Basic design of the PT schemes

1. General

A proficiency test is an inter-laboratory study to determine the performance of individual laboratories for specific tests and to monitor laboratory performance. Taking part in a proficiency testing scheme is a valuable part of a laboratory's quality control management.

Royal GD offers laboratories the possibility to participate in international proficiency testing schemes for antibody detection, molecular biology or bacteriology.

The list of PT's that are organized and the time schedule can be found on our website: www.gdanimalhealth.com/pts

1.1 Goal of the PTS

The goal of the PTS is to assess the performance of individual laboratories for specific tests, without giving a value judgement on the individual participant's performance. The report is focused on giving participants an insight in how their results compare to the results of the other laboratories.

1.2 Personnel involved in the design and operation of the PT scheme

The PTS team consists of:
Product specialist (the coordinator of the PTS)
Quality advisor
R&D scientists
Statistician
Technicians
Support staff

1.3 In-house activities and outsourcing

Tests for sample selection and quality control of PT sample material is performed by the GD laboratory, situated in the main building (Arnsbergstraat 7, 7418 EZ Deventer, The Netherlands).

For the distribution of the PTS samples, GD uses priority mail and express courier.
Standard priority mail will be sent via PostNL. PostNL's head office is located at: Waldorpstraat 3, 2521 CA Den Haag, The Netherlands
For shipment with express courier, GD uses FedEx. FedEx's head office is located at: 942 South Shady Grove Road in Memphis, USA

Some (equipment) maintenance contracts are outsourced.

2. Criteria and characteristics of PT schemes

2.1 Participants

Participants to the PTS may be: laboratories, research institutes, test kit manufacturers, et cetera.
Participation is possible from all over the world. In some countries we collaborate with distributors. The list of distributors are shown on the website of Royal GD: Our distributors

2.2 Characteristics of the PT's

Table 1 shows the characteristics and number of expected participants of the PT's which are organized yearly. Participation is possible for one or more test method(s). If more than one test methods are used, it is recommended to apply for more PT sets due to the limited sample volume.

Table 1: Characteristics per PT and expected numbers of participants:

