+	3+	3+	3+	100%
FMDVA-002	2+	2+	2+	2+	100%
FMDVA-003	1+	1+	1+	1+	100%
FMDVAS-001	3+	3+	3+	3+	100%
FMDVAS-002	2+	2+	2+	2+	100%
FMDVAS-003	1+	1+	1+	1+	100%
Sal-B-001	-	-	-	-	100%
Sal-B-002	-	-	-	-	100%
Sal-P-001	-	-	-	-	100%
Sal-P-002	-	-	-	-	100%
Tis-B-001	-	-	-	-	100%
Tis-B-002	-	-	-	-	100%
Tis-P-001	-	-	-	-	100%
Tis-P-002	-	-	-	-	100%

Diagnostic characteristics

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Conclusion

Sensitivity, specificity, and CI values were calculated by using https://www.medcalc.org/calc/diagnostic_test.php

1. Sensitivity

FMDV-positive samples in Korea, Vietnam, Myanmar

Sensitivity in bovine: 98.35% (n=595/605), (95% CI: 96.98% ~ 99.20%)

Sensitivity in swine: 99.1% (n= 544/549), (95% CI: 97.89% to 99.70%)

Total 98.7% sensitivity (n=1139/1154), (95% CI: 97.87% to 99.27%)

2. Specificity

FMDV-negative saliva in Korea (RT-PCR)

Specificity in bovine: 100% (n=92/92), (95% CI: 96.07% to 100.00%)

Specificity in swine: 99.5% (n= 398/400), (95% CI: 98.21% to 99.94%)

FMDV-negative tissue in Korea (RT-PCR)

Specificity in bovine: 100% (n=150/150), (95% CI: 97.57% to 100%)

Specificity in swine: 100% (n= 150/150), (95% CI: 97.57% to 100%)

Total 99.7% specificity (n= 790/792), (95% CI: 99.09% to 99.97%)

Reproducibility

Analytical reproducibility

***Conclusion*:** Using a series of products, researchers in three different laboratories tested the standard substance (O, A, Asia1 each strong, medium, weak positive sample, negative 4 samples, total 13 samples) twice a day for 5 days per Lot. The reproducibility test results were all determined to be consistent.

Three different labs tested reproducibility, and 100% of the results were consistent.

Diagnostic reproducibility

***Conclusion*:** Using a series of products, researchers in two different diagnostic laboratories tested the standard substance (O, A, each 2 strong, medium, weak positive samples, negative 4 samples, total 16 samples) twice a day for 3 days per Lot. There 2 different results in weak positive samples and all the other determined to be consistent.

Two different labs tested reproducibility, and 99.5% of the results were consistent.

Reference

Ku, B., Nah, J. & Ryoo, S., Sagong, M. & Kim, T. & Park, S-H. & Lee, J-W & Lee H J. & Wee, S-H. Development of rapid detection lateral flow strip kit for Foot-and-Mouth Disease virus serotypes O, A and Asia1 in clinical samples, 2017 Global FMD Research Alliance, p63, 2017

JACOBSON R.H. Validation of serological assays for diagnosis of infectious diseases, Rev. sci. tech. Off. int. Epiz., 17, p469-486, 1998

Annex 6. WOAH Procedure for Registration of Diagnostic Kits
Validation Abstract Sheet

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

Name of the diagnostic kit: Enferplex Bovine TB antibody test

Manufacturer: Enfer Scientific ULC

WOAH original approval number: 20190113

New procedure/approval number: 111824

Date of Registration: May 2023

Disease: Bovine tuberculosis

Pathogen Agent: *Mycobacterium bovis*

Type of Assay: Indirect chemiluminescent multiplex ELISA

Purpose of Assay:

Certified by the WOAH as fit for the detection of antibody to *Mycobacterium bovis* in bovine milk samples (May 2023) to be used as an ancillary test in conjunction with other methods for serological prevalence surveys, or diagnosis and management of *M. bovis* infection within herds, in particular for the following purposes:

1. To confirm, but not negate, diagnosis of suspect or clinical cases, including confirmation of positive screening tests in individual animals and in herds based on detection of antibodies in individual bovine milk samples excluding colostrum and first milk samples taken within 4 days of calving.

2. As a screening test to identify herds with *Mycobacterium bovis* infection based on detection of antibodies in bovine bulk tank milk samples excluding colostrum and first milk samples taken within 4 days of calving.

Species and Specimens

The test has been validated and approved for testing individual and bulk tank milk samples from cattle.

3. **Information on the kit**

   Please refer to the kit insert available on the WOAH Registry web page or contact manufacturer at:

Enfer Scientific ULC, Unit T, M7 Business Park, Newhall, Naas, Co. Kildare, Ireland.
Web: https://www.enfergroup.com/
Email: info@enfergroup.com
Tel: 00353 45 983800

4. **Summary of validation studies**

Analytical specificity

Individual milk samples

Analytical specificity was assessed using individual milk samples from bovine TB (bTB) free cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* **(**MAP), Bovine Viral Diarrhoea Virus (BVDV), and Infectious Bovine

Rhinotracheitis (IBR), Fasciola hepatica FH (FH), Bovine corona virus (BCV) and Bovine Respiratory Syncytial Virus (BRSV). The results are shown in Table 1.

Table 1. Analytical specificity of the Enferplex test using individual milk samples

Sample set	No. samples	Analytical specificity % high sensitivity setting										
		Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	Ag11
Map positive	129	99.2	97.7	100	99.2	100	97.7	99.2	99.2	100	97.7	97.7
BVDV positive	611	100	100	100	100	100	100	100	100	100	100	100
IBR gE positive	861	100	100	100	100	100	100	100	100	100	100	100
FH positive	286	99.7	100	99.7	99.7	99.7	100	100	100	100	100	100
BCV positive	536	99.6	100	99.6	99.8	99.8	100	100	100	100	100	100
BRSV positive	1096	99.7	100	99.7	99.8	99.8	100	100	100	100	100	100

The results show very high analytical specificity in individual milk samples from cattle infected with the listed pathogens.

Bulk tank milk samples

Analytical specificity was assessed using bulk tank milk samples from cattle naturally infected with MAP, BVDV, IBR, FH, BCV or BRSV. The results are shown in Table 2.

Table 2. Analytical specificity of the Enferplex test using bulk tank milk samples

Sample set	No. samples	Analytical specificity % high sensitivity setting										
		Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	Ag11
MAP positive	148	100	100	99.3	99.3	99.3	100	100	100	100	100	100
BVDV positive	52	100	100	100	100	100	100	100	100	100	100	100
IBR gE positive	1020	100	100	100	100	100	100	100	100	100	100	100
FH positive	158	100	100	100	100	100	100	100	100	100	100	100
BCV positive	1410	99.9	100	99.8	99.9	99.9	99.9	100	100	100	100	100
BRSV positive	1663	99.9	100	99.9	99.9	99.9	99.9	100	100	100	100	99.9

The results show very high analytical specificity in bulk milk samples from herds infected with the listed pathogens.

Conclusion: The specificity of the Enferplex Bovine TB assay was not adversely affected by MAP or other common pathogens of cattle when using individual milk samples or bulk tank milk samples from bTB negative animals.

Analytical sensitivity

Individual and bulk tank milk samples

Analytical sensitivity was estimated for each antigen in the test using endpoint titration of a strong positive individual anamnestic milk sample and a strong positive non-anamnestic bulk milk sample. The results show that the endpoint titres for the individual milk sample ranged from1:160 – 1:2560 across the 11 antigens in the test using individual milk, and 1/20 – 1/2560 using bulk tank milk.

Conclusion. The results show high endpoint titres and dynamic range of the test using individual anamnestic milk samples and good endpoint titres and dynamic range using non-anamnestic bulk milk samples.

Repeatability

Individual milk samples

To determine the within-run and between-run repeatability, three categories of milk sample were used: one milk sample negative against all 11 antigens; one milk sample dilution for each antigen giving weak positivity; one milk sample dilution for each antigen giving strong positivity. The samples were run in quadruplicate over 20 runs, split between 2 days and 2 operators. The mean, standard deviation (SD) and coefficient of variation (CV) of Relative Light Unit (RLU) values were calculated.

The % CV within-run and between run for weak positive samples ranged from 3.8 – 9.6% and for strong positive samples ranged from 1.4 and 3.9%. The mean values did not exceed 2 SDs over 20 runs of the test.

Bulk tank milk samples

To determine the within-run and between-run repeatability, three categories of milk sample were used: one bulk milk sample negative against all 11 antigens; one bulk milk sample for each antigen giving weak positivity; one bulk milk sample for each antigen giving strong positivity. The samples were run in quadruplicate over 20 runs, split between 2 days and 2 operators. The mean, standard deviation (SD) and coefficient of variation (CV) of RLU values were calculated.

The % CV within-run and between-run for weak positive samples ranged from 3.2 – 10.8% and for strong positive samples ranged from 1.4 and 4.0%. The mean values did not exceed 2 SDs over 20 runs of the test.

Conclusion: The Enferplex Bovine Tb antibody test showed very good within well and between run repeatability using individual and bulk tank milk samples.

