Validation Template for Point of Care (PoC) tests for Prohibited Matter in NSW

Veterinary point of care tests (PoCT) provide field-based or "pen-side" diagnostic information about disease states in animals. Validation is the process by which a test that has been properly developed, optimised and standardised, is assessed for performance against an intended purpose. Accreditation of a PoCT by the Australian Sub-committee of Animal Health Laboratory Standards (SCAHLS) or the World Animal Health Organisation (OIE) indicates fitness of a novel PoCT for an intended purpose. In the absence of such accreditation, novel PoCT need to be adequately validated to minimise the risk of a biosecurity event occurring.

The OIE chapter 1.1.6, 'Principles and methods of validation of diagnostic assays for infectious diseases', recognises several stages in the assay validation pathway:

- Stage 1) Analytical characteristics (sensitivity, ASe, and specificity, ASp);
- Stage 2) Diagnostic characteristics (sensitivity, DSe, and specificity, DSp);
- Stage 3) Reproducibility

An additional stage, field validation, has been proposed by Halpin et al. (2021) for PoCT which includes evaluation of test reliability and reproducibility under field conditions, the utility and of the test in the clinical pathway and the feasibility of conducting the test for the intended purpose. This validation pathway and the actions that must be undertaken for a PoCT to achieve provisional recognition and undergo field validation in NSW are detailed in Figure 1.

Due to significant trade, animal and human health risks, PoC testing for prohibited matter is prohibited in NSW. It is an offence under section 28 of the *Biosecurity Act 2015* to deal with prohibited matter, and clause 4 of the Biosecurity Regulation states that testing for the presence of biosecurity matter is a dealing. Field validation of a PoC for prohibited matter can therefore only be undertaken under an exemption order or prohibited matter permit for a specified purpose and by a specified party. Additional legislated requirements and responsibilities are outlined in the Point of Care Tests for Prohibited Matter policy. Note: PoCT containing prohibited matter as a reagent or control are outside the scope of this document. It is prohibited to develop, manufacture, import, distribute and use tests containing prohibited matter under the Act.

This form provides a template for the minimum information required to assess a non-SCAHLS/OIE accredited PoCT to achieve provisional recognition of the test in NSW. It is based on recommendations made in the OIE Terrestrial Manual, Chapters 1.1.6 'Principles and methods of Validation of diagnostic assays for infectious diseases,' SCAHLS 'Validation Template for Nucleic Acid Detection, Halpin et al 2021 'Perspectives and Challenges in Validating New Diagnostic Technologies,' the National Pathology Accreditation Council Australia 'Guidelines for Point of Care Testing.' Proposals will be reviewed by subject matter experts (both in terms of diagnostic test validation and NSW Biosecurity policy) who will provide recommendations to the Chief Veterinary Officer NSW for provisional recognition.

The OIE 'Standard Operating Procedure for OIE Registration of Diagnostic Kits,' and National Association testing Authorities (NATA) General Accreditation Guidance – Validation and Verification of Quantitative and Qualitative Test Methods should be used a reference when completing this dossier. Submissions should be forwarded for review to NSW DPI (animal.biosecurity@dpi.nsw.gov.au).

