Framework for the Certification Procedure of Commercial Diagnostic Reagents/Kits at SCIENSANO

Coordination of Diagnostic Control
NRLs of SCIENSANO

GUIDANCE DOCUMENT FOR THE CHECKLIST

This guidance document is based on:

- PRO/4.3/03: Titel/Titre: Certificering van (commerciële) diagnostische reagentia (kits) door het SCIENSANO / Certification de réactifs (commerciaux) de diagnostic (kits) par le SCIENSANO
- NF U 47-301: Méthodes d'analyse en santé animale. Dossier de présentation pour le contrôle de réactifs biologiques utilisés dans le domaine de la santé animale.
- OIE Procedure for Validation and Certification of Diagnostic Assays (2009). Manual of diagnostic tests and vaccines for terrestrial animals (chapter 1.1.4/5 on Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases)
- ISO 17025 (2005)

Scope (SCIENSANO PRO/4.3/03):

This document describes the framework proposed to certify (commercial) diagnostic reagents (kits) at the SCIENSANO. This framework will be applied to all (commercial) diagnostic reagents (kits) used in the Belgian official programs for animal disease control.

The certification procedure (phase 1) consists of an administrative and a technical evaluation by the SCIENSANO reference laboratory involved. During the administrative evaluation, the validation file of the manufacturer/supplier will be checked against administrative criteria, as determined, predefined and recorded by the expert committee and as communicated in the Official Publication. A manufacturer/supplier, who does not comply with the administrative minimum criteria, will not be allowed to the technical evaluation. During the technical evaluation, the SCIENSANO reference laboratory involved will check the requested diagnostic reagents from the manufacturer/supplier against the technical minimum criteria as determined and recorded by the expert committee and as communicated in the official publication and documents to be requested from SCIENSANO. Manufacturers/suppliers that succeeded phase 1 will be allowed to deliver batches for the Belgian Market. Each batch should be controlled by the SCIENSANO reference laboratory (phase 2).

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I.Framework Proposed

(Certification Procedure of Commercial Diagnostic Reagent)

A. Background: OIE Guidelines

The SCIENSANO framework for quality evaluation of diagnostic tests is based on the OIE Procedure for Validation and Certification of Diagnostic Assays (Manual of diagnostic tests and vaccines for terrestrial animals: Chapter 1.1.4/5: Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases; www.oie.int). This OIE Procedure follows the OIE Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Diseases, which states that test methods and related procedures must be appropriate for specific diagnostic applications in order for the test results to be of any relevance. In other words, the assay must be **'fit for purpose'.**

The intended purpose(s) of an assay have been broadly defined as:

1) Demonstrate freedom from infection in a defined population (country/ zone/ compartment/ herd) (prevalence apparently zero):
   a) 'Free' with and/or without vaccination,
   b) Historical freedom,
   c) Re-establishment of freedom after outbreaks.
2) Certify freedom from infection or agent in individual animals or products for trade/movement purposes.
3) Eradication of infection from defined populations.
4) Confirmatory diagnosis of suspect or clinical cases (includes confirmation of positive screening test).
5) Estimate prevalence of infection or exposure to facilitate risk analysis (surveys, herd health status, disease control measures).
6) Determine immune status of individual animals or populations (post-vaccination).

In practice it is possible that producers of (commercial) diagnostic assays claim a combination of these intended purposes. During evaluation (administrative and/or technical), sound arguments, to prove this claim, should be gathered and/or collected.

The OIE Standard further states that in order for a test method to be considered appropriate, it must be properly validated and that this validation must respect the principles outlined in the validation chapters of the *Terrestrial Manual*. While the OIE standard mainly deals with validation and fitness for purpose from a scientific perspective, it should also be noted that other factors might impact the relevance of an assay with respect to fitness for purpose. These factors include not only the diagnostic suitability of the assay, but also its acceptability by scientific and regulatory (national and international) communities, acceptability to the client, and feasibility given available laboratory resources. An inability to meet operational requirements of an assay also may make it unfit for its intended purpose. Such

requirements may include running costs, equipment availability, level of technical sophistication and interpretation skills, kit/reagent availability, shelf life, transport requirements, safety, biosecurity, sample throughput, test turn-around times, aspects of quality control and quality assurance.

For further information see:
http://www.oie.int/fr/normes/mmanual/2008/pdf/1.1.04_VALID.pdf
http://www.oie.int/vcda/eng/en_VCDA_registre.htm?e1d9

B. Framework for Certification of Diagnostic Reagents/Kits at SCIENSANO

Based on the OIE Guidelines, the OIE Stages of Validation and Certification of Diagnostic Tests see **Appendix A**), and the Register of diagnostic tests certified and validated by the OIE as '**fit for purpose'**, we determined which information manufacturers/suppliers should provide in Validation Files submitted for Administrative Evaluation, as well as Test Performance Parameters to be assessed during Technical Evaluation of Diagnostic Reagents/Kits at SCIENSANO.

The Steering Group SCIENSANO-FAFSC, as defined by PRO/4.3/03, will formulate the "Need for Certification" or the "Need for a Certification Tender", as the need to certify diagnostic reagents/kits may arise from the different partners involved in the official animal disease control programs.

For each approved and planned Certification a specific Expert Committee, as defined by PRO/4.3/03, will determine the purpose of the certification procedure (i.e. intended purpose of the reagent/kit) and the conditions for a reagent/kit to enter the Certification. Purpose and conditions will be specified in the Official Publication and relevant documents to be requested from SCIENSANO

The framework consists of a set of requirements in regards with the administrative and technical evaluation of diagnostic reagents/kits **during Phase 1 (new certification release)** of the Protocol of Certification (PRO/4.3/03), and (in future versions) technical evaluation of different batches/lots of reagents/kits **during Phase 2 (batch release by SCIENSANO)** of the Protocol of Certification (PRO/4.3/03).