Diagnostic characteristics

Threshold determination

Thresholds for the individual antigens were set empirically, targeting specificity at 98% for the high sensitivity setting and 99.5% for the high specificity setting of the test. The threshold for overall assay positivity was set based on a 2 – antigen rule, whereby the RLU signals from 2 or more antigens need to be above their individual antigen thresholds for the sample to be registered as “positive”. Sensitivity is maximised by taking the milk sample approximately 5-30 days after a SICCT test. The PPDb injection ‘boosts’ the antibody levels in animals which have been primed through *M. bovis* infection (‘boosted’ sample). If milk is taken outside this timeframe, then no boosting effect would be expected (‘non-boosted’ sample) and the sensitivity is somewhat lower.

Relative diagnostic sensitivity (DSn) and specificity (DSp) estimates

The performance levels indicated below were based on multiple batches of the Enferplex Bovine TB antibody test and reflect the biological diversity with respect to kit components (recombinant antigens, buffers, and conjugates, positive and negative controls). Relative diagnostic sensitivity was estimated using boosted individual milk samples from SICCT test positive animals and using non-boosted bulk tank milk samples from SICCT test positive herds in the UK and IE. Diagnostic specificity of individual milk samples was estimated using bTB free animals from the UK, and of bulk tank milk samples using herds from the UK, DK, DE, and NO that were deemed to be free of bTB.

Individual milk samples

Individual boosted milk samples from 305 SICCT test positive animals and from 1149 non-boosted and 195 boosted true negative reference animals from the UK were tested in the Enferplex Bovine TB antibody test. The results are shown in Table 3.

Table 3. Relative sensitivity of the Enferplex Bovine TB antibody test in individual milk samples using the high sensitivity setting

Test method under evaluation	Statistical variable	Target Species – cattle high sensitivity	Target Species – cattle high specificity
Relative diagnostic sensitivity SICCT test positive **Boosted**	N RSn CI	**305** **90.8%** **87.1-93.6**	**305** **87.2%** **83.0-90.6**
Relative sensitivity SICCT positive, bTB lesion positive Boosted	N RSn CI	**83** **95.2%** **88.3-98.1**	**83** **90.4%** **82.1-95.0**
Relative diagnostic specificity SICCT test negative and/or OTF status and Bovine TB history **Non-boosted**	N RSp CI	**1149** **99.7%** **99.2-99.9**	**1149** **99.8%** **99.4-100.0**
Relative diagnostic specificity SICCT test negative and/or OTF status and Bovine TB history **Boosted**	N RSp CI	**195** **98.5%** **95.6-99.5**	**195** **99.5%** **97.2-99.9**

The results show that the relative sensitivity was 90.8% and 87.2% using the high sensitivity and high specificity settings of the test respectively in boosted individual milk samples from SICCT test positive herds. In SICCT positive, lesion positive animals, the relative sensitivity was 95.2% and 90.5% using the high sensitivity and high specificity settings of the test respectively. The specificity was 99.7% using the high sensitivity setting and 99.8% using the high specificity setting of the test in bTB free herds. The relative specificity in boosted individual milk samples from bTB-free animals was 98.5% and 99.4% using the high sensitivity and high specificity settings of the test respectively.

Kappa agreement analysis between the Enferplex test and SICCT test results gave a Kappa value of 0.934 95% CI: (0.911-0.957) showing almost perfect agreement using boosted individual milk samples. Similarly, a Kappa value of 0.951 (95% CI: 0.911-0.973) was found between the Enferplex test and SICCT positive, lesion positive animals, indicating almost perfect agreement. Almost perfect agreement was observed using Kappa analysis between Enferplex antibody results and SICCT test status in boosted samples from SICCT test positive animals and boosted samples from bTB negative animals.

Likelihood ratio (LR) analysis was performed taking test outputs with a $LR^+ > 10$ or $LR^- < 0.1$ as good diagnostic evidence of the infection being either present or absent respectively (Caraguel & Colling, 2021). The Likelihood ratio (LR) for positive LR+ and LR^- were 347.8 (95% CI: 112.3-1077.5) and 0.092 (95% CI: 0.065-0.131) respectively for boosted samples from SICCT positive animals. The diagnostic odds ratio (DOR) was 3779.1 In boosted samples from SICCT positive animals with lesions, the LR+ and LR- were 364.5 (95% CI: 117.6 – 1129.8) and 0.048 (95% CI: 0.019-0.126) respectively. The DOR was 7544.5.

Analysis of paired milk and serum samples from 199 boosted SICCT test positive animals using Spearman's Rank correlation test gave coefficients ranging between 0.78 – 0.96 for the individual antigens used in the Enferplex test. The results thus showed good correlation between serum and milk samples. Analysis of paired serum and milk results using the McNemar discrimination test showed that the differences in proportions between serum and milk were not statistically significant at either the high sensitivity setting or the high specificity setting of the test. Similar high correlations between

serum and milk sample results were obtained when the number of antigens recognised by antibody was used instead of continuous data.

Conclusion: The results indicate that individual milk samples could be used instead of serum for the serodiagnosis of bTB using the Enferplex Bovine TB antibody test.

Bulk tank milk samples.

The relative diagnostic sensitivity and specificity of the Enferplex Bovine TB antibody test was estimated using bulk tank milk samples from bTB breakdown herds and bTB-free herds respectively.

Bulk tank milk samples from 235 SICCT positive herds and from 1792 true negative reference herds in the UK and Europe were tested in the Enferplex Bovine TB antibody test. The bulk tank milk samples from bTB positive herds were taken at the time of reading the SICCT test and were therefore non-boosted. The results are shown in Table 4.

Table 4. Relative sensitivity and specificity estimate of the Enferplex Bovine TB antibody test using non-boosted bulk tank milk samples

Test method under evaluation	Statistical variable	Target Species – cattle High Sensitivity	Target Species – cattle High Specificity
Relative diagnostic sensitivity SICCT positive	N	**247**	**247**
	RSn	**77.7%**	**71.7%**
	CI	**72.1-82.5**	**65.4-76.9**
Relative diagnostic specificity SICCT negative and/or OTF status and Bovine TB history	N	**1792**	**1792**
	RSp	**99.8%**	**99.9%**
	CI	**99.4-99.9**	**99.6-99.9**

The results show that the relative sensitivity was 77.7% and 71.7% using the high sensitivity and high specificity settings of the test respectively in non-boosted bulk tank milk samples from SICCT test positive herds. The specificity was 99.8% using the high sensitivity setting and 99.9% using the high specificity setting of the test in bTB free herds. Bulk tank milk samples were grouped depending on country of origin and the specificity obtained in the Enferplex Bovine TB antibody test compared. The results show that the specificity ranged between 99.0 – 100%, indicating that the diagnostic specificity of the Enferplex Bovine TB antibody test did not differ significantly between countries.

The relative sensitivity for bulk tank milk samples with low SICCT test prevalence (0.1 – 1.0%) was 74.1% using the high sensitivity setting of the test. No significant differences were noted in the Enferplex test relative sensitivity in relation to reactor prevalence, herd size or milk yield. Kappa agreement analysis between the Enferplex test bulk tank milk results and SICCT test results gave a Kappa value of 0.842 showing almost perfect agreement.

The likelihood ratio (LR) for positive (LR^+) and negative (LR^-) bulk tank milk samples were 348.0 and 0.223 respectively. The DOR was 1560. Test outputs with an $LR^+ > 10$ or $LR^- < 0.1$ are considered good diagnostic evidence of the infection being either present or absent respectively.

Conclusion: The results show that the Enferplex Bovine TB antibody test can be used to confirm the results of the SICCT test and as a screening test for bTB using non-boosted bulk tank milk samples.

Reproducibility

Evaluation panels of samples comprising negative, weak positive and strong positive individual milk and bulk tank milk samples were blinded and sent to the 3 independent laboratories for analytical reproducibility testing. Seven negative samples, 7 weak positive samples, and 7 strong positive samples were tested using two plates from two different kit batches and 1 technician in each laboratory. The results were sent to Enfer Scientific for un-blinding and analysis.

A series of linear mixed effect models were run with kit batch, laboratory, and sample considered. The results included the overall means, SD, CV, the 95% CI with the Upper and Lower Control Limits, an estimate of how much variation was due to these variables, and statistical analysis of the differences observed.