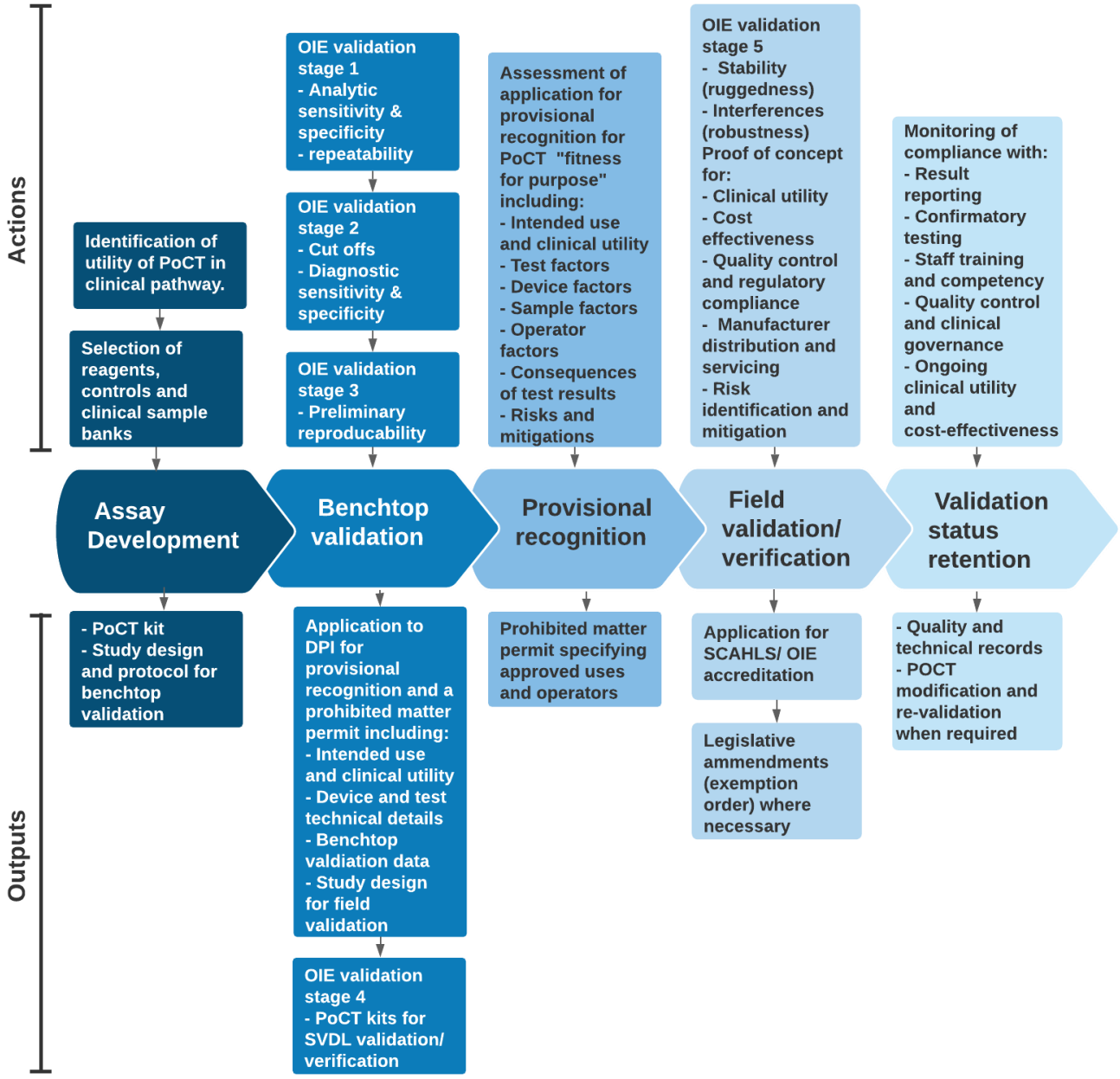

FIGURE 1: Adapted from Halpin et al. 2021, 'Fig. 1 The extended point-of-care test development and validation pathway, with additional field validation stage' and from the World Organisation for Animal Health Validation Pathway, Ch 1.1.6, Principles and methods of validation of diagnostic assays for infectious diseases.

1. Assay Development

1.1 Applicant details

Name:

Job Title:

Organisation:

Contact phone:

- ☐ 'Commercial in confidence'
- ☐ Not commercially sensitive

If yes, then it is up to the submitter to arrange appropriate formal agreements to allow the application to be reviewed by subject matter experts if considered necessary

1.2 Agreement to NSW POC test policy and procedures

I have read and understood the NSW Policy - Point of Care tests for prohibited matter in NSW, Procedure – Validation Point of Care tests for prohibited matter in NSW, Procedure – Use of Point of Care Tests for prohibited matter in NSW, Management of animal biosecurity in NSW, Surveillance for diseases of animals and aquatic pests, Endemic diseases of animals, Prohibited matter pests and diseases of animals and Procedure - Reporting notifiable pests and diseases of animals and biosecurity events. I accept that the information within this application will be reviewed by technical experts as determined by NSW Chief Veterinary Officer.

Signed by Applicant:

Date referred:

1.3 Test name, pathogen and antigen/antibody target

1.4 Intended purpose/s of assay

For the detection of *specify pathogen and antigen/antibody/biomarker target*

in *specify species and population type/s*

by *specify intended setting and user*

for the purpose of: *select all that apply*

- ☐ Investigation of clinical signs (confirmation of clinical cases)
- ☐ Contribute to eradication/control from defined population
- ☐ Population freedom (with/without vaccination)Population freedom (after outbreaks)
- ☐ Individual animal or product freedom from infection for trade or movement
- ☐ Prevalence estimate (risk analysis for surveys, herd health status, disease control measures
- ☐ Surveillance in apparently healthy animals
- ☐ Other, specify conditions

This test will be used as a: *select all that apply*

- ☐ Screening test.