C. Tasks during Certification

1) Preparing of minimum criteria document: **Ref. Lab.**
2) Planning of tenders: **Ref. Lab.**
3) Analysis of administrative files: **Ref. Lab.**
4) Collecting and certifying reference samples for technical validation: **Ref. Lab**
testing reference samples for minimum criteria: **Ref. Lab**
5) Statistical analysis of results: **Ref. Lab**
6) Internal discussion of results: **Ref. Lab + Diagnostic Control**
7) Prepare reports: **Ref. Lab**
8) Present to manufacturers and end users, invitations: **Ref.Lab + Diagnostic Control**

9) Publishing results/lists on website: **FAVV + SCIENSANO**

II. Administrative Evaluation (Phase I)

A. Required Contents of the Validation File submitted by the manufacturer / supplier for Administrative Evaluation of a diagnostic reagent/kit by the SCIENSANO when a new Certification is issued.

A.1. Required Administrative Information provided by Manufacturer

A.1.1 General Information

1.1.1. Name and contact details of the responsible supplier
1.1.2. Name and contact details of the manufacturer
1.1.3. Commercial name and denomination of the reagent/kit
1.1.4. Composition described in general terms comparable to terms mentioned on the package insert
1.1.5. Location(s) of manufacturing and quality control
1.1.6. Location(s) of conditioning
1.1.7. End-users (laboratories/governments) of the reagent/kit in Belgium and abroad
1.1.8. ISO-certified 9001 or satisfying the criteria of the ISO 9001

A.1.2 Presentation of diagnostic reagent/kit

1.2.1. Description of reagent type: ELISA (antibody, antigen, direct, indirect, competition, other) or PCR (PCR, RT-PCR, other), test kit composition (detailed information on all separate kit components), and of implementation technique (including schematic overview of protocol, incubation times, temperatures,...)
1.2.2. Fitness for purpose: according to OIE (Appendix A)
1.2.3. Scope: types of samples: species validated, matrices used (serum, milk, blood, other); Species and Matrix to be used with the diagnostic reagent/kit
1.2.4. Terms, conditions and duration of storage, shelf life of components and kit/batch
1.2.5. Labeling of reagent/kit/components
1.2.6. Package insert (leaflet): detailed user manual, critical steps of reaction, modalities of reading results (reader type), formulation/calculation of results (units, conversion factors), known interactions and cross-reactions, reference language and languages in which kit insert is translated
1.2.7. Equipment (e.g. extraction kit, PCR thermocycler, …) which is required and not supplied by manufacturer
1.2.8. Maximum number of reactions possible with one kit (excluding controls)
1.2.9. Modalities of disposal of reagent/kit/components: waste disposal/management
1.2.10. First date of release, references to release date, list of all modifications until present, including date, type and reason of each modification
1.2.11. Cost per reagent/kit and cost per sample tested (*euro*)

1.2.12. Format: strips and/or full plates. If strips (8 wells, 16 wells …)
1.2.13. Foresee a schematical overview of the assay (figure, visualization)
1.2.14. Type(s) of protocol (overnight, short procedure, other …)
1.2.15. Delivery modalities (ordering, delivery time, reservation of batches, emergency delivery, stock information) and technical support

A.1.3 Quality controls for finished products

The rules of control for the finished product, put in place by the manufacturer in view of the production of different batches/lots, are described:

1.3.1. Description of control objectives
1.3.2. Description of techniques for quality control
1.3.3. Detailed List, definition and description of acceptability criteria for batch release during in house Quality Control
1.3.4. Description of control certificate for 3 recent batches (copies to be attached)

A.1.4 Description of primary components and definition of a 'batch'

1.4.1. Primary Components
1.4.2. Chemical Components
1.4.3. Biological Components

Detailed description of all primary or active constituents and components (e.g. enzymatic substrate, colorant, oligonucleotides, peptides, proteins, antigens, strains, antibodies, dilution buffer, conjugate (Mab? Pab? Clone? References …) others), especially those that could influence the results obtained, or that could present a biological or chemical risk, in order to facilitate risk appreciation.

1.4.4. Definition of 'batch components'
1.4.5. Process of 'batch' identification
1.4.6. Components which are interchangeable or non-interchangeable between kits/batches

The manufacturer is required to supply a detailed and clear definition of what combination of components exactly constitutes a 'batch'. A description of the process of batch (lot) identification for each reagent or component entering each kit is requested. Each finished kit is defined by a batch/lot number, which is unique for each combination of component batches/lots. The manufacturers have to indicate components that according to them can or cannot be used with components of other batches/lots. Interchangeability of component or reagent lots/batches has to be represented the technical validation information. Proof of interchangeability should be provided under 2.1.3 for all the validation parameters asked for: *CV for repeatibility and reproducibility ; *same (negative or positive) result for reference samples.

Regardless of the manufacturer's definition of a 'batch', the SCIENSANO will formulate its own definition of what constitutes an acceptable 'batch'. The SCIENSANO definition will be one of the administrative minimum criteria and which will be described in the administrative dossier of each separate certification.

A.2. Technical Validation Information provided by Manufacturer

The technical validation information is a list of all parameters to be evaluated by the manufacturer during the validation of a reagent/kit, and which have to be provided to the SCIENSANO in order to facilitate the comparison between different reagents/kits. Part of these parameter results may be obtained by the manufacturer through internal assays, and part may be obtained by clearly identified reagent/kit users, in the scope of reagent/kit evaluation/validation.

A.2.1 Validation of Batches and Components

2.1.1. Specify production batches for which the validation parameters are supplied
2.1.2. Specify protocol(s) for which the validation parameters are supplied
2.1.3. Specify interchangeable components for which the information below is supplied

Certification evaluation is performed with a minimum of 3 specified production batches. When several technical protocols are proposed by the manufacturer, the parameters described below have to be validated for each protocol, and the manufacturer has to supply assay results indicating that regardless of the protocol, the test results (detectability, sensitivity, etc.) are identical. The producer has to specify for which production batches, which protocols and which kit components the validation parameters are supplied.

