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Diagnostics
Diagnostic

PM 7/122 (1) Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories*

Specific scope

This EPPO Standard specifies:

- the requirements for a laboratory to be able to organize interlaboratory comparisons for plant pest diagnostic laboratories
- the requirements for the development and operation of interlaboratory comparisons for plant pest diagnostic laboratories.

This EPPO Standard was developed taking into account elements included in ISO 17043 *Conformity assessment–General requirements for proficiency testing* (ISO, 2010) and generally follows the structure of this Standard, however, accreditation for the organization of interlaboratory comparison activities is not covered.

Specific approval

First approved in 2014-09.

1. Introduction

Interlaboratory comparisons, i.e. proficiency testing (PT) and test performance studies (TPS), the latter being also referred to as ring tests or collaborative trials, have become an essential aspect of laboratory practice in all areas of testing and their use is increasing internationally. Proficiency tests may be organized at the request of NPPOs, and/or producers associations. Participation in proficiency tests is often a requirement in the accreditation process. Test performance studies provide added value to the validation process.

Typical objectives for interlaboratory comparisons are presented below with a distinction made between those relevant for proficiency testing and test performance studies (Table 1).

Characteristics of interlaboratory comparison in diagnostics for quarantine pests

The number of laboratories performing diagnostics for quarantine pests is relatively low in EPPO member countries compared to other areas of testing (e.g. chemical analysis). The regional capacity to organize interlaboratory comparison is consequently limited.

In most interlaboratory comparisons the samples are simultaneously distributed to participants for concurrent testing. After completion of the testing, the results are returned to the organizer of the interlaboratory comparison to be evaluated.

Some interlaboratory comparisons are sequential and involve the sample (e.g. a mounted slide for an insect or fungus) being circulated successively from one participant to the next (i.e. sequential participation), and occasionally circulated back to the organizer for rechecking. This could be the case when it is not possible to prepare enough homogenous samples (e.g. limited number of specimens of insects).

All test results in laboratories performing tests for quarantine pests are given in qualitative terms (test positive or negative or undetermined). It is recognized that some tests will generate quantitative data (e.g. optical density for ELISA, number of cells for IF, Ct values for real-time PCR[1], measurements for morphological features, etc.). However, such

*Organization of interlaboratory comparisons by plant pest diagnostic laboratories is a new developing area, consequently the Standard will be revised in 2016 based on experience following its use in laboratories until this date.

[1]It should be noted that Ct, Cq or Cp, are equivalent.

Table 1 Objectives for interlaboratory comparisons

	PT	TPS
a) Evaluation of the accuracy of the results produced by laboratories for specific tests and monitoring laboratories' continuing performance	X	(X)*
b) Identification of problems in laboratories which should lead to the initiation of actions for improvement. For example, these may be related to inadequate test procedures, effectiveness of staff training and supervision, or verification of equipment	X	
c) Establishment of the comparability of tests	(X)	X†
d) Establishment of the effectiveness of a test	(X)	X
d) Provision of additional confidence to laboratory customers	X	(X)
e) Identification of interlaboratory differences	X	
f) Education of participating laboratories based on the outcomes of such comparisons	X	
g) Establishment of uncertainty levels		X
h) Evaluation of the performance characteristics of a test	(X)‡	X

X, main objective; (X), possible objective.
*It should be noted that in a test performance study, participant laboratories are assumed to be competent to undertake the test in question. However, participation in a test performance study may provide independent demonstration of laboratory competence, provided that the results obtained by the laboratory are accurate.
†When several tests are included in the TPS.
‡When several laboratories use the same test in a PT and have been shown to be proficient.

quantitative data is used to assign a qualitative value to the test result (positive/negative/undetermined).

The statistical procedures which may be applied to qualitative data are somewhat limited. This limitation is also noted in animal health testing (OIE, 2013).The choice of statistical analysis will in part be determined by the type of data generated by the test in question.

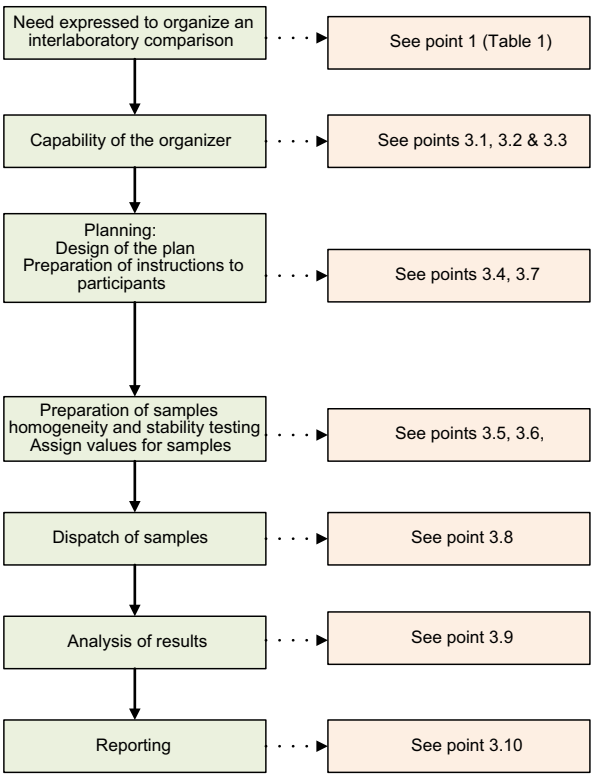

Fig. 1 Different steps to be followed when organizing an interlaboratory comparison.