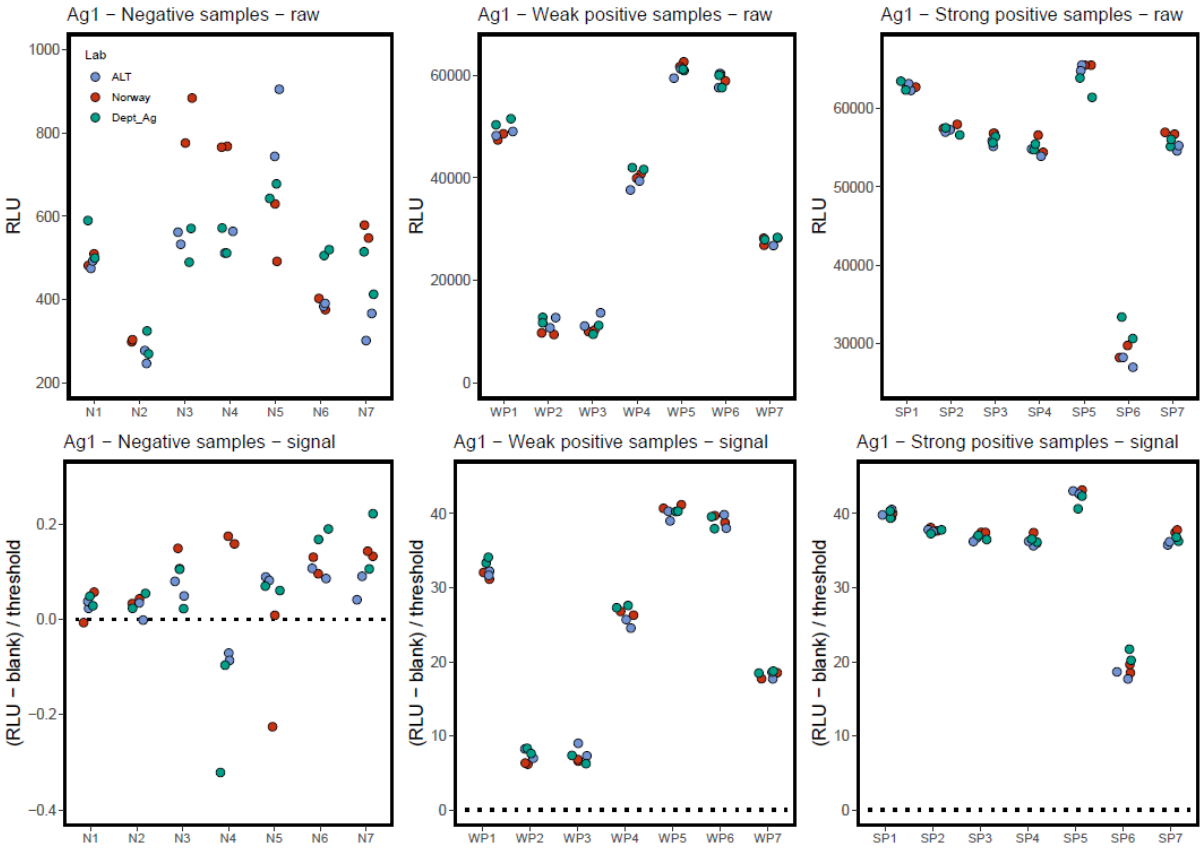
Analytical reproducibility

Individual milk samples

The results show that the CVs for negative samples varied extensively, reflecting the fact that a high proportion of the S/CO ratios were close to or below zero. The results showed that most of the S/CO ratio responses with WP and SP samples had CVs less than 10%. There were 31 results where the CVs were >10%. Of these, 23/31 were associated with responses that were below threshold for the individual antigens and would be deemed to be negative responses for those antigens. The CVs of the remaining 9 were 10.4, 10.4, 11.2, 12.0, 12.1, 12.4, 12.6, 13.3, and 15.9%. Analyses using mixed linear models showed that for the WP and SP samples, 98 – 100% of the variation was due to the sample and none was due to the kit or laboratory.

An example of the individual milk reproducibility RLU and S/CO data for Ag 1 obtained by three laboratories (colour coded in duplicate) is shown in Figure 1. The cut-off for S/CO ratio is 1.

Figure 1. Individual milk reproducibility data for Ag1.



Conclusion: The Enferplex Bovine TB antibody test thus shows good analytical reproducibility between kits and laboratories when testing individual milk samples.

Diagnostic reproducibility

The diagnostic reproducibility results for individual milk samples are shown in Table 5.

Table 5. Summary of diagnostic reproducibility testing using the 2 Ag rule

Samples	Number positive/tested		
	Laboratory 1	Laboratory 2	Laboratory 3
Positive control	2/2	2/2	2/2
Negative control	0/2	0/2	0/2
Blinded Negatives	0/7	0/7	0/7
Blinded weak positives	7/7	7/7	7/7
Blinded strong positives	7/7	7/7	7/7
Blinded weak positives	7/7	7/7	7/7
Blinded strong positives	7/7	7/7	7/7

The results show complete concordance between the 3 laboratories. The results demonstrate high reproducibility of the Enferplex Bovine TB antibody test when used in 3 different laboratories with 2 different kit batches using individual milk samples.

Analytical reproducibility

Bulk tank milk

The results show that the CVs for negative samples varied extensively, reflecting the fact that a high proportion of the S/CO ratios were close to or below zero. The results showed that most of the S/CO ratios above threshold with WP and SP bulk milk samples had CVs less than 10%. Higher CVs were associated with samples below mean value threshold. There were 17 results where the CV% was >10%. Of these, only 2/17 were above 20% (20.8%; 26.8%).

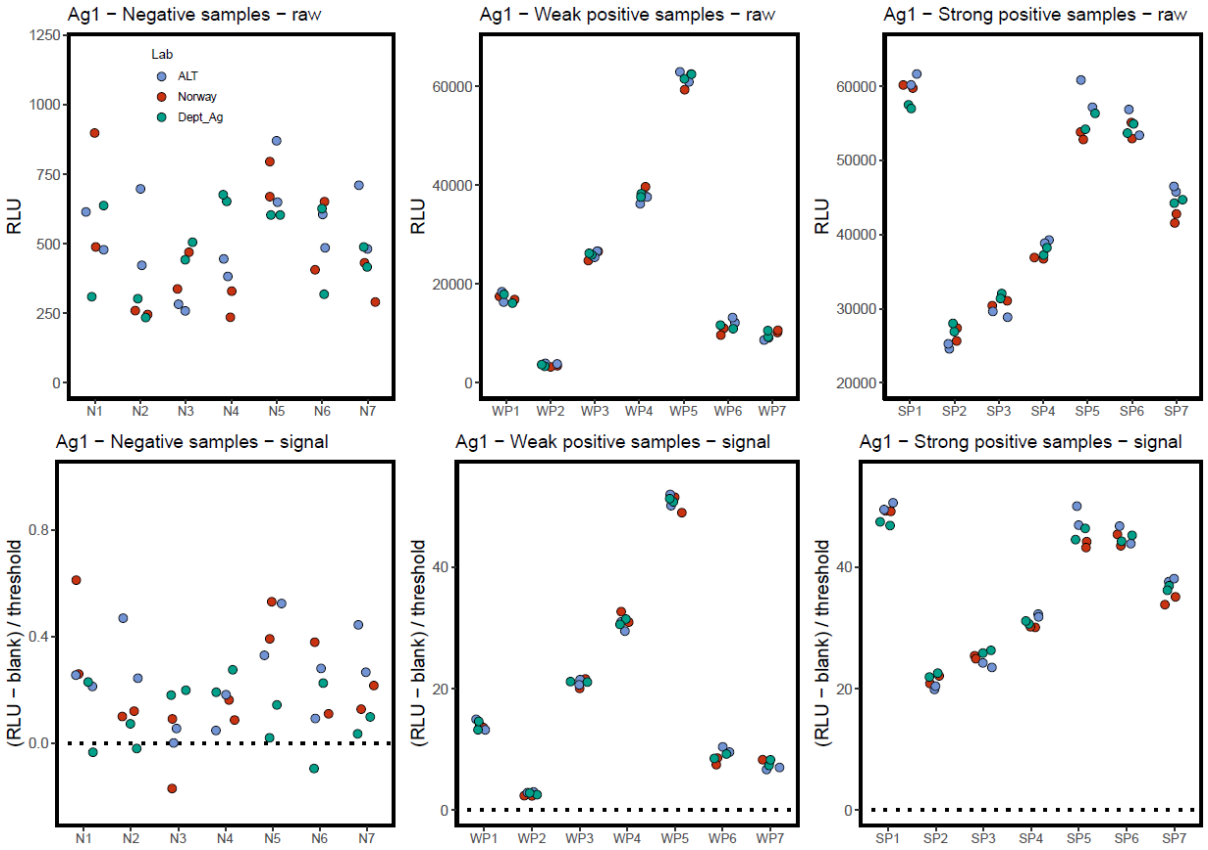
Analysis using mixed linear models showed that for the WP and SP samples, 85 – 100% of the variation was due to the sample and none was due to the kit or laboratory. The Enferplex Bovine TB antibody test thus shows good reproducibility between kits and laboratories using non-boosted bulk tank milk samples.

Diagnostic reproducibility

Bulk tank milk

Bulk tank milk diagnostic reproducibility was assessed in three independent laboratories and the results sent to Enfer Scientific for un-blinding and analysis. The results showed complete concordance between the 3 laboratories using different 2 kits. Representative plots of the raw RLU values and S/CO ratios obtained for Ag1 using negative, weak positive, and strong positive bulk tank milk samples are shown in Figure 2. The duplicate values from each lab for each sample colour coded for laboratory are shown. The cut-off for S/CO ratio is 1.

Figure 2. Bulk tank milk reproducibility for Ag 1.



Conclusion: The Enferplex Bovine TB antibody test thus shows good analytical reproducibility between kits and laboratories when testing bulk tank milk samples.

The diagnostic reproducibility results for bulk tank milk samples are shown in Table 6.