Code	Name	Sample material	Sample volume	Test method	Expected numbers of participants*
VLDIA171	PTS Salmonella porcine antibody detection	lyophilized serum	150 µl	ELISA	20-25
VLDIA172	PTS IBDV antibody detection (Gumboro)	lyophilized serum	150 µl	ELISA, AGP or VNT	55-60
VLDIA219	PTS NDV antibody detection	lyophilized serum	150 µl	ELISA or HI	90-100
VLDIA225	PTS IBV antibody detection	lyophilized serum	150 µl	ELISA, AGP or HI	60-65
VLDIA226	PTS Mycoplasma (Mg/Ms) antibody detection	lyophilized serum	250 µl	ELISA , HI or RPA	85-95
VLDIA232	PTS AIV antibody detection	lyophilized serum	250 µl	ELISA, AGP, or HI (H5, H7 and H9)	85-95
VLDIA233	PTS Salmonella poultry antibody detection	lyophilized serum	150 µl	ELISA or RPA	45-50
VLDIA234	PTS PRRSV antibody detection	lyophilized serum	200 µl	ELISA or IPMA	85-95
VLDIA235	PTS SRLVs (MVV/CAEV) antibody detection	lyophilized serum	300 µl	ELISA or AGP	30-35
VLDIA255	PTS aMPV antibody detection (TRT)	lyophilized serum	150 µl	ELISA or VNT	25-30
VLDIA277	PTS ARV antibody detection (REO)	lyophilized serum	150 µl	ELISA or AGP	35-40
VLDIA285	PTS PCV2 virus detection	lyophilized serum, medium	500 µl	PCR	30-35
VLDIA286	PTS BVD virus and antigen detection	lyophilized serum, tissue, EDTA / ear notch	1000 µl	antigen ELISA or PCR	50-55
VLDIA290	PTS PRRS virus detection	lyophilized serum	1000 µl	PCR	75-80
VLDIA292	PTS ILT antibody detection	lyophilized serum	150 µl	ELISA, AGP or VNT	35-40
VLDIA294	PTS APP antibody detection	lyophilized serum	150 µl	ELISA or CFT	15-20
VLDIA295	PTS SIV antibody detection	lyophilized serum	150 µl	ELISA or HI	25-30
VLDIA296	PTS IBV virus detection	lyophilized kidney suspension and medium	500 µl	PCR	25-30
VLDIA303	PTS Mycoplasma (Mg/Ms) bacteria detection	lyophilized medium	200 µl	PCR	50-55
VLDIA313	PTS EDS antibody detection	lyophilized serum	150 µl	ELISA or HI	30-35
VLDIA314	PTS IBD virus detection (Gumboro)	lyophilized bursa suspension	1000 µl	PCR	20-25
VLDIA323	PTS Bacterial identification - cattle round 1	lyophilized culture	500 µl	Culture or PCR	50-55
VLDIA323	PTS Bacterial identification - poultry	lyophilized culture	500 µl	Culture or PCR	30-35
VLDIA323	PTS Bacterial identification - companion animals	lyophilized culture	500 µl	Culture or PCR	30-35
VLDIA323	PTS Bacterial identification - cattle round 2	lyophilized culture	500 µl	Culture or PCR	50-55
VLDIA323	PTS Bacterial identification - swine	lyophilized culture	500 µl	Culture or PCR	25-30
VLDIA324	PTS Antibiotic susceptibility of bacterial strains - cattle round 1	lyophilized culture	500 µl	Antibiotic susceptibility test	45-50
VLDIA324	PTS Antibiotic susceptibility of bacterial strains - poultry	lyophilized culture	500 µl	Antibiotic susceptibility test	25-30
VLDIA324	PTS Antibiotic susceptibility of bacterial strains - companion animals	lyophilized culture	500 µl	Antibiotic susceptibility test	20-25
VLDIA324	PTS Antibiotic susceptibility of bacterial strains - cattle round 2	lyophilized culture	500 µl	Antibiotic susceptibility test	40-45
VLDIA324	PTS Antibiotic susceptibility of bacterial strains - swine	lyophilized culture	500 µl	Antibiotic susceptibility test	15-20
VLDIA327	PTS AI virus detection	spiked FTA card	1 FTA card	PCR	55-60
VLDIA328	PTS ND virus detection	spiked FTA card	1 FTA card	PCR	20-25
VLDIA329	PTS CAV antibody detection	lyophilized serum	150 µl	ELISA	25-30
VLDIA333	PTS IBR antibody detection (serum)	lyophilized serum	1000 µl	ELISA	25-30
VLDIA334	PTS IBR antibody detection (milk)	lyophilized milk	1000 µl	ELISA	20-25
VLDIA336	PTS MAP antibody detection (serum)	lyophilized serum	150 µl	ELISA	40-45
VLDIA337	PTS MAP antibody detection (milk)	lyophilized milk	250 µl	ELISA	35-40
VLDIA340	PTS Mycoplasma Hyopneumoniae antibody detection	lyophilized serum	250 µl	ELISA	20-25
VLDIA344	PTS PCV2 antibody detection	lyophilized serum	200 µl	ELISA	10-15
VLDIA345	PTS Mycoplasma Hyopneumoniae bacteria detection	spiked FTA card	1 FTA card	PCR	15-20
VLDIA347	PTS Swine Influenza virus detection	spiked FTA card	1 FTA card	PCR	15-20

**The expected numbers of participants are based on the number of participants over the last 5 years.*

2.3 Characteristics of PT samples

PT samples are produced according to ISO17043 requirements. Before approval of the PT item, homogeneity and stability are analyzed, as described in more detail in chapter 4.1.