- ☐ Detection of a group of pathogens. For example, the pan-pesti virus assay that detects several agents within the genus *Pestivirus* such as BVDV 1, BVDV 2, BDV, CSF
- ☐ Detection of a single pathogen e.g. CSF assay
- ☐ Subtyping
- ☐ Adjunct test (this is an assay that is NOT used for diagnosis but is used for further characterisation)
- ☐ Other, specify

1.5 Type of POC assay: *select all that apply*

- ☐ PCR nucleic acid test:
  - Real time PCR hydrolysis probe-based
  - Real time PCR intercalating dye (or similar)
  - Conventional PCR with sequencing
  - Conventional PCR without sequencing
  - Other, specify
- ☐ Isothermal nucleic acid test:
  - nucleic acid sequence-based amplification (NASBA),
  - transcription-mediated amplification (TMA),
  - strand displacement amplification (SDA),
  - loop-mediated isothermal amplification (LAMP),
  - helicase-dependent amplification (HDA),
  - recombinase polymerase amplification (RPA),
  - rolling-circle amplification (RCA)
  - Other, specify
- ☐ Electrochemiluminescence test
- ☐ Immunochromatography test
- ☐ Mobile enzyme linked immunosorbent assay
- ☐ Electrochemical/colorimetric aptamer test
- ☐ Biosensor chip (specify technologies employed eg. microfluidics, surface plasmon resonance (SPR), white light reflectance spectroscopy (WLR), etc.
- ☐ In-house design (provide any relevant references in 2.2)
- ☐ Published assay or adaptation of same (provide reference in 2.2)
- ☐ Commercial kit
- ☐ Other, specify

NOTE: If an assay is submitted that is designed to provide absolute quantitation of gene expression (eg genome equivalents) additional direction must be sought from NSW DPI, prior to submission of the validation dossier, on the type of data that may be appropriate for showing that the assay is fit for the purpose of reporting quantitative values for diagnostic samples.

1.6 Justification for POC test in diagnostic pathway

- *A description of the existing clinical pathway (equivalent laboratory test turnaround time/cost/availability) for individuals/institutions/NSW Government where known.*
- *How the clinical pathway will be altered by incorporating the test device*
- *Consequences of correct test results to individual animals and owners, wider industry, communities and the environment*
- *Consequences of incorrect test results to individual animals and owners, wider industry, communities and the environment*
- *Intended risk-mitigation measures (eg. published guidelines, in-house training for users, internal quality control support, etc.)*

1.7 Technical description

Device factors:

- *Size and weight of test device*
- *Power source of test device (including details about mains supply, battery life, charging time etc)*
- *Associated equipment required to perform test (device/cartridges/other consumables)*
- *Shelf life of reagents and consumables*
- *Details on methods and means of calibration, internal quality control and device maintenance*
- *Description of how/if results are presented to operator or provided directly to a laboratory for review (include whether units are the same as current laboratory test, device retention of results)*
- *IT System interoperability and Bluetooth/Wifi connectivity*
- *Turnaround time for a single test*
- *Maximum throughput of test device (number of tests able to be performed over a given time period)*

Costs, distribution and servicing

- *Availability and continuity of supply of reagents, controls and consumables for sampling, testing, maintenance, calibration and quality control*
- *Plan for ongoing technical support for devices/ tests*
- *Cost per device, cost of extra equipment needed to perform test/store test, consumables, any other costs eg, including capital, costs, other fixed, costs, variable, costs and professional costs. Specify which costs and what proportion will be covered by the POCT manufacturer during field validation and ongoing.*
- *Reccomended price of test charged to individual sample/animal/owner and what proportion of costs will be covered by the POCT manufacturer during field validation and ongoing.*
- *Desired support from NSW Government in POC validation/implementation*
- *Proportion of test costs that will be covered by the POCT manufacturer during field validation.*

Sample factors

- *Type of sample/s to be used and intended method of collection (discuss invasiveness, risk to subject, risk to sampler/handlers)*
- *Expected robustness of test to sample contamination/ interference*

Operator factors:

- *Level of technical ability required for sample collection, processing, running of test, calibration and maintenance of device*
- *Level of operator training required*

1.8 Benchtop validation protocol

Attach protocol as Appendix 1, as it would appear in an ANZSDP or Institutional QA document prepared in compliance with ISO17025.

Protocol must include:

- *The selection of specimens/tissues*
- *Method of preparation/treatment of samples*
- *Requirement for measurement precision*
- *Method of conducting POC test (Note: the criteria used to define positive/negative/indeterminate results, including the threshold settings and cut offs (if applicable), for all data presented in this validation dossier, must be those specified in the protocol provided as Appendix 1 to the dossier.*
- *Cut-off and threshold values and data interpretation.*
- *Details of all quality controls used routinely in assay runs to ensure reliability of sample preparation procedures, absence of contamination and integrity of results.*

1.9 References

Provide relevant references including those on which the test was based, as well as any publications (published or submitted) resulting from the current validation work.