Table 6. Summary of diagnostic reproducibility testing using the 2 Ag rule

Samples	Number positive/tested		
	Laboratory 1	Laboratory 2	Laboratory 3
Positive control	2/2	2/2	2/2
Negative control	0/2	0/2	0/2
Blinded Negatives	0/7	0/7	0/7
Blinded weak positives	7/7	7/7	7/7
Blinded strong positives	7/7	7/7	7/7
Blinded weak positives	7/7	7/7	7/7
Blinded strong positives	7/7	7\7	7/7

The results show complete concordance between the 3 laboratories. The results demonstrate high reproducibility of the Enferplex Bovine TB antibody test when used in 3 different laboratories with 2 different kit batches using bulk tank milk samples.

Reference

Caraguel, C.G.B. & Colling A. (2021). Diagnostic likelihood ratio – the next generation of diagnostic test accuracy measurement. Rev. Sci. Tech. Off. Int. Epiz. 40(1): 299-309.

Annex 7. WOAH Procedure for Registration of Diagnostic Kits
Abstract Sheet

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

Name of the diagnostic kit: BOVIGAM® - *Mycobacterium bovis* Gamma interferon test kit for cattle

Manufacturer: Prionics Lelystad B.V.

WOAH Approval number: 20150110

Date of Registration: May 2015

New Procedure/approval number: 051319

Date of Registration of the extension: May 2023

Disease: Bovine Tuberculosis

Pathogen Agent: *Mycobacterium bovis* and other mycobacteria belonging to the tuberculosis complex (e.g. *M. caprae*)

Type of Assay: Indirect ELISA assay

Purpose of Assay: For the detection of cell-mediated immune response to infection with *Mycobacterium bovis* and other mycobacteria belonging to the tuberculosis complex on analysis of whole blood specimens in cattle, buffalo (*Syncerus caffer*), goat, water buffalos (*Bubalus bubalis*) and provisionally for sheep for the following purposes:

1. Historical freedom
2. Re-establishment of freedom after outbreaks
3. Certify freedom from infection or agent in individual animals or products for trade/movement purposes
4. Eradication of infection from defined populations
5. Confirmatory diagnosis of suspect or clinical cases (includes confirmation of positive screening test)
6. Estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control)
7. Ancillary test for eradication of Tuberculosis

Species and Specimen: Cattle, buffalo (*Syncerus caffer*), goats, water buffalo (*Bubalus bubalis*) and provisionally for sheep - blood-based in vitro laboratory test.

The assay has been further validated for the detection of IFNγ in plasma obtained from stimulated blood samples of suspected water buffalos (*Bubalus bubalis)*. Application for extension of the claim to water buffalo (*Bubalus bubalis*) for BOVIGAM® - *Mycobacterium bovis* Gamma interferon test kit for cattle, hereinafter referred to as BOVIGAM, registered at WOAH (approval number: 20150110) proposed 2021.

This abstract is updated to include the relevant data obtained with samples from water buffalo (*Bubalus bubalis*) to support the claims for diagnostic test characteristics as per the WOAH guidelines.

1. Information on the kit

Please refer to the kit insert available on the WOAH Registry web page or contact the manufacturer at:

Website link: thermofisher.com

Email address: info.nl.prionics@thermofisher.com

2. Summary of validation studies

Analytical characteristics

Analytical sensitivity

BOVIGAM is adjusted to detect 80 pg/ml of recombinant bovine IFN-ɣ.

Whole blood stimulation: Analytical sensitivity of the stimulation part cannot be evaluated as the detection limit depends on the bovine Tb status of the tested animal. In principal whole blood samples between 1.5 ml and 250 µl have been tested and were assessed as suitable for the diagnosis of bovine Tb. The effect of lymphocyte count on reliability and detection limit is unknown. Lymphocyte counts may vary from cattle to cattle. The minimum number required for a reliable result has not been established.

Analytical specificity

Recombinant bovine IFN-ɣ, α and β were assayed in BOVIGAM at biologically active concentrations of 1, 10 and 1000 ng/ml, respectively. BOVIGAM did not detect Interferon-α and –β samples. Reactivity of purified protein derivative from *Mycobacterium bovis* (PPDB) and purified protein derivative from *Mycobacterium avium* (PPDA) stimulated whole blood samples derived from cattle infected with *M. tuberculosis*, *M. africanum*, *M. microti*, *M. canetti*, *M. pinnipedi*, *M. caprae*, who belong to the tuberculosis complex mycobacteria, lead to true positive results in BOVIGAM and cannot be cross-reactive or false positive.

***Repeatability data*:**

Within run repeatability data 1 (2015):

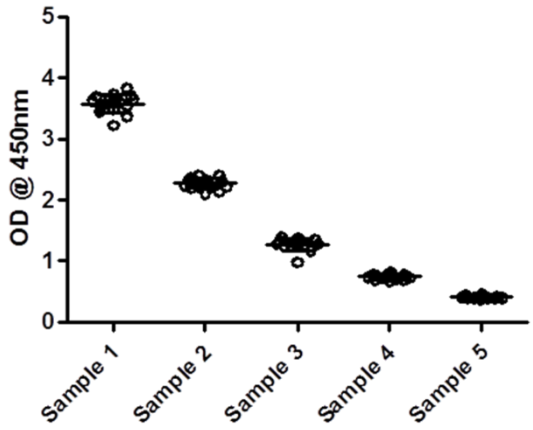
Aim: To demonstrate that the BOVIGAM ELISA has minimal well-to-well variation.

Methods: The within-run repeatability of the BOVIGAM ELISA was estimated by assaying 5 different concentrations of recombinant bovine IFN-γ in 16 replicates using a single test-kit lot (lot number 633261701). Each IFN-γ sample had an analyte concentration within the operating range of the assay.

Results: Figure 1 shows the optical density readings of the 16 replicates for each of the five concentrations of recombinant bovine IFN-γ. Horizontal lines and error bars represent the mean and standard deviation, respectively. As detailed in table 1, the coefficient of variation was less than 10% for all five samples.

Conclusions: The BOVIGAM ELISA displays excellent interwell repeatability for detecting bovine IFN-γ at different concentrations across the operating range of the assay.

Figure 1:



Within run repeatability data 2 (2021):

Aim: To demonstrate that the BOVIGAM ELISA has a minimal well-to-well variation with samples from water buffalo (*Bubalus bubalis*).

Methods: The repeatability was estimated on 4 plasma samples, selected from a panel of 3 field samples from different animals covering the operating range of the assay, one strong, one medium, and one weak, and then a negative field sample; each sample was tested in triplicate: within (intra) assay variation was assessed from the three replicates of each sample in one run (one operator);

Results: The experiment was carried out with samples stimulated with PBS, PPDA and PPDB.

Stimulation with PBS:

sample	operator	day	N Obs	Mean OD	CV%
sample 1	operator 1	day 1	4	0.051	2.773
		day 2	4	0.058	3.539
		day 3	4	0.057	5.165
	operator 2	day 1	4	0.055	4.855
		day 2	4	0.053	1.541
		day 3	4	0.059	4.523
sample 2	operator 1	day 1	4	0.045	1.297
		day 2	4	0.049	3.844
		day 3	4	0.053	6.715
	operator 2	day 1	4	0.044	4.402
		day 2	4	0.049	1.944
		day 3	4	0.052	1.850
sample 3	operator 1	day 1	4	0.069	3.225
		day 2	4	0.077	3.353
		day 3	4	0.084	3.972
	operator 2	day 1	4	0.069	1.393
		day 2	4	0.074	8.049
		day 3	4	0.091	3.836
sample 4	operator 1	day 1	4	0.040	6.027
		day 2	4	0.041	7.180
		day 3	4	0.041	3.050
	operator 2	day 1	4	0.039	5.252
		day 2	4	0.043	7.316
		day 3	4	0.044	8.089

Stimulation with bovine PPD:

Sample	operator	day	N Obs	Mean OD	CV%
sample 1	operator 1	day 1	4	0.073	1.118
		day 2	4	0.092	3.733
		day 3	4	0.081	1.558

Sample	operator	day	N Obs	Mean OD	CV%
	operator 2	day 1	4	0.073	0.687
		day 2	4	0.087	2.816
		day 3	4	0.095	1.328
sample 2	operator 1	day 1	4	0.239	0.714
		day 2	4	0.229	0.549
		day 3	4	0.231	0.903
	operator 2	day 1	4	0.235	1.120
		day 2	4	0.231	0.740
		day 3	4	0.234	0.642
sample 3	operator 1	day 1	4	1.121	0.263
		day 2	4	1.122	0.223
		day 3	4	1.118	0.231
	operator 2	day 1	4	1.109	0.725
		day 2	4	1.117	0.267
		day 3	4	1.107	0.585
sample 4	operator 1	day 1	4	3.210	0.256
		day 2	4	3.227	0.882
		day 3	4	3.228	0.399
	operator 2	day 1	4	3.210	0.275
		day 2	4	3.228	0.456
		day 3	4	3.218	0.222

Stimulation with avian PPD:

sample	operator	day	N Obs	Mean OD	CV%
sample 1	operator 1	day 1	4	0.084	2.572
		day 2	4	0.084	2.632
		day 3	4	0.100	2.160
	operator 2	day 1	4	0.098	2.961
		day 2	4	0.105	2.333
		day 3	4	0.121	2.338
sample 2	operator 1	day 1	4	0.102	2.531
		day 2	4	0.090	1.442
		day 3	4	0.091	2.374
	operator 2	day 1	4	0.097	2.280
		day 2	4	0.091	1.427
		day 3	4	0.092	1.411
sample 3	operator 1	day 1	4	0.720	0.593

sample	operator	day	N Obs	Mean OD	CV%
		day 2	4	0.708	0.960
		day 3	4	0.729	0.453
	operator 2	day 1	4	0.718	0.927
		day 2	4	0.713	0.380
		day 3	4	0.719	0.309
sample 4	operator 1	day 1	4	0.999	1.193
		day 2	4	1.001	1.756
		day 3	4	0.988	0.486
	operator 2	day 1	4	0.990	1.610
		day 2	4	1.002	1.019
		day 3	4	1.010	2.454

All CV% (Percentage of Coefficient of Variation) observed post testing, shown in the above tables are below 10%.