The selection of PT samples includes samples which are representative for samples found in the field. The values can vary from negative to weak positive and strong positive samples.

Both antigen and antibody samples may either originate from the field or may from animal experiments using vaccines or pathogen strains. Samples originating from the field may contain multiple strains.

For each sample, a qualitative assigned value is determined. This value is based on tests performed in the GD laboratory, which do not serve as a gold standard tests. Therefore, the assigned value is not a sample criterium. The assigned value of the sample will be shared in the final report.

PT samples for antibody and antigen detection are all inactivated.
The sample volume is sufficient to perform at least one test in duplicate.

3. Participation

3.1 Registration

Participants can register via our general application form (from GD's website) or via the personalized invitation, which is sent via email.
After submission of the application form, the participant will receive a confirmation email.
The contact person's email address in the application form will be used for all communication and documents related to the PT, such as emails with tracking numbers of the PTS samples, the online reporting tool and the final report and certificate.

When submitting the application form, the participant agrees to:
1. the Royal GD General Terms and Conditions for Diagnostics & PTS
2. mentioning of the participant's laboratory name in the final report of the PTS
In case of participation with more test methods, it is recommend to use multiple sample sets, which can be purchased using the application form.

3.2 Distribution of PT samples

Shipment

PT samples are shipped according to the time schedule. PT samples for antibody detection are shipped using priority mail (EU) or express courier service (outside EU). PT samples for molecular biology are shipped using an express courier service. PT samples for bacteriology are shipped using an express courier service under UN3373 biological substances category B. All samples are shipped at room temperature. After arrival, PT samples must be stored at 2-8°C.

Importation of PT samples

The participant is responsible for import documents which allow reception of the samples. The participant shall deliver the necessary documents for import to the PT team, upfront to the distribution of the PT samples.

Information sent with the samples

When the samples are sent, the participant will receive an email with link to the results form. The email will also contain a login code and a password. If the samples are sent with an express courier, the participant will receive a tracking number to track the shipment.

The invoice for participation will also be sent upon shipment of the samples.

Along with the samples, the participant will receive an information letter, including:

- Number of samples and storage conditions
- Instructions for reconstitution of the samples, if applicable
- Instructions for reporting test results, a link to the report form and user name and password
- Test methods which may be used
- Deadlines for the specific PT
- The items which will be included in the report
- Sample receipt form

3.3 Handling and Testing of the PT samples

The PT items consist of biological material and should be treated accordingly. Disposal shall be performed according to local regulations.

Storage

All samples have to be stored upon arrival at 2-8 °C until testing.

Samples for bacteriology must be tested within 3 days after reconstitution.
Samples for all other test methods must be tested within 5 days after reconstitution.

Reconstitution of lyophilized samples

Lyophilized samples, must be reconstituted by adding the required amount of sterile water (at room temperature).

Testing

The sample set consists of coded samples that have to be tested in duplicate (in different test runs) using a method that is shown in table 1. The samples should be tested under normal routine test conditions.

Samples for PCR testing, should be extracted first, using an extraction method. After RNA or DNA extraction, a PCR must be performed in duplicate using the same extraction eluate.

Ear notches and samples for bacteriology are not tested in duplicate.

3.4 Reporting test results

Each participant will receive an email containing a link to GD's digital reporting system, including a user name and password. The password is also the unique participation code and is only known by the participant and the PT team.

The following results should be reported:

- Date of reception of the samples
- Date of execution of the tests
- Name of test kit/method and lot number
- Cut off of the test
- Qualitative results
- Quantitative results

After submission of the test results, the participant will receive an email with a confirmation of the submitted results and a request to check these carefully and correct these if necessary. The participant is at all times responsible for correct submission of the test results.

3.5 Results per test method

- Results from indirect ELISAs should be reported as actual titres (if appropriate) or S/P (Sample-To-Positive) values (maximum 3 digits) if appropriate, and a qualitative test result using the cut off that is routinely used by the participating laboratory.