2. Outcomes of benchtop validation

2. 1 Analytical Characteristics (OIE Validation Stage 1)

2.1.1 Analytical specificity (ASp)

ASp evaluates the differentiation of target agent from a range of other non-target but related infectious agents and other diseases with similar clinical presentations. ASp evaluation is qualitative and should reflect the intended purpose of assay (as indicated in Background Summary above) and whether the assay is designed to be selective, exclusive or inclusive (OIE Ch 1.1.6).

The panel of samples should contain:

- *well-characterised isolates of the target pathogen, including isolates from a variety of geographical areas and hosts*
- *related organisms and pathogens which cause similar clinical syndromes.*

Selectivity - the extent to which a method can detect and quantify the target analyte in the presence of non-specific reactants, such as interferents and degradants (e.g matrix components, inhibitors of enzymes in the reaction mix). Assessment of ASp should include use of relevant matrices for intended purpose, such as tissue, blood, inhibitory substances.

Exclusivity (confirmatory assay e.g. AIV H5-assay) – detects a genomic sequence unique to the targeted organism. Specificity testing should include known organisms or strains of the target organism that may cross-react. For example, an assay to detect AIV H5-subtypes should be assessed for cross reaction with non-H5 AIV subtypes.

Inclusivity (screening assay e.g. AIV matrix assay) – this is the capacity of an assay to detect several strains or serovars of a species, several species of a genus, or a similar grouping of closely related organisms or antibodies thereto. It characterises the scope of action for a screening assay. Specificity testing should include target lineages, strains, species to be detected and then the assay should be evaluated for capacity to exclude related organisms such as non-pathogenic strains of intended target and pathogens that cause similar clinical syndromes.

DATA PRESENTATION: Insert table providing description of:

- *related infectious agents tested (agent, strain, isolate, subtype or serotype, if applicable)*
- *no. of replicates (if done), tissues assessed and result interpretation (positive/negative/indeterminate)*
- *in the case of cross-reacting samples that give indeterminate/positive results include the mean assay output, replicate output values (if done)*

Provide comment on off-target interference and indicate gaps in the data, such as missing targets and reason for omission (e.g. due to lack of access to agent or agent not relevant to testing context).

2.1.2 Analytical Sensitivity

Synonymous with limit of detection, ASe is estimated using a dilution-to-extinction study whereby serial dilutions of a quantified target analyte are made in an appropriate sample matrix. The dilution series must extend to at least one dilution past end-point (negative/not detectable). There are two common approaches noted in OIE Ch 2.2.3:

1. *A dilution series of the target pathogen diluted in sample matrix (not buffer). OIE recommend comparing a 'new' method with a standard method to yield a comparative measure of existing and new methods.*
2. *A dilution series of plasmid or other synthetic construct containing the target diluted in sample matrix and amount of the target detectable by the test method.*

Other options include performing a dilution series (in appropriate matrix) on a positive field sample (instead of spiked sample) or amplifying and purifying an amplicon corresponding to the PCR gene target from a field sample and performing a dilution series on this to determine the LOD.

Whichever method is used, the matrix used for the dilution series must be appropriate for the target tissue type and must be clearly stated.

Several methods are available to quantitate the starting concentration of the analyte, such as Qubit, nanodrop, TCID50, EID50, etc. Provide details of whichever method is used to establish the starting concentration of the analyte.

DATA PRESENTATION: Insert table providing description of:

- *analyte (e.g. virus, plasmid sequence, field sample);*
- *matrices/diluent used (e.g. buffer, negative DNA, negative tissue etc.);*
- *concentration (ng/ul, genome equivalents, TCID50 etc.) and method of quantitation (e.g. nanodrop, Qubit, tissue culture etc)*
- *dilution series (and expected concentration based on starting or highest concentration);*
- *Test output values (eg. Ct, titre, etc) for each replicate &/or mean output vale and Standard Deviation (SD) of replicates or coefficient of variation;*
- *briefly describe criteria used to define positive/negative/indeterminate results and end-point. (For example: each dilution was tested in triplicate, all replicates must show 100% or 0% response to be classified as positive or negative, respectively; dilutions with disparate replicates will be classified as indeterminate; end-point is last dilution with all replicates classified as positive);*
- *Plot mean output value vs log dilution and include trendline, equation and R2-value and test efficiency (%)*