A.2.2 Validation Parameters

For clear definitions and information on possible methodologies, see Appendix E. For each separate certification, the minimum criteria document will list those validation parameters that are also minimum criteria. The validation information has to consist of:

2.2.1. Epidemiological information of the validation studies

This includes: validated species, validated matrices, sample size (min.-max.), methodology of sample size calculation, design prevalence used to substantiate freedom of disease, population determination (infection/vaccination status of the animals), and calculation of diagnostic sensitivity/specificity. Sufficient samples must be used, in order be representative for the target population and which correspond to the domains and limits of use (specified in the package insert). The results for these samples have to be obtained by one or more recognized techniques and have to be

specified. SCIENSANO usually demands a minimum of 300 samples to be used in any study that is used to calculate Diagnostic Sensitivity and/or Diagnostic Specificity.

2.2.2. Determining the cut-off(s) / threshold(s) for positivity

The thresholds have to comply with national and /or international requirements when these exist. Raw data dilutions/preparation of dilutions, OD values, and calculated results) must be provided by the manufacturer in order to verify the cut-off(s) thresholds considered.

2.2.3. Analytical sensitivity (detectability):

Synonyms: analytical sensitivity = detectability = limit of detection (LOD) = detectielimiet/detecteerbaarheid = limite de detection/détectabilité ;

The lower limit of detection is determined with reference materials (certified, internal, external), when these exist and are available. When no reference material exists, a control sample from the supplier can be used, on the condition that this sample is described in detail (how this control sample is produced, made, bought, references).

When a reagent can be used for more than one matrix or animal species, the sensitivity of the measurement in each of these usable matrices (serum, plasma, milk…) or species has to be determined in studies. The results are presented and have to show the variation of the measured signal in relation to the variation of the quantity of analyte to be measured, as well as matrix effects on the measured signal. The manufacturer will include graphs of the dilutions used to calculate analytical sensitivity, as well as a graph/figure on antibody response after experimental infection, indicating range of days post infection the antibodies can be detected.

2.2.4. Analytical specificity (cross-reactivity)

Where relevant, predefined samples (strains, peptides …) should be collected and chosen for their known ability to interfere with the measurement.

The cross reactivity is determined with reference materials (certified, internal, external), when these exist and are available. When no reference material exists, a control sample from the supplier can be used, on the condition that this sample is described in detail (how this control sample is produced, made, bought, references).

When a reagent can be used for more than one matrix or animal species, the cross reactivity in each of these usable matrices (serum, plasma, milk…) or species has to be determined in studies. The results are presented for OD, calculated results and titers.

2.2.5. Diagnostic sensitivity + 95% confidence limits (CI)

Synonyms: diagnostic sensitivity = gevoeligheid = sensibilité

Sufficient samples representative of the population are used. In general, 300 to 1000 samples are needed and this will be a minimum criterion, as described in the minimum criteria document of each certification. The manufacturer is required to describe the situation in regards with prevalence and vaccination policies in the country where those samples were acquired. A detailed description of samples used and definition of infectious status is also required. The manufacturer will state how the DSe was calculated: with or without gold standard.

2.2.6. Diagnostic specificity + 95% confidence limits (CI)

Synonyms: diagnostic specificity = specificiteit = spécificité

Sufficient samples representative of the population are used and some samples are chosen for their known ability to interfere with the reaction. In general, 300 to 1000 samples are needed and this will be a minimum criterion, as described in the minimum criteria document of each certification. The manufacturer is required to describe the situation in regards with prevalence and vaccination policies in the country where those samples were acquired. The manufacturer will state how the DSp was calculated: with or without gold standard.

2.2.7. Precision: repeatability and reproducibility + 95% confidence limits (CI)

Synonyms : (1) precision = precisie = fidélité ; (2) repeatability = maximum precision = répétabilité = précision maximale = herhaalbaarheid = maximale precisie ; (3) Intra-laboratory reproducibility = Intermediate Precision = Réproductibilité Intra-laboratoire = Fidélité Intermédiaire = Intra-laboratorium Reproduceer-baarheid = Intermediaire Precisie

Definition: precision = repeatability + intra-laboratory reproducibility

Repeatability (samples tested in 1 batch, on 1 day, by 1 person, in 1 laboratory), and intralaboratory reproducibility (samples tested in a minimum of 3 different batches, on different days, by different persons, in 1 laboratory) are presented by the manufacturer. Raw data must be provided by the manufacturer. Results of Proficiency Tests (PT's) can be included and presented.

The manufacturer will describe the batches, species, matrices, number and status of samples, dilutions, number of repeats, number of plates for which CV's are determined. CV's will be given for separate samples as well as a total CV per plate.

2.2.8. Robustness (variations to which testkit is sensitive)

Synonyms: robustness = robustesse = robuustheid

Robustness under normal conditions is presented for all evaluation results obtained by the manufacturer and users, for a minimum of 3 batches. This includes

information about where tests were performed and which exact normal conditions were tested (e.g. temperature range). When a technical protocol produces ranges, e.g. in duration or incubation temperature, tests are conducted for extreme use conditions, and the results are presented.

2.2.9. Agreement between tests

For test agreement, the Kappa statistic for agreement between the kit under evaluation and other reference kits is presented.

2.2.10. Predictive values

Predictive values: probability the animal is/not diseased, based on a positive/negative test result. The manufacturer will describe the predictive values in the concerning species, samples and matrices, as well as the reference test(s) which was (were) used.