Conclusions: The BOVIGAM ELISA displays excellent interwell repeatability for the detecting bovine IFN-γ at different concentrations across the operating range of the assay on water buffalo (*Bubalus bubalis*) stimulated plasma samples.

Between-run repeatability data 1 (2015):

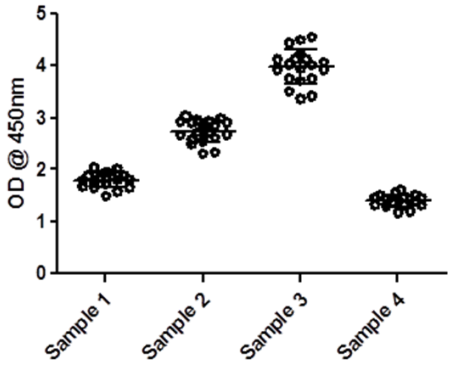
Aim: To demonstrate that the BOVIGAM ELISA has minimal between-run variation.

Methods: Four samples of bovine whole blood culture supernatants were aliquoted and stored frozen at -80°C. The antigens used for the stimulation of bovine whole blood generate samples 1, 2, 3 and 4 were avian tuberculin purified protein derivative (PPD-A), staphylococcal Enterotoxin B (SEB), early secreted antigen target 6kD protein (ESAT-6)/ culture filtrate protein 10 kD (CFP-10) peptide cocktail and Rv3615c peptide cocktail respectively. These samples were then used to assess the between-run repeatability of the BOVIGAM ELISA. Each sample was assayed in triplicate in a total of 19 runs, performed on 5 separate days by 2 different operators.

Results: Figure 2 shows the optical density readings of the four bovine whole blood culture supernatants run on 19 occasions. Horizontal lines and error bars represent the mean and standard deviation, respectively. As detailed in table 2 the coefficient of variation was less than 10% for all four samples.

Conclusions: The BOVIGAM ELISA displays excellent between-run repeatabilitydetecting of bovine IFN-γ in supernatants from bovine whole blood assays.

Figure 2: The BOVIGAM ELISA has minimal between-run variation.



Whole blood stimulation on cattle reactor variances of repeatability: Values differ between days less than 20%

Whole blood stimulated samples with pokeweed variances of repeatability: Values differ between days less than 6%

Between-run repeatability data 2 (2021):

Aim: To demonstrate that the BOVIGAM ELISA has a minimal test-to-test variation with samples from water buffalo (*Bubalus bubalis*).

Methods: The repeatability was estimated on 4 plasma samples, selected from a panel of 3 field samples from different animals covering the operating range of the assay, one strong, one medium, and one weak, and then a negative field sample; each sample was tested in triplicate: between (inter) assay variation was assessed by comparison of results from 2 operators testing the panel of samples (each in triplicate) over 3 days.

Results: The experiment was carried out with samples stimulated with PBS, PPDA and PPDB.

Stimulation with PBS:

sample	N Obs	Mean	CV%
sample 1	24	0.055	6.229
sample 2	24	0.049	8.127
sample 3	24	0.077	11.428
sample 4	24	0.041	7.013

Stimulation with Bovine PPD inter-assay

sample	N Obs	Mean	CV%
sample 1	24	0.083	10.648
sample 2	24	0.233	1.638
sample 3	24	1.116	0.641
sample 4	24	3.220	0.486

Stimulation with Avian PPD inter-assay

sample	N Obs	Mean	CV%
sample 1	24	0.099	13.321
sample 2	24	0.094	5.221
sample 3	24	0.718	1.089
sample 4	24	0.998	1.574

All CV observed are below 15%.

Conclusion: The BOVIGAM ELISA displays excellent inter-test repeatability for detecting bovine IFN-γ at different concentrations across the operating range of the assay on water buffalo (*Bubalus bubalis*) stimulated plasma samples.

Diagnostic Characteristics

Threshold determination

Each country has to determine its own unique cut-off adopted to the regional cattle TB situation in the country.

Diagnostic sensitivity (DSn) and specificity (DSp) estimates

BOVIGAM		**Target Species**				
		Cattle	**Buffalo (*Syncerus caffer*)**	**Goats**	**Sheep**	**Water buffalo (*Bubalus bubalis)***
Diagnostic sensitivity*[1] (classical statistics with PPDs)	N DSn CI	8879 84.6% (95%CI = 73.0-95.5%)	2514 81.6-91.9%	472 58-100%	4 100%	458 94.7% (95 CI: = 92.3-96.5%)
Diagnostic specificity*[2] (classical statistics with PPDs)	N DSp CI	10966 97.4% (95%CI = 87.5-99.6%)	608 86.2-99.4%	140 96-100%	3 100%	489 98.5% (95 CI = 98.5%-96.9%)
Diagnostic sensitivity*[3] (Bayesian analysis with PPDs)	N DSn CI	4937 33.9-68.8%[+] n.a.	n.a.	n.a.	n.a.	n.a
Diagnostic specificity*[4] (Bayesian analysis with PPDs)	N DSn CI	4937 87.9-99.8%[+] n.a.	n.a.	n.a.	n.a.	n.a
Diagnostic sensitivity*[5] (Esat-6/CFP10)	N DSn CI	771 52.2%-85% n.a.[$]	n.a.	n.a.	4 100% n.a.	n.a
Diagnostic specificity* (Esat-6/CFP10)	N DSn CI	2039 94%-98.9% n.a.[$]	n.a.	n.a.	3 100% n.a.	n.a

* Different cut-offs may apply; [$] Specificity and sensitivity estimates based on several studies thus a 95% CI is not given here; [+]Depending on test assumption

*1-2 cattle → The following different cut-offs have been applied for these studies

Criterion No.	**Criterion**
Criterion 1:	BOD_COD > 0 and BOD_AOD > 0;
Criterion 2	BOD/COD > 1.25 and BOD_AOD > 0;
Criterion 3:	BOD/COD > 1.5 and BOD_AOD > 0;
Criterion 4:	BOD_COD P0.05 and BOD_AOD > 0;
Criterion 5:	If BOD = 0.1, then BOD/COD > 1.5 and BOD_AOD > 0. If BOD > 0.1, then BOD_COD > 0.05 and BOD_AOD > 0;
Criterion 6:	BOD_AODP0.1;
Criterion 7:	BOD_COD P0.1 and BOD/AODP 1.8;
Criterion 8:	BOD_COD P0.1 and BOD/AODP 1.25;
Criterion 9:	BOD/AODP1.8;
Criterion 10:	BOD_COD P0.05 and BOD/AODP 1.8 (”criterion 4 if BOD/AODP1.0);
Criterion 11:	BOVIGAM: BOD_COD P0.1 and BOD_AOD > 0;
Criterion 12:	BOD_COD P2(COD) and BOD_AODP0.05;
Criterion 13:	BOD_COD P0.1 and BOD_AODP 0.1;
Criterion 14:	BOD_AODP0.04.

- **BOD**: Mean optical density value of the plasma from the bovine PPD-stimulate blood.
- **AOD**: Mean optical density value of the plasma from the avian PPD-stimulated blood.
- **COD**: Mean optical density value of the plasma from blood incubated with phosphate-buffered saline (nil antigen control).

*1-2 buffalo → The following different cut-offs have been applied for these studies

Criterion No.	Criterion
Criterion C1:	BOD-AOD P0.05 and BOD-AOD > 0;
Criterion C4:	ODbovine readings < 0.385 are interpreted as test negative, and ODbovine readings≥0.385 are interpreted as test positive
Criterion 5:	ODbovine – ODavian > 0.20 and if ODfortuitum – ODnil < 0.15, provided that ODnil <0.25. In cases where ODfortuitum – ODnil > 0.15 the buffalo was classified as a multiple reactor (MR).

*1-2 goats → The following different cut-offs have been applied for these studies

Criterion No.	Criterion
Criterion C2:	IFN-c assay. Standard interpretation: Goat positive if bovine PPD OD minus no antigen sample ODP0.1 and bovine PPD OD > avian PPD OD. Severe interpretation: Goat positive if bovine PPD OD minus no antigen sample ODP0.05 and bovine PPD OD > avian PPD OD.

*1-2 cattle → The following different cut-offs have been applied for these studies

Criterion No.	Criterion
Criterion C3:	OD indices (ODI): ratio of the OD for stimulated cultures compared with the OD for control cultures. An ODI>2 is regarded as positive.