2.1.3 Repeatability

Repeatability is the measure of agreement between results both within and between runs, using the same test method in one laboratory (OIE Ch 2.2.3). Repeatability is estimated by evaluating variation in results of replicates (OIE Ch 1.1.6). OIE recommend the following procedure (see Ch 1.1.6 and 2.2.3):

- *select a panel of 3-5 samples covering the operating range of the assay*
- *test each sample using the entire procedure, including independent nucleic acid extraction*

- *assess within (intra) assay variation using (at least five) replicates of each sample in one run (one operator)*
- *assess between (inter) assay variation by testing the panel of samples over several days, using 2 or more operators, minimum of 20 runs.*

Although the OIE recommendations (below)are very thorough, they may also be impractical, in terms of sample preparation (a minimum of 100 homogeneous aliquots of each sample in the panel are needed) and deployment of personnel/reagent resources. Alternative approaches that may be appropriate (depending on the specifics of the disease and test being validated) are included.

1. *Repeatability must test the entire assay process, including sample processing, as this can be a source of significant variability. The general acceptable approach is: select a panel of 3 field samples (or experimental infections) from different animals covering the operating range of the assay, one strong, one medium and one weak; 30-40 (homogeneously mixed) aliquots of each sample will be required.*
2. *test each sample in triplicate using the entire procedure, including independent sample preparations of each replicate*
3. *within (intra) assay variation is assessed from the three replicates of each sample in one run (one operator)*
4. *between (inter) assay variation is assessed by comparison of results from 2 or more operators testing the panel of samples (each in triplicate) over several days, minimum of 10 runs.*

Alternative 1: Where samples from different animals (field or experimentally infected) are not available, a single field/experimental sample may be used and diluted in suitable negative matrix to create a minimum of 3 samples covering the analytical range of the assay (essentially strong, medium and weak). The volume of each dilution must be sufficient to permit replicate testing, 30-40 (homogenously mixed) aliquots of each sample are required. Thereafter the method of testing follows points 2-4 above.

Alternative 2: Where no field or experimental-infection samples are available, one (or more) isolates (e.g. cultured virus) can be spiked into suitable negative tissue to create a panel of 3 samples, one strong, one medium and one weak, covering the analytical range of the assay. The volume of each dilution must be sufficient to permit replicate testing, 30-40 (homogenously mixed) aliquots of each sample are required. Thereafter the method of testing follows points 2-4 above.

Optional: In addition to the protocol above, repeatability testing of the assay alone can be assessed by using plasmid or a suitable synthetic construct. Plasmid of known copy number is spiked into negative DNA followed by serial 10-fold dilutions (in negative DNA) to extinction (below the level of detection estimated from initial copy number). Each dilution is then tested in replicate (3-5 reps). This could be performed by a single operator and repeated independently by 2 or more operators for minimum of 5 runs. Each operator must independently prepare dilution series for each run.

DATA PRESENTATION: Insert data providing description of:

- *number and type of analytes used in panel (e.g. virus, plasmid sequence);*
- *number of replicates and relevant preparation data, including matrix (diluent) used;*
- *sample preparation method*
- *Assay output values for each replicate & mean output value +SD of replicates or coefficient of variation;*
- *criteria used to define positive/negative/indeterminate results*

2.2 Diagnostic characteristics (OIE validation stage 2)

2.2.1 Diagnostic specificity (DSp) and sensitivity (DSe)

The diagnostic performance of an assay is commonly measured as sensitivity (DSe) and specificity (DSp) or combined measures of these that estimate likelihood ratios of positive and negative results. Diagnostic specificity is determined by the proportion of samples from known uninfected reference animals that test negative in an assay. Diagnostic sensitivity is determined by the proportion of samples from known infected

reference animals that test positive in an assay. Methods and statistical models to estimate DSe and DSp will depend on several factors including the availability or absence of existing reference (standard) test/s for comparative analyses, the identification of suitable negative populations and the availability of confirmed positive samples.

The OIE test validation pathway provides for provisional recognition of candidate tests where critical benchmark parameters have been adequately assessed (ASe, ASp and repeatability) and preliminary assessments of DSp, Dse and reproducibility have been performed on well-characterised samples (Ch 1.1.6). To ensure proper evaluation of diagnostic characteristics it is essential to provide detailed and transparent documentation of the animal populations, case definition and analysis model used.

2.2.2 Cut-offs/thresholds

The cut-off points (decision limits) for defining positive, negative and indeterminate results must be clearly defined in the protocol in Appendix 1. It may be appropriate to categorise as positive/suspicious/indeterminate any sample that produces a typical curve, regardless of test output value. The test protocol may also include suitable approaches (algorithms) to address suspicious/indeterminate results within the context of the clinical history and presentation.