2.2.11. Accuracy

Synonyms: (1) accuracy = juistheid = justesse ; (2) proficiency test = interlaboratory assay = essai d'aptitude = interlaboratoriumproef

Results of different Proficiency Tests (PT's) have to be included and presented (z-score, conclusions of PT trials, interlaboratory reproducibility ...).

2.2.12 Publications

The manufacturer will attach a copy of any scientific peer-reviewed papers, where the authors have used the reagent under evaluation. The main results will be presented and summarized.

A.3. Annex

A.3.1 Annexes

On the "Annex" sheet, a list of annexes is demanded some of which are compulsory. Extra documents may be added as desired. The addition of validation trial set-ups and practical descriptions are recommended. These documents need to be clearly marked and submitted to us under a separate folder "Annexes" in the email, disc and paper Dossiers (3x in total).

A.3.2 Feedback

On the "Annex" sheet manufacturers can also give general feedback and practical suggestions about the checklist layout (not-compulsory).

III. Technical Evaluation (Phase I)

During the technical evaluation, the SCIENSANO National Reference Laboratory (NRL) involved will check the submitted diagnostic reagents from the manufacturer/supplier against the **Technical Minimum Criteria** as defined and communicated in the minimum criteria document.

These technical minimum criteria will be different for each Certification. First, the '**fitness for purpose'** of the reagent/kit within an official disease control program will be defined by the Steering Group and the Expert Committee. The primary minimal technical criteria can then be determined in function of the defined purpose, by choosing the appropriate criteria from list **(A).** Diagnostic reagents/kits will be checked against these 'A'-criteria at the SCIENSANO RL.

Secondary criteria such as feasibility criteria in list **(B)** and operational criteria in list **(C)** may be taken into account by the end-users for the final choice of reagent/kit, and can be assessed with questionnaires **(Appendices B-D**), but are not part of the official technical evaluation within the certification procedure at SCIENSANO (phase 1).

A. Primary 'Fitness for Purpose' Criteria:
- Analytical sensitivity (detectability)
- Analytical specificity (cross reactivity and nonspecific reactions)
- Maximum precision = repeatability: CV with 95% confidence limits (CI)
- Intermediate precision = intra-laboratory reproducibility: CV with 95% confidence limits (CI)
- Proficiency test: reproducibility: CV with 95% confidence limits (CI)
- Threshold/Cut-off determination - methodology
- Diagnostic sensitivity (DSe) estimate with 95% confidence limits (CI) (nr. samples ~ NRL)
- Diagnostic specificity (DSp) estimate with 95% confidence limits (CI) (nr. samples ~ NRL)
- Agreement between tests: kappa statistic
- Quality control and quality assurance (ISO 9001 production)

B. Secondary Acceptability/Feasibility Criteria:
- Reagent/Kit acceptability by scientific and regulatory communities
- Acceptability to the client
- Feasibility given available laboratory resources (Hereto, a questionnaire for the technicians of SCIENSANO exists in 3 languages; this questionnaire can be adapted from kit to kit)

C. Secondary Operational Criteria
- Running costs / costs per test
- Equipment availability
- Level of technical sophistication and interpretation skills
- Kit/reagent availability
- Shelf life
- Production time and delivery capacity
- Transport requirements - delivery time (in time of crisis).
- Safety, and Biosecurity
- Sample throughput/automatization
- Test turn-around times

IV. Appendices

A. OIE Stages of Validation

STAGE 1 VALIDATION

1. Optimization and standardization of reagents
    a) Linear operating range of the assay
    b) Calibration against reference reagents
        i) *International standards*
        ii) *In-house standards*
2. Repeatability
3. Determination of analytical specificity and sensitivity

Deliverables:
- Analytical sensitivity
- Analytical specificity
- Repeatability intra-assay (different runs within a short time delay = 1 day = 1 technician)
- Repeatability inter-assay (different runs with a same batch = different days (at least 10, = different technicians)
- Repeatability inter-lot (different runs with at least 3 different batches)

STAGE 2 VALIDATION

1. Determining assay performance characteristics after establishment of a standard assay method and reagent criteria: **by use of reference animal populations**
    a) Infected or exposed and uninfected or non-exposed reference animals
    b) Reference animal status determined by other assays
    c) Experimentally infected or vaccinated reference animals
    d) Reference animals – Status unknown
2. Threshold determination (detection limit)
3. Assay performance estimates
    a) Number of reference animals required
    b) DSe and DSp estimates based on reference animals with defined infection status
    c) DSe and DSp estimates based on animals with infection status not defined
4. Comparison and harmonization of assays

Deliverables:
- Threshold/Cut-off determination
- Diagnostic sensitivity (DSe) estimate with 95% confidence limits (CI)
- Diagnostic specificity (DSp) estimate with 95% confidence limits (CI)
- Agreement between tests: kappa statistic (golden standard)

STAGE 3 VALIDATION

Establishing reproducibility and augmenting repeatability estimates of the assay, through conduction of (international) ring trials and proficiency tests (PTs)

Deliverables:
- Reproducibility
- Results of (international) proficiency tests (PTs)

STAGE 4 VALIDATION

1. Program implementation
2. Monitoring validity of assay performance
    a) Interpretation of test results - factors affecting assay validity
    b) Maintenance of validation criteria
    c) Enhancement and extension of validation criteria

Continuous validation through ongoing PT's and training programs.

B. Easy-to-Use Questionnaire English (for SCIENSANO laboratory technicians)

FILL IN FORM
GUIDELINES FOR TECHNICIANS TO EVALUATE THE CRITERIUM 'EASY TO USE' FOR ELISA ESSAY'S:

NAME OF THE KIT: …….

NAME OF THE PRODUCER: …….