*3-4 cattle, baysian analyis → a specific cut-off did not apply as it is a Bayesian analysis; details see using latent class analysis to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions Tracy A. Clegg, Anthony Duignan, Clare Whelan, Eamonn Gormley, Margaret Good, John Clarke, Nils Toft, Simon J. More Veterinary Microbiology 151 (2011) 68–76

*5-6 cattle, ESAT6/CFP10→ The following different cut-offs have been applied for these studies

Criterion No.	Criterion
Criterion 1:	Esat6/CFP10 > 0.1
Criterion 2:	PPDB-PPDA > 0.1 And PPDB - Nil > 0.1
Criterion 3:	PPDB-PPDA > 0.1 And Esat6/CFP10 > 0.1 (confirmatory
Criterion 4:	bPPD - PBS ≥0.05 and bPPD greater than aPPD
Criterion 5:	Prionics PC-EC- Nil > 0.1 (confirmatory)

*5-6 Sheep, ESAT6/CFP10→ The following different cut-offs have been applied for these studies

Criterion No.	Criterion
Criterion C3:	An ODI>2 is regarded as positive.

Comparative performance 1 (2015)

	Diagnostic sensitivity	Diagnostic specificity
Skin Test - CCT	80%*	96.8%*
Skin Test – CFT/SCT	84%*	99.50%*

Comparative performance 2 (2021), on water buffalo (*Bubalus bubalis*)

The comparison study was carried out on 489 positives samples with intradermal test SICCT test and Bovigam kit

Test	Diagnostic Sensitivity
SICCT	88.3%
Bovigam	94.7%

Agreement and discrepancies

High agreement between BOVIGAM and the conventional bio-assay for bovine IFN-ɣ could be observed. BOVIGAM demonstrates a higher sensitivity than the bioassay. comparative cervical tuberculin/caudal-fold tuberculin/Single cervical tuberculin Skin tests: Bovine and/or Avian Tuberculin PPDs are administered intradermally and are thus *in vivo* diagnostics. In TB cattle, injection of bovine tuberculin PPD results in an immunological response at the site of injection. This is referred to as the Delayed Type Hypersensitivity (DTH) reaction and is observed as local inflammation and swelling of the skin (lesion). The thickness of the skin is measured with callipers 72 hours following injection. Avian tuberculin PPD is used to control for unspecific reactions. A full set of T-cells can be stimulated. BOVIGAM is an *in vitro* test to stimulate whole blood samples with PPDs or other specific Antigens. A marker concentration, IFN-ɣ is measured. Predominantly, CD4+ cells are stimulated. Proportion of agreement is about 70% as the immune response behind the test system is different and other sub population of TB positive animals can be recognized with each test. In the table below several studies are displayed summarizing the proportion of agreement between skin test applications and BOVIGAM.

Proportion of agreement between different skin test assays and BOVIGAM.

Author	Species	Skin test	BOVIGAM®	Proportion of agreement	Kappa (k)
Lopes et al., 2012	Cattle N= 350	CCT	According PI	79.4% to 85.3%	0.546 to 0.663
Antognoli et al., 2010	Cattle N= 900	CCT	According PI	n.a.	0.45 (95%CI 0.28 – 0.62)
Goosen et al., 2013	Buffalo N= 82	SCT	According PI Or South Africa specific for buffalo	63% 64%	n.a. n.a.
Kalis et et, 2003	Cattle N= 1631	SCT	According PI**	85.7%	0.41
Schroeder, 2014	Cattle N=541	CCT	According PI	95.1%	0.501

Reproducibility

Experiment 1 (2015):

To investigate the reproducibility of the BOVIGAM ELISA when performed in different laboratories.

Methods: Given that it is technically impractical to send freshly drawn blood samples to laboratories located in different countries to perform the whole blood stimulations, we have confined the analysis of reproducibility to the detection of IFN-γ using the BOVIGAM ELISA. Whole blood samples from 21 animals (16 SICCT skin test positive natural field rectors, 3 BCG-vaccinated/*M. bovis* infected and 2 non-vaccinated/non-infected controls) were incubated with PPD-A, PPD-B, ESAT-6/CFP-10 peptide cocktail and Rv3615c peptide cocktail. These stimulations were set up in multiple wells, which allowed for the pooling of replicate samples to create a panel of identical aliquots, which were then subsequently tested in the BOVIGAM ELISA at the laboratories listed above. A different BOVIGAM ELISA kit batch was used in each laboratory (VISAVET kit# 6632600201, Luddington kit# 6332601801, Weybridge kit# 6332601701). Each animal was then scored as test positive or negative using three different readout systems: (i) the standard comparative readout of bovine PPD minus avian PPD (B-A), (ii) responses to the ESAT-6/CFP-10 peptide cocktail (E/C), or (iii) responses to the ESAT-6/CFP-10 peptide cocktail and/or the Rv3615c peptide cocktail (E/C ± Rv).

Results: The test results generated by three independent laboratories for 21 animals using either (i) B-A, (ii) E/C, or (iii) E/C ± Rv3615c are shown in table 18.

Table 18: Agreement of test results from three independent laboratories.

	B-A			E/C			E/C and/or Rv3615c		
I.D.	**VISAVET**	**Luddington**	**Weybridge**	**VISAVET**	**Luddington**	**Weybridge**	**VISAVET**	**Luddington**	**Weybridge**
S1	N	N	N	N	N	N	N	N	N
S2	Y	Y	Y	Y	Y	Y	Y	Y	Y
S3	Y	Y	Y	Y	Y	Y	Y	Y	Y
S4	Y	Y	Y	N	N	N	N	N	N
S5	Y	Y	Y	Y	Y	Y	Y	Y	Y
S6	Y	Y	Y	Y	Y	Y	Y	Y	Y
S8	Y	Y	Y	N	N	N	**Y**	**N**	**Y**
S9	Y	Y	Y	Y	Y	Y	Y	Y	Y
S10	Y	Y	Y	Y	Y	Y	Y	Y	Y
S11	Y	Y	Y	Y	Y	Y	Y	Y	Y
S12	Y	Y	Y	Y	Y	Y	Y	Y	Y
S13	Y	Y	Y	N	N	N	Y	Y	Y
S14	Y	Y	Y	Y	Y	Y	Y	Y	Y
S15	Y	Y	Y	Y	Y	Y	Y	Y	Y
S16	Y	Y	Y	Y	Y	Y	Y	Y	Y
S17	Y	Y	Y	Y	Y	Y	Y	Y	Y
S20	N	N	N	N	N	N	N	N	N
S21	N	N	N	N	N	N	N	N	N
S23	N	N	N	N	N	N	N	N	N
S24	Y	Y	Y	N	N	N	N	N	N
S25	Y	Y	Y	N	N	N	N	N	N

Table 18: Y = test positive response, N = test negative response, B-A = the standard comparative readout of bovine PPD minus avian PPD, E/C = responses to the ESAT-6/CFP-10 peptide cocktail, E/C and/or Rv3615c = responses to the ESAT-6/CFP-10 peptide cocktail and/or the Rv3615c peptide cocktail

Complete test agreement (100%) was seen across all three laboratories when using either B-A or E/C as readouts. Furthermore, 100% test agreement was also observed between Weybridge and VISAVET laboratories when using E/C ± Rv3615c as a readout. The only discrepancy in test results occurred when comparing E/C ± Rv3615c results from Luddington laboratory with either Weybridge or VISAVET (highlighted in red), where sample S8 tested negative in the former laboratory but positive in the two latter laboratories. This resulted in a test agreement of 95.24% (kappa value of 0.8966, interpreted as very good agreement) between Luddington and either Weybridge or VISAVET when comparing E/C ± Rv3615c results.

Conclusions:

These results demonstrate the high reproducibility of the BOVIGAM ELISA when used at different laboratories, with different kit batches and with a variety of different readout systems.

Experiment 2 (2015):

To investigate the variability of results obtained at different laboratories using sample tubes from the same animal drawn at the same time.

Methods: 316 blood samples were submitted in parallel to AHVLA Luddington and also to a second laboratory (either AHVLA Weybridge or AHVLA Sutton Bonnington) for blood stimulations and IFN-γ ELISA. These consisted of 285 samples from the IFN-γ Specificity Trial and 31 samples from SICCT skin test positive animals. Each sample was tested for IFN-γ production against a medium (negative) control, PPD-A, PPD-B, and SEB (positive control) according to the relevant SOPs.

Results: All controls were within the ranges specified by the SOP. For the B-A readout, positive results were determined by subtracting the response to avian tuberculin from that to bovine tuberculin; those of 0.1 or more were considered positive. The agreement between the two sites is 96.52% (results summarized in the table below).

Summary of test agreement for the B-A responses.

		Second Laboratory		
		Test negative	**Test positive**	**Total**
Luddington	Test negative	275	5	280
	Test positive	6	30	36
	Total	281	35	316

A similar analysis was carried out for responses to the ESAT-6/CFP-10 peptide cocktail, where a total of 287 blood samples were submitted in parallel to AHVLA Luddington and AHVLA Weybridge. These consisted of 284 samples from the IFN-γ Specificity Trial and 3 samples from animals positive to SICCT skin test positive animals. Positive results were determined by subtracting the response to the negative control from the response to the peptide cocktail; those of 0.1 or more were considered positive. The agreement between the two sites is 94.43% (results summarized in the table below).