2.2.3 Sample numbers, case definition and analysis model

The required number of known positive and negative samples will depend on the likely values of DSe and DSp of the candidate assay and the desired confidence level and permitted error margin. For all intended purposes, it is essential that the case definition, used to identify positive populations, is clearly stated. Tables of the theoretical sample numbers required to achieve the desired confidence are available from several sources including OIE Ch 1.1.6, however, it is recommended that an epidemiologist and statistician be consulted prior to data collection to ensure suitable reference samples and analysis models have been identified especially where no standard test exists and/or positive samples are in limited supply.

The selection of suitable animal populations can be problematic for both endemic diseases (identifying true negative populations) and exotic or rare diseases (identifying true positive populations). Obtaining adequate numbers of suitable populations is crucial for estimating DSe and DSp the associated confidence intervals for these. It is recommended that an epidemiologist and statistician be consulted prior to data collection to determine the most appropriate animal populations, numbers and analysis models to use to determine diagnostic characteristics. A network approach using sample panels may be required to obtain statistically robust results where field samples are limited. For example, an antibody ELISA for Hendra virus (HeV)- infected horses was evaluated using using sera from 19 infected and 477 non-infected horses and an additional six panels of well-characterised sera were tested to estimate the ASe, ASp, repeatability and reproducibility in a network approach. Network laboratories provided additional baseline data from HeV-negative horses, which resulted in robust estimates for DSp in non-infected populations.

Data presentation

When results of the candidate assay are compared to a reference test data should be presented in 2x2 table with a confidence interval (ideally CI95%). Latent class and other models (eg. Bayesian Assurance Method) may also be used where there is no reference test for comparison, or to compare candidate assays to an existing assay without assuming the existing test is 'perfect'.

Note

In the following areas, indicate the purpose being assessed, provide as much population information as possible to establish the 'fitness' of the assay in the clinical context.

Sections 2.2.4-2.2.7 should be replicated to provide data for each specified 'intended purpose' and corresponding (relevant) animal population/s.

2.2.4 Intended purpose of POC test *(Number of)*

- ☐ Investigation of clinical signs (confirmation of clinical cases)
- ☐ Contribute to eradication/control from defined populations
- ☐ Population freedom (with/without vaccination)
- ☐ Population freedom (after outbreaks)
- ☐ Individual animal or product freedom from infection for trade or movement
- ☐ Prevalence estimate (risk analysis for surveys, herd health status, disease control measures)
- ☐ Surveillance in apparently healthy animals
- ☐ Other, specify conditions

2.2.5 Define population/s used during benchtop validation (should be reflective of intended populations for field validation)

Provide as much relevant population information as possible to support the fitness of the assay for the stated clinical purpose. Including, for example species, samples/tissue types, whether samples are derived from experimental infections (specify isolate/strain used for infection), field samples (specify isolate/strain detected), wild or farmed, numbers of animals in each category, sex/age (if known), pregnancy status (if known), vaccination status (specify isolate/strain used).

2.2.6 Analysis model

Describe the analysis model used. Eg comparison of new assay to a reference method, Bayesian Latent Class Analysis (describe a priori assumptions etc).

Insert 2x2 table or other analysis model used to determine Diagnostic characteristics

2.2.7 Statement on fitness for purpose

Provide conclusions regarding the fitness-for-purpose of the candidate assay for the intended purpose/s. Provide specific recommendations on the populations and circumstances in which the test will be validated in the field.

2. 3 Preliminary Reproducibility (OIE Validation Stage 3)

Reproducibility is a measure of the ability of a test method to produce consistent results for the same samples tested in different laboratories, preferably in different regions or countries, using the identical assay (protocol, reagents and controls) (OIE Ch 1.1.6). For POC tests, specific aspects of reproducibility, ruggedness and robustness, are assessed during field validation.

Provisional recognition may be granted for POC tests without undertaking thorough benchtop reproducibility studies under specific circumstances (eg. accelerated recognition of a novel POC during an emergency response). However, it is strongly reccomended to undertake and include at least preliminary estimates of POC test reproducibility in this dossier. Additionally, a validation package (including testing kits, protocol, etc.) should be supplied to the NSW State Veterinary Laboratory (SVDL) at the Elizabeth Macarthur Agricultural Institute (EMAI) for test verification and generation of preliminary reproducibility data.

Apart from providing POC test manufacturers and regulatory bodies with increased confidence of the fitness of the test for its intended purpose during field validation studies, generation of preliminary data on ruggedness and robustness of the test under different laboratory conditions may allow some reduction in the sample size required in the field diagnostic accuracy study under a seamless and adaptive analytical model (eg. Bayesian assurance method).