	QUESTION ?	YES*	NO*
1	Are ELISA kit instructions (instruction leaflet) available in different languages (French, English, Dutch,…)?		
2	Are kit instructions (instruction leaflet) easy to understand by an experienced technician?		
3	Is the ELISA kit available in strip format?		
4	The Positive and Negative control of the kit are ready to use (= no need for predilution or dilution step)?		
5	The conjugate reagent of the kit is ready-to-use?		
6	The substrate reagent of the kit is ready-to-use?		
7	The stop-solution (if there is one) of the kit is ready to use?		
8	Is there need for a (pre)dilution step for the samples?		
9	Is the (pre)dilution step well and detailed described (= specifically describing how to prepare the final dilution (= x µl of sample in x µl of dilution buffer) in the ELISA plate) in the instruction leaflet?		
10	If there is a (pre) dilution step for the samples, is it the same (pre)dilution step as for the positive and negative ELISA controls?		
11	The final volumes to add at each step of the ELISA (samples, conjugate, substrate, and stop-solution) are the same?		
12	The conjugate solution is coloured?		
13	The substrate solution is coloured?		
14	The temperature for incubation at each step of the ELISA (samples, conjugate, substrate, and stop-solution) is the same?		
15	If incubation should be done at room temperature, is this room temperature detailed described (= x°C) in the instruction leaflet?		
16	Is there need for additional reading at a second wavelength (correcting for background)?		
17	Is the time when the ELISA plate should be read, after adding the stop solution, detailed described (=x min) in the instruction leaflet?		
18	Is it possible to complete the ELISA (begin to end) within 4 hours?		
19	Is it possible to automatize the ELISA (robot)?		
20	The calculations for validation of the ELISA and the results are easy to understand and to execute?		

* If YES/NO are not significant to use put NR (not relevant).

C. Easy-to-Use Questionnaire français (for SCIENSANO laboratory technicians)

SOUMISSIONS
GUIDE A L'ATTENTION DES TECHNICIENS POUR EVALUER LE CRITERE 'FACILITE D'EMPLOI DE LA TROUSSE ELISA

NOM DE LA TROUSSE : …….

NOM DU PRODUCTEUR : …….

	QUESTION ?	OUI*	NON*
1	Les instructions de la notice de la trousse sont-elles disponibles en plusieurs langues (Français, Anglais, Flamand,…) ?		
2	Les instructions de la notice sont-elles faciles à comprendre ?		
3	La trousse est-elle disponible sous forme de 'strips' (barrettes) ?		
4	Les témoins Positif et Négatif de la trousse sont-ils faciles à utiliser (= pré-dilution ou dilution non nécessaire) ?		
5	Le réactif 'Conjugué' est-il prêt à l'emploi ?		
6	Le réactif 'Substrat' est-il prêt à l'emploi ?		
7	La solution d'arrêt (s'il y en a une) est-elle prête à l'emploi ?		
8	Une étape de pré-dilution des échantillons est-elle nécessaire ?		
9	L'éventuelle étape de pré-dilution des échantillons est-elle clairement et suffisamment décrite dans la notice d'instruction (en particulier la manière d'obtenir la dilution finale sur la plaque Elisa) ?		
10	S'il y a une étape de pré-dilution des échantillons, est-elle la même que celle pour les témoins de la trousse ?		
11	Les volumes finaux à ajouter à chaque étape de réalisation du test sont-ils les mêmes ? (échantillon, conjugué, substrat, solution d'arrêt)		
12	Le réactif 'Conjugué' est-il coloré ?		
13	Le réactif 'Substrat' est-il coloré ?		
14	La température d'incubation est-elle la même pour chaque phase de réalisation du test ELISA ?		
15	Si l'incubation doit être réalisé a la température ambiante d'incubation est-elle clairement et suffisamment décrite dans la notice d'instruction (°C)?		
16	Le test ELISA nécessite-t-il une lecture à double longueur d'onde (pour éviter les bruits de fond) ?		
17	La durée pour la lecture l'ELISA, âpres l'ajout de la solution d'arrêt, est-elle clairement et suffisamment décrite dans la notice d'instruction (x ± y min)?		
18	L'ELISA (Début à la fin) est-il réalisable en 4 heures ?		
19	Les temps d'incubation sont-ils compatibles avec une planification aisée de réalisation des tests (possibilité de réaliser des tests avec des robots)?		
20	Les calculs des critères de validation du test et des résultats (pos-neg) sont-ils simples à effectuer et à interpréter ?		

Si QUI ou NON n'est pas possible écrit NA (non applicable)

D. Easy-to-Use Questionnaire Nederlands (for SCIENSANO laboratory technicians)

INVULFORMULIER
RICHTLIJNEN VOOR TECHNICI OM KRITERIUM 'GEBRUIKSVRIENDELIJKHEID' TE EVALUEREN VOOR COMMERCIELE ELISA KITS

NAAM VAN DE KIT: …….

NAAM VAN DE PRODUCENT: …….