- Results of blocking ELISAs should be reported in % inhibition or S/N ratio and a qualitative test result using the cut off that is routinely used by the participating laboratory.

- Agar gel precipitation test (AGPT) results should be reported as qualitative results (negative/positive or suspect/inconclusive) only.

- Hemagglutination Inhibition test (HI) results should be reported as log2 titres and as qualitative results (negative/positive) in the columns provided. Please also report the amount (HAU) and strain of antigen.

- Rapid (or Serum) Plate Agglutination (SPA/RPA) test results should be reported as log2 titres and as qualitative results (negative/positive) in the columns provided. Please also report the manufacturer of the antigen.

- Virus neutralisation test (VNT) results should be reported as log2 titres and as qualitative results (negative/positive) in the columns provided. Please also report the strain that is used as antigen.

- Immunoperoxidase monolayer assay (IPMA) results should be reported as titres and as qualitative results (negative/positive) in the columns provided on the form. Please report the type of PRRSV strain used as antigen.

- Complement Fixation Test (CFT) results should be reported as titres and as qualitative results (negative/positive/anticomplementary) in the columns provided on the form. Please report the type of antigen used in the test.

- PCR can be reported as conventional or RT-PCR. Quantitative results should be reported as Ct -Value. Qualitative results should be reported as positive/negative. For some PT, the detected strain should be reported.

- Culture results should be reported by entering the used agar(s), the incubation conditions, and the used identification test(s). Furthermore, identification of the bacterial species should be reported, both the number of different species and the identification result(s).

- Antibiotic susceptibility test results should be reported by entering the used method for susceptibility testing (for example tablet, disk, MIC), the tested antimicrobial agents (including concentrations), the inhibition zone or MIC value, the broth,and the atmosphere. The final result should be reported as resistant, intermediate or susceptible.

4 Statistical analyses

4.1 Homogeneity and stability

The homogeneity and stability of sample sets used in a PTS are mostly based on quantitative results of GD tests.

Blocking ELISAs (S/N Ratio) are assessed qualitatively, when the quantitative results fail to meet the predefined criteria. The S/N value of the positive results are usually very low (S/N below 0.2) and variation at this level distant from the cut-off is not meaningful which makes the calculation of CV values illogical.

For the criteria of homogeneity and stability, a distinction is made between poultry antibody determinations with log2 titers and other test systems.

Homogeneity for poultry antibody determinations with log2 titers:
Before a specific sample is used in the PTS, the mean log2 titer is calculated from 20 test results. A sample is considered homogeneous if the titers of all individual measurements differ by a maximum of +/- 1 (log2) step from the mean titer of the 20 measurements.

Homogeneity for other tests systems:
For each sample the coefficient of variation (CV%) is calculated once before the specific sample is used in the PTS, from twenty measurements using the following formula: (stdev / mean value) * 100. The CV% is acceptable if lower than the maximum CV% of the test repeatability (CVr).

Note: If the coefficient of variation (CV%) of a sample does not meet the acceptance criteria and the assay is a blocking ELISA, the results are interpreted qualitatively. This requires that all 20 individual measurements have the same qualitative outcome (either positive or negative). If a sample have both positive and negative results, the data are nonetheless evaluated quantitatively.

Stability for poultry antibody determinations with log2 titers:
For each sample the mean log2 titer is calculated from in total six measurements before and during the participants'

test period (three different moments). A sample is considered stable during the test period if the titers of all individual measurements differ within a maximum of +/- 2 (log2) steps from the mean titer of the 6 measurements.

Stability for other test systems:
For each sample the coefficient of variation (CV%) is calculated from in total six measurements before and during the participant test period (three different moments), using the following formula: (stdev / mean value) * 100.
The CV% is acceptable if lower than the maximum CV% of the test reproducibility (CVR).