It is recommended that an epidemiologist and statistician be consulted prior to data collection to ensure benchtop reproducibility studies have been designed to generate data of sufficient quality and quantity that it may be used to streamline field studies where desired.

When undertaking a standard reproducibility study, at least three blinded laboratories should test the same panel of 10-20 samples. About 25% of the samples should be negative and the remainder positive, with concentrations covering the operating range of the assay. The panel composition should reflect the inherent variability of the target pathogen (eg.different serotypes) and circulating or geographically relevant strains.

In addition to the reproducibility panel, further evidence of reproducibility can be provided by use of an external quality control (EQC), such as the network quality control (NQC) issued to the LEADDR network. Ideally this should be prepared as a weak control to accrue log-term data on assay reproducibility and laboratory proficiency.

Any samples used for benchtop reproducibility studies must be homogeneous and stable for the period of use, whether that is a short period of time for a reproducibility study, or extended use for an external quality control. For this purpose, it is useful to use samples prepared by an accredited proficiency testing provider in accordance with ISO17043, or where this is not possible, to have evidence that the samples are homogeneous and stable and thus fit-for-purpose.

Provide details of on-going assay performance in participating laboratories via reproducibility studies, satisfactory performance of relevant proficiency testing and/or external quality assurance data.

3. Study Design for Field Validation/ verification

Field validation for POC tests allows verification of the intra and inter-operator impacts on the reproducibility of diagnostic test characteristics under a range of realistic field conditions (ruggedness) and sample types (robustness). It also provides proof of concept for the utility and cost-effectiveness of the POC test in the clinical pathway and an opportunity to assess and refine risk management/mitigation strategies related to both true and false test results.

The ruggedness or "stability" of a POC test describes the effect of factors such as temperature (throughout the cold chain), relative humidity and storage duration on the test device, reagents and consumables and the impact this has on the diagnostic characteristics (DSe and DSp) of the test.

The robustness of a POC test describes the interference of expected contaminants (eg. soil, faeces, commensal microflora) and variation in sample matrices (tissue type, effect of sampling artefacts, use of non-sterile water or reagents) on the diagnostic characteristics (DSe and DSp) of the test.

The prevalence of the disease in the target population interacts with the diagnostic characteristics of the POC test through positive and negative predictive values (NPV and PPV respectively) and needs to be considered in sample size calculations. The positive predictive value is the percentage of truly positive test results among all test-positive results (ie. the confidence that a positive test result indicates a truly infected individual). When disease prevalence drops (eg. during an effective disease eradication campaign), there will be an increased likelihood of false-positive test results among all positive test results unless the DSp is 100%. If the intended use of the test to identify truly infected animals (eg. investigation of clinical signs, diagnosis for eradication/control from defined population) or the consequences of a false positive result are high (eg. for exotic diseases with significant trade consequences), the POC test would only be deemed fit for purpose if it had a high DSp under field conditions, or where the use of other information (e.g. clinical history) would increase the relative prevalence of a rare disease in the tested population.

The NPV is the percentage of truly negative test results among all test-negative results (ie. confidence that a negative result indicates a truly non-infected individual) and its value is not appreciably affected when the prevalence of infection is low (e.g. <10%). POC tests with high DSe or the use of two or more tests' results

interpreted in parallel (where any positive-test result makes the individual positive) can increase the NPV. A high NPV is desirable for tests intended to screen apparently healthy populations and demonstrate freedom from disease or where the consequences of a false negative are high (eg. high impact zoonotic pathogens where a false negative test result may increase the risk of human exposure). These principles can be applied to individual animals or can be extended to multiple epidemiological units (i.e. herds and clusters of herds in the same geographical area).

Attach a proposed field validation protocol as Appendix 2, as it would appear in an ANZSDP or Institutional QA document prepared in compliance with ISO17025. Provide as much relevant information as possible to support the fitness of the assay for the stated clinical purpose.