	VRAAG ?	JA*	NEE*
1	Zijn de ELISA kit instructies (bijsluiter kit) geschreven in verschillende talen (Frans, Engels, Nederlands, …)?		
2	Zijn de ELISA kit instructies (bijsluiter kit) éénvoudig te begrijpen door een ervaren technicus?		
3	Is de ELISA ook in "stripformaat" beschikbaar?		
4	Zijn de positieve en negatieve kitcontroles gebruiksklaar (=ready to use)?		
5	Is het conjugaat van de kit gebruiksklaar?		
6	Is het substraat van de kit gebruiksklaar?		
7	Is de stopoplossing (indien er één aanwezig is) van de kit gebruiksklaar?		
8	Is er een (voor)verdunningstap nodig voor de stalen?		
9	Is deze (voor)verdunningstap voor de stalen gedetailleerd beschreven in de bijsluiter van de kit (gespecificeerd hoe de verdunning precies (x µl staal in y µl verdunningsvloeistof) dient gemaakt te worden om de finale verdunning te bereiken)?		
10	Indien er een (voor)verdunningstap voor de stalen is, is deze dezelfde als de (voor)verdunningstap van de positieve en negatieve controles van de kit?		
11	Zijn de te gebruiken (finale) volumes dezelfde bij elke stap van de ELISA (stalen, conjugaat, substraat en stopoplossing)?		
12	Is het (de) conjugaat(oplossing) gekleurd?		
13	Is het (de) substraat(oplossing) gekleurd?		
14	Is de incubatietemperatuur dezelfde bij elke stap van de ELISA (stalen, conjugaat, substraat en stopoplossing)?		
15	Indien de incubatietemperatuur bij kamertemperatuur dient te gebeuren, is deze kamertemperatuur specifiek beschreven (x °C) in de bijsluiter van de kit?		
16	Moet op het einde van de ELISA op een dubbele golflengte (nm) worden afgelezen (controle achtergrondkleuring)?		
17	Dient het aflezen van het resultaat, na toevoegen van stopoplossing, te gebeuren na een gedetailleerde beschreven tijdsperiode (x + ± min) in de kitinstructie?		
18	Is de ELISA (begin tot einde) uitvoerbaar binnen een tijdsperiode van 4 uur?		
19	Is er mogelijkheid tot automatisatie voor het uitvoeren van de ELISA (robot)?		
20	Zijn de berekeningen voor de validatie van de ELISA en de resultaten éénvoudig uit te voeren en te begrijpen?		

*Indien JA /NEE niet van toepassing zijn schrijf NVT.

E. Quality Evaluation of Diagnostic Reagents/Test Kits: Definitions for Checklist

(Dohoo, 2009; Jacobson, 1996)

E.1. Early Validation and Standardization Requirements

1. Feasibility studies
2. Control samples
3. Standardization
4. Checkerboard titrations

E.2. Definitions for Test Performance Parameters and related terms

1. Analytical sensitivity = Detectability = Limit of detection = Detectielimiet/ Detecteerbaarheid = Limite de detection/ Détectabilité

- *Analytical sensitivity = Limit of detection:* lowest concentration of analyte the test can detect or should detect, but in numbers that cannot be estimated accurately.
- *Detectielimiet:* de laagste concentratie van een analyt dat kan worden gedetecteerd door een test, maar in aantallen die niet nauwkeurig kunnen worden geschat.
- *Limite de détection*: la plus petite concentration d'analyte qui peut être détectée, mais en nombres qui ne peuvent être estimés exactement.

2. Analytical specificity = cross reactivity and nonspecific reactions

- *Analytical specificity*: capacity of test to react to only one analyte, measure of cross-reactivity and nonspecific reactions
- *Cross reactivity*: check all relevant species and/or strains, subtypes other than the one in consideration.
- *Nonspecific reactions*: false positive reaction not due to immunity against or infection with a pathogen or other relevant species and/or strains, subtypes other than the one in consideration.

3. Diagnostic Sensitivity = Gevoeligheid = Sensibilité

- *Diagnostic Sensitivity:* ability of the test to correctly detect animals known to be infected (or in certain cases: vaccinated), i.e. the proportion of infected animals that test positive.
- *Gevoeligheid:* het deel van het totale aantal positieve monsters (geïnfecteerde of in bepaalde gevallen gevaccineerde dieren) die volgens de gebruikte methode correct wordt ingedeeld.
- *Sensibilité:* la fraction du nombre total d'échantillons positifs (animaux infectés ou dans certains cas vaccinés) correctement classée dans la méthode utilisée.

4. Diagnostic Specificity = Specificiteit = Spécificité

- *Diagnostic Specificity:* ability of the test to detect non-infected and non-vaccinated animals correctly, i.e. the proportion of non-infected animals that test negative.
- *Specificiteit:* het deel van het totale aantal negatieve monsters (niet geïnfecteerd en niet gevaccineerd) die correct volgens de gebruikte methode wordt ingedeeld.
- *Spécificité:* la fraction du nombre total d'échantillons négatifs (pas infecté et pas vaccine) correctement classée dans la méthode utilisée.

5. Accuracy = Juistheid = Justesse

- *Accuracy:* ability of the test to give a true measure of the analyte, average of results from repeat tests should give values close to the true value – measure of inaccuracy.

- *Juistheid:* de mate van overeenstemming tussen de gemiddelde waarde die is verkregen uit een lange reeks testresultaten en een aanvaarde referentiewaarde. De juistheid wordt doorgaans uitgedrukt als bias.
- *Justesse:* étroitesse de l'accord entre la valeur moyenne obtenue à partir d'une large série de résultats d'essai et une valeur de référence acceptée. La justesse est généralement exprimée en termes de biais.

4. Precision = Precisie = Fidélité

- *Precision:* consistency of the test results (regardless of correct or not), variability between test results of the same sample, tested several times under specified conditions. Precision consists of repeatability (maximum value of precision) and the intra-laboratory reproducibility (intermediate value of precision).
- *Precisie:* is de mate van spreiding in meetresultaten die verkregen wordt door de methode meerdere malen onder vastgelegde condities op hetzelfde monster uit te voeren. De precisie omvat de bepaling van de herhaalbaarheid (maximum waarde van precisie) en de intra-laboratorium reproduceerbaarheid (intermediaire waarde van precisie).
- *Fidélité:* est le calcul de la dispersion des résultats de mesure obtenus en effectuant une méthode à plusieurs reprises dans des conditions déterminées sur un même échantillon. Soit la détermination de la répétabilité (valeur de fidélité maximale) et de la reproductibilité intra-laboratoire (valeur de fidélité intermédiaire).