Summary of test agreement for ESAT-6/CFP-10 responses.

		Weybridge		
		Test negative	**Test positive**	**Total**
Luddington	Test negative	268	6	274
	Test positive	10	3	13
	Total	278	9	287

Experiment 3 (2015)

In a further trial in France, reproducibility has been tested between laboratories (table below).

	Laboratoire Départemental de l'Hérault, Montpellier, Carmargues		Laboratoire Départemental D`Analyses Agriculture et Vétérinaire; Coulounieix-Chamiers Dordogne	Laboratoire Départemental de la Côte-d'Or, Dijon	
Batch Number	6332603001	6332604201	6332603701	6332602701	6332603401
Mean Ref Material	19.65%	19%	20.43%	22.56%	20.05%
Standard deviation	1.82	2.71	2.69	1.97	1.47
%CV	9.23%	14.56%	13.17%	9.0%	7.0%

These results demonstrate the high reproducibility of the BOVIGAM ELISA when used at different laboratories, with different kit batches and with a variety of different readout systems at different days.

Experiment 4 [2021, on water buffalo (*Bubalus bubalis*)**]**

Methods

For the estimation of reproducibility, 32 serum samples from 32 buffalo heads were selected, 16 of which were positive and 16 negative. The tests were performed by two different laboratories (IZSME-Salerno, IZSUM-Perugia).

For results expressed on a nominal scale (negative, positive) the Kappa statistical index can be used to quantify the degree of agreement, beyond the case, between the results of a test. The kappa varies from 0 (no agreement) to 1 (perfect agreement) (Fleiss, 1981; Landis & Koch 1977). For qualitative evaluation, reproducibility has been defined as the degree of agreement between different laboratories on the same sample. It was calculated on 32 samples from two different laboratories with the Fleiss Kappa.

Results

	Bovigam criterion		
id_samples	**Expected**	**lab 1**	**lab 2**
1	NEG	NEG	NEG
2	NEG	NEG	NEG
3	NEG	NEG	NEG
4	NEG	NEG	NEG
5	NEG	NEG	NEG
6	NEG	NEG	NEG
7	NEG	NEG	NEG
8	NEG	NEG	NEG
9	NEG	NEG	NEG
10	NEG	NEG	NEG
11	NEG	NEG	NEG

12	NEG	NEG	NEG
13	NEG	NEG	NEG
14	NEG	NEG	NEG
15	NEG	NEG	NEG
16	NEG	NEG	NEG
17	POS	POS	POS
18	POS	POS	POS
19	POS	POS	POS
20	POS	POS	POS
21	POS	POS	POS
22	POS	POS	POS
23	POS	NEG	POS
24	POS	POS	POS
25	POS	NEG	POS
26	POS	POS	POS
27	POS	NEG	POS
28	POS	POS	POS
29	POS	POS	POS
30	POS	POS	POS
31	POS	POS	POS
32	POS	POS	POS

For the Bovigam criterion, the kappa was equal to 0.81 (IC95% 0.61-1.00), indicating an almost perfect agreement between the laboratories; 3 discrepancies were observed on 32 samples. The proportion of agreement observed was 90%. The null hypothesis that this value is equal to 0 (non-correlation) gave back a p-value<0.001, indicating that the value of K obtained is significantly different from 0.

Conclusion: The BOVIGAM ELISA displays excellent inter-laboratory reproducibility on water buffalo (*Bubalus bubalis*) stimulated plasma samples.

Application

Some reference laboratories use BOVIGAM as an ancillary test for animals tested negative in the skin test within a herd that presented some positive skin tests(e.g. Ireland, UK). Some reference laboratories use BOVIGAM as a confirmatory test of animals which has been tested positive in skin test (e.g. Bavaria). Mexican and one Laboratory in France (for bull fighting herds) use BOVIGAM as primary test for tuberculosis diagnostic in cattle.

BOVIGAM has been used several million times since its introduction in 1988, mostly in routine laboratories. Typical laboratories have used this test to analyze several hundred samples per day. Minimum turn-around time for the test is 4 hours for the ELISA and 16-24 hours for the stimulation of whole blood samples.

References

Wood, P. R., Corner, L.A., Rothel, J.S., Baldock, C., Jones, S.L., Cousins, D.B., McCormick, B.S., Francis, B.R., Creeper, J., Tweddle, N.E. (1991) Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis." Aust Vet J 68: 286-90.

R. de la Rua-Domenech , A.T. Goodchild , H.M. Vordermeier , R.G. Hewinson ,K.H. Christiansen , R.S. Clifton-Hadley Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, c-interferon assay and other ancillary diagnostic techniques; Research in Veterinary Science 81 (2006) 190–210)

Vordermeier; M. and Ewer, K; Specificity Trial of the BOVIGAM® IFN-Gamma Test in GB Cattle; TB Research Group, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB Funded by Defra under surveillance project SB4021 April 2006

Using latent class analysis to estimate the test characteristics of the □-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions Tracy A. Clegg, Anthony Duignan, Clare Whelan, Eamonn Gormley, Margaret Good, John Clarke, Nils Toft, Simon J. More Veterinary Microbiology 151 (2011) 68–76

Annex 8. WOAH Procedure for Registration of Diagnostic Kits
Abstract Sheet

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 6 to 10 February 2023

_____

Name of the diagnostic kit: BIONOTE® Rapid MERS-CoV Ag Test Kit

Manufacturer: BioNote, Inc.

Procedure /Approval number: 20160212

Date of Registration: May 2016

Date of Renewal: May 2023

Disease: Middle East Respiratory Syndrome

Pathogen Agent: Middle East Respiratory Syndrome (MERS)

Type of Assay: Immunochromatographic assay

Purpose of Assay: Certified by WOAH fit for the qualitative detection of Middle East Respiratory Syndrome Coronavirus antigens from nasal swabs in dromedary camels for the following purposes:

- Detection of MERS CoV infected herds (herd test) with acutely infected animals with high virus loads;
- When used as a supplemental test, to estimate prevalence of infection to facilitate risk analysis s, e.g. surveys, herd health schemes and disease control programs

Species and Specimen: Nasal swabs in dromedary camels

1. Information on the kit

Please refer to the kit insert available on the WOAH Registry web page or contact manufacturer at:
Website link: www.bionote.co.kr
Email address: bionote@bionote.co.kr

2. Summary of validation studies

Analytical specificity

Conclusion: The BRM kit does not have cross-reactivity with camel coronaviruses (DcCoV UAE-HKU23), COVID-19 (SARS-CoV-2), and other coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, RbCoV HKU14, Ty-Bat CoV HKU4).

Table 1 Analytical Specificity

Viruses			BRM kit result
Alpha coronavirus		Human coronavirus 229E (HCoV-229E)	Negative
		Human coronavirus NL63 (HCoV-NL63)	Negative
Beta coronavirus	**Embecovirus**	Human coronavirus OC43 (HCoV-OC43)	Negative
		Rabbit coronavirus HKU14 (RbCoV HKU14)	Negative
		Dromedary camel coronavirus UAE-HKU23 (DcCoV UAE-HKU23)	Negative

Viruses			BRM kit result
	Sarbecovirus	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Negative
	Merbecovirus	Middle East respiratory syndrome coronavirus (MERS-CoV)	Positive
		Tylonycteris bat coronavirus HKU4 (Ty-Bat CoV HKU4)	Negative

Analytical sensitivity

Conclusion:
Experiment 1. BIONOTE® Rapid MERS-CoV Ag Test Kit (BRM kit) detected up to 3.125 ng/ml of recombinant nucleocapsid antigen of MERS CoV.

Experiment 2. Negative camel nasal swabs, collected from Central Veterinary Research Laboratory (CVRL) in Dubai, UAE, and MERS-CoV Culture Fluid were used for the Limit of detection test. The MERS-CoV Culture Fluid was diluted into 2-fold steps and tested simultaneously with the the UpE and Orf1b real-time RT-PCR (Corman et al. (2012)). In experiments performed using MERS-CoV Culture Fluid, BRM kit can detect up to $1.63x10^2$ $TCID_{50}$/mL, corresponding to an UpE CT value of 32.51 and ORF1b CT value of 34.93 according to molecular analysis performed concurrently.

Repeatability

Within run variation was assessed using quadruplicates of 5 inhouse samples (one strong, one medium, one weak and two negative samples) in four runs by one operator. Between run variation was assessed using triplicates of 5 inhouse samples in 30 runs by 3 operators on separate days. Batch-to-batch variation was assessed using 5 inhouse samples by 1 operator on one day.

***Conclusion*:** CV values were all below 5% in the Within run, Between run, and batch-to-batch variation.

Diagnostic Characteristics

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Conclusion: BIONOTE® Rapid MERS-CoV Ag Test Kit is a qualitative test. The presence of the purple line on both the control(C) and test(T) position is considered to be the threshold determination. The test sample is positive when two lines (C line and T line both) appear and negative when only the C line appears. Lines consist of immuneo-reaction of the gold conjugate and target analytes. Gold conjugate consist of colloidal gold and MERS CoV antibody. The threshold is determined by the analytical sensitivity as 10^5 $TCID_{50}$ (50% Tissue Culture Infective Dose).