As a minimum the protocol will include:

- *Intended use of POC test in the field*
- *Population the POC will be used on in the field including species, samples/tissue types, stage of clinical disease, wild or farmed, numbers of animals in each category, sex/age (if known), pregnancy status (if known), vaccination status (specify isolate/strain used), etc.*
- *Prevalence of disease in the target population*
- *Sample size and requirements for confirmatory testing during field validation*
- *The selection of specimens/tissues*
- *Method of preparation/treatment of samples, reagent storage, etc.*
- *Requirement for measurement precision*
- *Method of conducting POC test and intended user including safety data sheets, safe handing practices for all samples, reagents, controls, calibration procedures, maintenance and troubleshooting procedures, quality control (QC) and external quality assurance (EQA) procedures and quality record sheets.*
- *Cut-off and threshold values and data interpretation including critical alert limits, known interferences and reference ranges.*
- *Details of all quality controls used routinely in assay runs to ensure reliability of sample preparation procedures, absence of contamination and integrity of results.*
- *Intended analysis model*

3.1 Statement on fitness for purpose

Provide conclusions regarding the fitness-for-purpose of the candidate assay for the intended purpose/s. Provide specific recommendations on the populations and circumstances in which the test will be validated in the field.

3.2 PoCT operator training and assessment

In order to ensure the safety of PoCT operators and the delivery of accurate and reliable results, PoCT manufacturers will be required to provide training and assessment and ongoing technical support for operators. Records of competency must be kept and supplied for review by authorised officers and known or suspected issues that may alter the safety or quality of the POC test to an authorised officer immediately. This includes use of PoCT for any purpose other than that for which it has been validated.

Attach a proposed operator training and assessment protocol as Appendix 3. Provide as much relevant information as possible to support the safety of the operator, maintenance of internal quality control (QC) and external quality assurance (EQA) and the ability to generate reliable and accurate results.

As a minimum the protocol will include description of planned delivery of training and assessment in:

- *the ability to demonstrate appropriate use of the device.*
- *pre-analytical requirements such as sample collection,*
- *reagent storage requirements,*
- *safe work and infection control practices.*

- *the ability to identify results that fall outside of reference ranges.*
- *device maintenance.*
- *an understanding of Quality Control (QC) and Quality Assurance Program (QAP)*
- *confidentiality of patient and client information*
- *general biosecurity duty*
- *methodology, frequency and timing of competency assessments*

3.3 Animal ethics

Animal ethics approval has already been achieved for the study protocol for field validation?

- ☐ Yes
- ☐ No

If no, please provide details of the state of the application. If there is sufficient reason to believe that animal ethics approval for the listed study protocol is not imminent, the PoCT application will not be reviewed until AEC approval has been achieved.

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4. Record of assessment

4.1 List of experts

List of experts consulted in the approval process and their institutional affiliation and area of expertise/background

4.2 Assessment Result

The NSW Government working group may make one of the following recommendations to the NSW Chief Veterinary Officer (CVO) based on their review of the validation dossier:

1. *Provisionally recognise the assay as fit for one or more of the intended purpose/s; recommend CVO grant prohibited matter permit or exemption order under the following conditions (see 4.3.1) OR*
2. *Do not provisionally recognise the assay as fit for one/more intended purpose/s. Further validation data (see 4.3.2) needed for reconsideration of the application by the working group.*

4.3 The NSW POC working group make the following recommendation:

4.3.1 Validation acceptable:

For the detection of *specify pathogen and antigen/antibody/biomarker target*

in *specify species and population type/s*

by *specify intended setting and user*

for the purpose of: *select all that apply*

- ☐ Investigation of clinical signs (confirmation of clinical cases)
- ☐ Contribute to eradication/control from defined population
- ☐ Population freedom (with/without vaccination)
- ☐ Population freedom (after outbreaks)
- ☐ Individual animal or product freedom from infection for trade or movement
- ☐ Prevalence estimate (risk analysis for surveys, herd health status, disease control measures
- ☐ Surveillance in apparently healthy animals
- ☐ Other, specify conditions

This test will be used as a: *select all that apply*

- ☐ Screening test.
- ☐ Confirmatory test
- ☐ Detection of a group of pathogens. For example, the pan-pesti virus assay that detects several agents within the genus *Pestivirus* such as BVDV 1, BVDV 2, BDV, CSF

- ☐ Detection of a single pathogen e.g. CSF assay
- ☐ Subtyping
- ☐ Adjunct test (this is an assay that is NOT used for diagnosis but is used for further characterisation) e.g. sequencing
- ☐ Other, specify

With the following conditions of use

eg. specific species, specific number of animals (herd/individual), specific location of testing, etc.

4.3.2 Validation not acceptable

Specify the reasons for rejecting the application and any additional data required by the working group for re-consideration of validation

4.4 Date of Decision and Reporting

Decision: *dd/mm/yyyy*

Reported: *dd/mm/yyyy*

4.5 Next steps *select all that apply*

- ☐ Chair working group forward record of assessment process to NSW CVO
- ☐ Chair working group forward record of assessment process to submitter
- ☐ Preparation of AHC issues paper
- ☐ Preparation of exemption order
- ☐ Preparation of prohibited matter permit