5. Repeatability = Maximum Precision = Répétabilité = Précision Maximale = Herhaalbaarheid = Maximale Precisie

- *Repeatability = Maximum value of precision:* the precision obtained by running the same samples in replicate, in 1 assay, in 1 batch, in 1 laboratory, on 1 day, by 1 person. Variation of factors is minimal or non-existent, value of precision is maximal.
- *Répétabilité = valeur de fidélité maximale:* est la fidélité obtenue par la même méthode avec le même échantillon, le même analyste, sur le même appareil de mesure et à des moments aussi rapprochés que possible. Variation de facteurs est minimale ou inexistante, valeur de fidélité est maximale.
- *Herhaalbaarheid = maximale precisie:* precisie verkregen met dezelfde methode, hetzelfde monster, dezelfde analist, dezelfde meetapparatuur, op zo dicht mogelijk bij elkaar gelegen tijdstippen. Variatie van factoren is minimaal of onbestaande, waarde van precisie is maximaal.

6. Intra-laboratory reproducibility = Intermediate Precision = Réproductibilité Intra-laboratoire = Fidélité Intermédiaire = Intra-laboratorium Reproduceer-baarheid = Intermediaire Precisie

- *Intra-laboratory reproducibility = Intermediate value of precision:* the precision obtained by running the same samples in 1 laboratory under different settings:

different lots or/and different persons or/and different days. Variation of factors is maximal, value of precision is intermediate.
- *Reproductibilité Intra-laboratoire = Valeur de fidélité intermédiaire:* est la fidélité obtenue par une même méthode sur un même échantillon qui est effectuée par différents analystes et/ou à différents moments et/ou utilisant des lots différents. Variabilité de facteurs est maximale, valeur de fidélité est intermédiaire.
- *Intra-laboratorium Reproduceerbaarheid = Intermediaire waarde van precisie:* de precisie verkregen met dezelfde methode, op hetzelfde monster en uitgevoerd door verschillende analisten en/of op verschillende tijdstippen en/of met verschillende loten. Variatie van factoren is maximaal, waarde van precisie is intermediair.

7. Proficiency Test = Essai d'aptitude

- *Proficiency Test:* test results obtained by running the same samples in assays in different labs
- *Essai d'aptitude:* essai réalisé en parallèle, sur des matériaux identiques ou semblables, par deux ou plusieurs laboratoires dans des conditions prédéterminées.
- *Proficiency test :* beproeving uitgevoerd in parallel, op dezelfde of soortgelijke materialen, door twee of meer laboratoria onder vooraf bepaalde voorwaarden.

8. Relative trueness = Relatieve juistheid = Accord Relatif

- *Relative Trueness:* the degree of correspondence of the results of the method under evaluation to those obtained using a recognized reference test/method.
- *Relatieve juistheid:* de graad van overeenkomst tussen de resultaten van een methode onder evaluatie en de resultaten die verkregen worden door gebruik te maken van een erkende referentietest/-methode.
- *Accord relatif:* degré de correspondance entre les résultats de la méthode à évaluer et ceux obtenus par un test/une méthode de référence connu(e).

9. Reliability

- *Reliability:* ability of test to distinguish between individuals, variability of a test result related to the variability between individuals.

10. Cut-off / Threshold

- *Cut-off = Threshold:* Interpreting test results on a continuous scale, by selecting a cut point.

11. Predictive values (pos./neg.)

- *Predictive values:* probability the animal is/not diseased, based on a positive/negative test result.

12. Likelihood Ratio

- *Likelihood Ratio:* ratio of probability pos./neg. result among diseased / probability of pos./neg. among non-diseased.

13. Reference Test = Standard of Comparison = Referentiemethode/-test = Méthode/ test de référence

- *Reference Test = Standard of Comparison:* relative standard test / method, to compare tests and to estimate Se/Sp of a test under evaluation, and to characterize reference material.
- *Referentiemethode/-test:* grondig bestudeerde methode / test waarvan de nauwkeurigheid en de precisie duidelijk en precies werden bewezen en die dus gebruikt kan worden om de nauwkeurigheid van andere methoden voor dezelfde meting te evalueren, in het bijzonder door de karakterisering van referentiemateriaal toe te laten. Normaal gaat het om een nationale of internationale standaardmethode.
- *Méthode/test de référence:* méthode / test dûment étudiée dont l'exactitude et la précision ont été clairement et exactement démontrées et qui peut donc être employée pour évaluer l'exactitude d'autres méthodes pour la même mesure, en particulier en permettant la caractérisation de matériel de référence. Normalement il s'agit d'une méthode standard nationale ou internationale.

14. Robustness = Robustesse = Robuustheid

- *Robustness:* sensitivity of test to variation in working conditions, under normal and extreme use conditions, and for each different protocol.
- *Robustesse:* sensibilité d'une méthode d'analyse aux variations des conditions d'expérience qui peut s'exprimer par une liste des échantillons, des analytes, des conditions d'entreposage, des conditions d'environnement et/ou de préparation de l'échantillon pour lesquels la méthode peut être appliquée telle quelle ou moyennant certaines modifications mineures. Pour toutes les conditions d'expérience qui, dans la pratique, sont sujettes à des variations (par exemple: stabilité des réactifs, composition de l'échantillon, pH, température), on indiquera toutes les variations pouvant influencer le résultat de l'analyse.
- *Robuustheid:* de gevoeligheid van een analysemethode voor veranderingen in de proefomstandigheden, die kan worden uitgedrukt als een lijst van de monstermaterialen, analyten, opslagcondities, omgevings- en/of monster-voorbereidingscondities waarmee de methode zoals beschreven of met gespecificeerde kleine wijzigingen kan worden toegepast. Voor alle proefomstandigheden die in de praktijk aan fluctuaties onderhevig zouden kunnen zijn (bijvoorbeeld stabiliteit van de reagentia, samenstelling van het monster, pH,

temperatuur) dienen alle afwijkingen die invloed zouden kunnen hebben op het resultaat van de analyse te worden aangegeven.