Note: If the coefficient of variation (CV%) of a sample does not meet the acceptance criteria and the assay is a blocking ELISA, the results are interpreted qualitatively. This requires that all six individual measurements have the same qualitative outcome (either positive or negative). If a sample have both positive and negative results, the data are nonetheless evaluated quantitatively.

Homogeneity and stability in comparison with the results of the participants:
The homogeneity and stability of the sample set are also assessed by comparing our results with those of the participants, either solely qualitatively or both quantitatively and qualitatively, according to the ISO 13528:2015 guidelines. In the decisive qualitative comparison, a difference of 20 percentage point (percentage of positive samples) between the homogeneity test samples, stability test samples and PTS participants' results is allowed.

For some PT, no comparison with the PT participants is possible, due to the diverse nature of the test and/or an insufficient number of participants with the same test system as the organiser. In this case, only a comparison between the homogeneity and stability test samples is performed, allowing 20 percentage point difference between the results of the homogeneity and stability samples.

4.2 The assigned value
The assigned value of the PT sample is based on the qualitative result of 20 tests, performed in the Royal GD laboratory.

The assigned value is reported as P, N, P/N or N/P

P = all tests were positive
N = all tests were negative
P/N = sample tested both positive and negative, mostly positive
N/P = sample tested both negative and positive, mostly negative

4.3 Other statistical analyses
The applied statistical calculations are based on the standard ISO 5725.
If there are at least 6 test results from a particular test system, the following statistical calculations are performed:
- Z-scores
- Trueness of the test results of a laboratory
- The within-laboratory reproducibility sd
- The between-laboratory reproducibility sd

Before the statistical calculations are done for a group of laboratories using a particular test system, the values of outliers are adjusted using robust analysis (algorithms A and S). Outliers are thus not excluded from the calculations of the mean results of a group of laboratories using a particular test system, but taken into account using the adjusted value.

If there are less than 6 test results from a particular test system, the between-laboratory reproducibility sd is not relevant and therefore only the within-laboratory reproducibility sd is calculated.

For more details on the statistical analyses please see document

5 Contents of the report
When the PT is finished, each participant receives a certificate of participation and a final report.

The final report contains:

- Introduction
- Description of the samples, including assigned value
- Homogeneity and stability of the samples
- List of participants (name of company/institute and country)
- Overview of the results
- Discussion and conclusions
- Annex with anonymized results

An example of a final report can be found here

6 Non-disclosure of information

The results of all participants are anonymized before analysis and are shown in the final report using the participation codes. The participation list is only visible for participants and organizer.

7 Handling of errors or problems

Recommendations or procedures for potential errors or problems can be found in the table below. In cases of other errors or problems, please contact the PT team.

Error or problem	Recommendation or procedure
Samples were not received in good order (e.g. too late or damaged).	Please contact the PT team.
Samples have not been reconstituted correctly or do not dissolve properly.	Please contact the PT team.
User name and password of the sample form have not been received.	Please contact the PT team.
Participant wishes to submit results of more than one test.	In the online results form, results of one test can be submitted. Please contact the PT team for specific instructions for submitting results of more than one test.
Results have not been submitted in time.	The submission deadline can be found in the PT time schedule. Before the deadline, the participant will receive a reminder to report the results. **Results cannot be submitted after the submission deadline**. In this case, the participant will receive the report with the results of other laboratories, so an inter-laboratory comparison is still possible.
Results were not submitted correctly.	Participants are responsible for correct submission of results. After submission, the participant receives a copy of the form, with the request to carefully check the submitted results. In case of false entries, the form can be submitted again. The latest submission will overwrite the previous submission.
Results were not submitted in duplicate.	Results will be analyzed and reported, but the within-lab reproducibility cannot be calculated.
Results were reported with incorrect test units (e.g. S/P instead of titre).	If it is clear which test unit was reported, in most cases, the PT team can calculate the correct test unit. If this is not possible, the test results will be shown in the table “others” and between-lab reproducibility will not be calculated.
The final report has not been received according to the PT time schedule.	Please contact the PT team.