Table 2a Relative diagnostic sensitivity (DSn) and specificity (DSp) estimates

Test method under evaluation		Target Species
Diagnostic sensitivity	N DSn CI	*(66)* *(93.9%)* *(85.20-98.32%)*
Diagnostic specificity	N DSp CI	*(523)* *(99.6%)* *(98.63-99.95%)*

Table 2b 2x2 table for relative DSn and DSp

Summary		UpE and Orf1A rRT-PCR		Total
		POS	**NEG**	
BRM kit	*POS*	*62*	*2*	*64*
	NEG	*4*	*521*	*525*
Total		*66*	*523*	*589*

Reproducibility

Analytical reproducibility

Reproducibility was assessed at three sites using a blinded coded reference panel. The panels were tested using three different lots in 21 runs at 3 different sites by an operator each day for three days. Each site ran positive and negative reference panels for each day of testing.

Conclusion: The CVs of the between site assay reproducibility is 3~11%.

Diagnostic reproducibility

The scope of this interlaboratory comparison was to determine the reproducibility of the Real-Time PCR and the BRM kit to detect MERS-CoV in real nasal swab samples collected in transport media in three participating laboratories.

[Test Date]: October 2015

[Test site] Three laboratories participated in the International Inter-laboratory Comparison on the BRM kit. (Participants also tested samples by Real Time PCR and results are shown for information only.)

1. Abu Dhabi Food Control Authority (ADFCA)

Location: United Arab Emirates
City: Abu Dhabi
Level of expertise : highly trained technician
Accreditation status : ISO 17025

2. King Faisal University Laboratory (KFU)

Location: Kingdom of Saudi Arabia
City: Al-Hasa
Level of expertise : highly trained technician
Accreditation status : ISO 17025

3. Molecular Biology & Genetics laboratories (MBG)

Location: United Arab Emirates
City: Dubai
Level of expertise : highly trained technician
Accreditation status : ISO 17025

[Materials]

1. Test panel information

The panel consisted of 6 positive and 4 negative samples. Samples were prepared from samples with known history. Samples were aliquoted in portions of 300µl and stored in 2ml vials. Test samples were prepared from nasal swabs from MERS positive and negative camels.

2. Shipping conditions

The samples were dispatched to the participants on the month of October 2015. Each participant received one box containing the test materials (Ten 2ml vials containing 300µl of each sample).
Samples were frozen and shipped with dry ice to the laboratories.

[Result]

BIONOTE® Rapid MERS-CoV Ag Test Kit

Samples were analyzed by each lab using BRM kit and Real-Time PCR. BRM kit results of three participants are illustrated in Table 3 below.

Table 3. BRM kit results of three participants

Sample No.	Targeted Results (Original)	KFU, Saudi Arabia	MBG LAB	VLD- ADFCA
1	Positive	Positive	Positive	Positive
2	Positive	Positive	Positive	Positive
3	Negative	Negative	Negative	Negative
4	Positive	Positive	Weak Positive	Positive
5	Positive	Positive	Weak Positive	Positive
6	Negative	Negative	Negative	Negative
7	Positive	Positive	Positive	Positive
8	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative
10	Positive	Positive	Positive	Positive

Real-Time PCR test

Samples were also analyzed by the 3 participants using real time PCR. ADFCA (Abu Dhabi, UAE) real-time PCR results are based on UPE and Roche MERS-CoV qPCR kit in which the Orf la gene is targeted. KFU, (Saudi Arabia) real-time PCR results are based on UPE and CDC MERS-Co V qPCR kit in which the N2 gene is targeted. MBG, (Dubai, UAE) real-time PCR results are based on 2nd Derivative Max Analysis. Qualitative and quantitative Real-Time PCR results of each participant are given in table 4 below.

It was concluded that the "No CT value" result was clearly negative. When CT values exceeded 35, interpretations were different for each laboratory, but when other PCRs were performed, interpretations were made along with the results. Because according to CDC, MERS CoV-positive samples must test positive for two separate genetic targets (e.g. upE and N2 or N2 and N3 or upE and N3, etc.), both targets must be positive to be interpreted as positive.

Table 4. Real-Time PCR results of three participants

	KFU, Saudi Arabia			MBG LAB		VLD- ADFCA		
Sample No.	Real-Time PCR-Result	CT Value UPE	CT Value N2	Real-Time PCR-Result	2nd Derivative Max Analysis	PCR-Result	CT Value UPE	CT Value ORF1a
1	Positive	21.33	16.65	Positive	19.59	Positive	23.65	24.1
2	Positive	16.01	15.97	Positive	19.61	Positive	23.34	23.84
3	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
4	Positive	19.95	18.16	Positive	21.2	Positive	24.8	24.68
5	Positive	25.9	19.03	Positive	21.15	Positive	24.89	24.51
6	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
7	Positive	20.06	19.86	Positive	19.22	Positive	23.16	23.26
8	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
9	Negative	No Ct	39.95*	Inconclusive**	>35	Negative	No Ct	No Ct
10	Positive	22.16	18.95	Positive	20.84	Positive	24	23.87

** Sample 9 gave an inconclusive Ct value of 39.95 in N2 qPCR, but no Ct in upE and therefore, it was considered as negative by KFU.*

***For MBG lab the Ct value cut off is 35; any amplification beyond 35 is reported as inconclusive.*

[Conclusion]

Interlaboratory comparison testing of the BRM kit with a panel consisting of 6 MERS positive and 4 MERS negative samples in 3 different laboratories showed 100% concordance of results for the BRM kit using KFU and VLD molecular assays as reference tests. Results from MGB assay were excluded because no negative results were produced in this assay.

Additional testing

Further testing of spiked samples of 12 positive and 18 negative camel nasal swab samples was performed by the BRM kit, MERS-CoV RT-PCR, MERS-CoV real-time PCR and DcCoV UAE-HKU23 real-time PCR . The relative specificity and sensitivity of the rapid MERS-CoV Ag test kit compared to the qPCR were 100% (18/18) and 91.7% (11/12), respectively (Lau, Susanna Kar-Pui, et al., 2022).

Table 5. 2x2 table

		MERS-CoV N Real-time PCR		
		Positive	**Negative**	**Total**
Rapid MERS-CoV Ag test kit	**Positive**	11	0	11
	Negative	1	18	19
	Total	12	18	30
Sensitivity		91.7%		
Specificity		100%		

Conclusion

The BRM kit is shown to be less sensitive than the real-time PCR assays. Samples with viral load below the detection limit of the BRM kit are likely to test negative in the BRM kit. It is a common observation that antigen tests can be markedly less sensitive than real-time PCR tests. MERS-CoV-2-infected camels can shed a low level of viral RNA for an extended period (several weeks). Nonetheless, infectious virus can only be detected mainly in the first week after infection (Adney et al., EID 2014).

In summary, the BRM kit can detect a positive sample with a high viral load and would be useful as a screening assay for a prompt identification of highly infectious camels, thereby allowing timely risk management (e.g. quarantine). As this antigen test might fail to detect some MERS-positive camels that have low viral load (e.g. those at early onset), a negative test result cannot completely exclude MERS-CoV infection. The BIONOTE test has an estimated **diagnostic window of 1~7 days (as opposed to the real-time PCR 1-35 days)**. Samples that are taken beyond this time point are likely to be negative in the Bionote test (see also detailed protocol for the sampling, storage and transport of specimens in kit information).

When using the BRM test kit, the diagnostic algorithm as provided in the instructions for use should be followed. If the test is negative and the animal is showing clinical signs, then further investigations are required. This could be explained due to having low virus titer below the detection limit of the rapid antigen test. In this case, further investigations will include re-testing of negative camels at 2-3 days intervals to detect viral antigen as the viral antigen is likely to increase shortly after infection. We set the monitoring interval as 2~3 days, because the rapid antigen test could detect MERS-CoV antigen in 7 days after onset of infection.

Reference

WOAH Terrestrial Manual (2021)

Song, Daesub, et al. "Development and validation of a rapid immunochromatographic assay for detection of Middle East respiratory syndrome coronavirus antigen in dromedary camels." Journal of clinical microbiology 53.4 (2015): 1178-1182.

Lau, Susanna Kar-Pui, et al. "Evaluation of a Rapid Immunochromatographic Middle East Respiratory Syndrome Coronavirus Antigen Detection Assay." Infectious Microbes & Diseases 4.4 (2022): 175-177.

Corman, V. M., et al. "Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction." Eurosurveillance 17.39 (2012): 20285.

Adney, Danielle R., et al. "Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels." Emerging infectious diseases 20.12 (2014): 1999. (DOI: http://dx.doi.org/10.3201/eid2012.141280)

https://www.cdc.gov/coronavirus/mers/lab/lab-testing.html

Acknowledgement

Most of the validation studies were organised and performed by the Veterinary Laboratories Division of Abu Dhabi Food Control Authority (ADFCA), United Arab Emirates. BioNote, Inc.. Thanks to Dr. Salama, the director of Veterinary Laboratories Division, Animal Wealth Sector, ADFCA, UAE for continuous support for this work.