15. Uncertainty of Measurement = Meetonzekerheid = Incertitude de mesure

- *Uncertainty of measurement:* is a parameter, associated with the result of a measurement, that characterizes the
dispersion of the values that could reasonably be attributed to the measurand.
- *Meetonzekerheid:* parameter, verbonden aan het resultaat van een meting, die de spreiding aanduidt van de waarden die redelijkerwijs toegekend kunnen worden aan de measurand.
- *Incertitude de mesure*: paramètre, associé au résultat d'une mesure, qui caractérise la dispersion des valeurs qui peuvent raisonnablement être attribuée au mesurande

16. Validation = Validation = Validatie

- *Validation:* the process of establishing evidence that provides a high degree of assurance that a testkit accomplishes its intended requirements. This involves acceptance of fitness for purpose with end users and other product stakeholders.
- *Validation:* est la confirmation par examen et l'apport de preuves objectives du fait que les prescriptions particulières en vue d'une utilisation prévue déterminée sont remplies. La validation doit être aussi étendue que l'impose la réponse aux besoins pour l'application ou le domaine d'application donné. - *Validatie:* bevestiging door onderzoek en levering van objectief bewijs dat aan bepaalde eisen voor een specifiek beoogd gebruik wordt voldaan. De validatie moet zo uitgebreid zijn als nodig is om te voldoen aan de eisen van de aangegeven toepassing of het aangegeven toepassingsgebied.

17. Reference Material (reference sample + standard sample) = Referentiemateriaal (referentiestaal + standaardstaal) = Matériel de Référence (échantillon de reference + échantillon standard) (SCIENSANO definition)

- *Reference Material* = reference sample + standard sample.
- *Matériel de Référence* = échantillon de référence + échantillon standard.
- *Referentiemateriaal* = referentiestaal + standaardstaal.

- *Reference Sample:* a sample with known accurate characteristics and which has been recognised (inter)nationally.
- *Referentiestaal:* staal die bekende nauwkeurige kenmerken heeft en die nationaal of internationaal erkend is.
- *Echantillon de Référence:* échantillon ayant des caractéristiques connues avec précision et étant reconnu au niveau national ou international.

- *Standard Sample:* a sample with known status and which has been tested at least 10 times by the reference laboratory
- *Standaardstaal:* staal met gekende status en minstens 10 keer getest door het referentielaboratorium.
- *Echantillon Standard:* échantillon à statut connu et testé au moins 10 fois par le laboratoire de référence.

18. Test agreement

- *Test agreement:* how well 2 tests agree for same sample, or how well 2 raters (people) agree for the same test result (kappa statistics).

E.3. Requirements to obtain Test Performance Parameters

1. **Analytical sensitivity**

   Test dilution series of known samples (reference sera) in end-point dilution analysis. Does the kit detect international standard serum as described by OIE (if available)?

2. **Analytical specificity**

   Test nearest relevant neighbors, similar pathogens, cross-reactions, use known samples from animals infected by related pathogens

3. **Accuracy**

- Need a 'measure of inaccuracy' to correct test results
- Running the test on reference samples with a known quantity of the analyte.
- Field samples previously tested by a reference method or golden standard
- Spiked samples (with known quantity of analyte)
- Correct calibration equipment
- Following SOPs
- Always including control/reference samples in each test run
- Normalized results: z-score from PT, kappa-statistic

4. **Precision**

- Performing repeated tests on known samples and using averages of test results
- Correct calibration equipment
- Following SOPs
- normalized results: repeatability and reproducibility

5. **Precision and Agreement for quantitative tests**

- Coefficient of variation: CV = σ/μ , based on repeat runs of same test, averaged or for each sample separately
- (Pearson correlation coefficient: PCC: to compare continuous results of 2 sets of test results)
- Concordance correlation coefficient: CCC: to compare continuous results of 2 sets of test results (CCC= accuracy parameter * PCC)
- Limits of agreement plots: Bland-Altman plots
- Intra-class correlation coefficient: ICC (= Var indiv / var indiv + measurement error)

6. Precision and agreements for qualitative tests

- Categorical results (dichotomous or multiple classes)
- Cohen's kappa-statistic (2x2 table)
- McNemar's X2 test for paired data to compare proportions of positives

7. Diagnostic sensitivity and diagnostic specificity

- 2x2 table calculations
- Use a panel of known positive and negative samples, from reference animals with known history and infection status
- Source animals as close to target population as possible
- Usually 300-1000 minimum sample size
- Give 95% confidence intervals

8. Cut-off / Threshold

- Frequency distributions of diseased and non-diseased animals (μ +/- 2* or 3*σ)
- ROC analysis: construct ROC curve: Se vs. 1-Sp and select max. (Se+Sp) as cut-off
- Construct Se-Sp plot
- use only uninfected animals
- Intrinsic cut-off from sera drawn randomly from target population, no prior knowledge of infection status
- 2 cut-offs: grey zone/borderline – confirmation test

9. Predictive values

- Positive and negative PV: need estimate of prevalence in target population

10. Reference / Standard of comparison / Gold Standard

- Other ELISA, PCR, isolation with known Se / Sp
- Results from experimentally infected / vaccinated animals

11. Repeatability

- Use of normalized results from many runs of replicate samples (within plate)
- 10-20 runs minimum

12. Proficiency tests

- Several laboratories using the same test (protocol, reagents, controls)
- Testing 10-20 samples, which represent the full range of expected concentrations/titres

E.4. Table of Test Parameters

Test Parameter	Value	95% CI
Analytical sensitivity		
Analytical specificity		
Accuracy		
Precision		
Repeatability = maximum precision		
Intermediate precision = Reproducibility		
Interlab reproducibility = minimum precision		
Reliability		
Cut-off / Threshold		
Reference / Standard of comparison / Gold Standard sensitivity		
Reference / Standard of comparison / Gold Standard specificity		
Diagnostic sensitivity		
Diagnostic specificity		
Positive predictive value		
Negative predictive value		