Unlike, ISO 17043 *Conformity assessment – General requirements for proficiency testing* (ISO, 2010), confidentiality requirements are not included in a separate paragraph but underlined in 3.2, 3.4.1 and 3.10.

The flow diagram presented in Fig. 1 summarizes the different steps to be followed when organizing an interlaboratory comparison.

2. Definitions

Only definitions specifically relevant to this Standard are included. Other definitions are included in PM 7/76.[2]

Participant: laboratory, organization or individual that receives samples for interlaboratory comparison and submits results for review by the organizer of the interlaboratory comparison.

3. Technical Requirements

3.1 General

The feasibility (e.g. biological and logistical) of the interlaboratory comparisons should be evaluated by the organizer.

Appropriate technical competence is required for organizers of an interlaboratory comparison as described below:

- *Organizers of proficiency testing* should have appropriate technical competence on the organism and the test(s) performed or access to such competence. Outsourcing of such competence should be documented;
- These requirements are also valid for *organizers of test performance studies*, with the exception that the organizer should not outsource technical competence on the organism and the test(s) performed;

[2]Published on page 335–337 of this issue of the EPPO Bulletin.

- In both cases complementary competence may be required for statistical analysis; in such situations the organizer should have documented access to such competence.

3.2 Personnel

Members of the organizer's staff and, when relevant, sub-contractors, should be designated as responsible for specific tasks on the basis of their competence. A list of the main tasks for the organization of an interlaboratory comparison is given below (more details on these tasks are provided in subsequent sections of this Standard):

Coordination of the interlaboratory comparison:

- Plan the testing schemes (including the distribution of information);
- Supervise the technical operations and the statistical analysis;
- Analyse the results of the interlaboratory comparison and prepare a report;
- Assure confidentiality to the participants;
- Ensure communication with the participants;
- Authorize the issuance of the report;

Operating and technical operations:

- Select appropriate samples and when relevant equipment and reagents;
- Prepare, handle and distribute samples and when relevant equipment and reagents;
- Operate specific equipment (e.g. lyophilizer);
- Conduct measurements to determine stability and homogeneity, as well as assigned values and estimate the measurement uncertainty.

Statistical analysis:

- Select the appropriate statistical approach;
- Operate the data processing system;
- Conduct statistical analysis;
- Evaluate the performance of participants (PT), or of the tests (TPS).

As far as possible it should be avoided that one person is responsible for several tasks. If the organizer (and when relevant a sub-contractor) of the interlaboratory comparison is also a participating laboratory, the staff involved in the test preparation should not participate in the interlaboratory comparison unless appropriate precaution is taken (e.g. randomization and labeling of the samples are done by another person in the laboratory).

Requirements for competence and training of staff are included in PM 7/84 *Basic requirements for quality management in plant pest diagnosis laboratories* (EPPO, 2007).

3.3 Equipment and facilities

The organizer should ensure that conditions are appropriate for the preparation and operation of the interlaboratory comparison. This includes facilities and equipment:

- To prepare, test (homogeneity, stability see point 3.5.1), handle, store and dispatch samples and when relevant reagents;
- To process data, communicate, and retrieve materials (e.g. stability samples, slides) and records.

The organizer should ensure that cross-contamination between test samples is avoided:

- Environmental conditions in the laboratory and related to equipment that may compromise the operation of the interlaboratory comparison should be monitored (e.g. monitoring insects, spores,. . .);
- The work area should be appropriately decontaminated as required;
- Areas where incompatible activities take place are clearly separated, see PM 7/84 *Basic requirements for quality management in plant pest diagnosis laboratories* (EPPO, 2007) section accommodation of environmental conditions.

The organizer should ensure that the equipment required for the operation of the interlaboratory comparison is appropriately maintained, controlled (e.g. temperature) and calibrated (see PM 7/84). Particular care should be taken for equipment that is used to confirm homogeneity and stability, to define assigned values and to store samples/materials.

Where potentially hazardous samples, chemicals and materials are used, facilities should be available to ensure their safe handling, decontamination and disposal.

Necessary action should be taken to correctly handle quarantine pests (information on confinement conditions are provided in the Appendix of PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests,* EPPO (2006)). These conditions also apply to samples that are produced at sites away from the organizer's permanent facility (e.g. test fields for producing inoculated samples, soil).

3.4 Planning an interlaboratory comparison

The plan should describe the objectives and provide a detailed description of the interlaboratory comparison.

When developing such a plan the organizer should identify the critical processes which directly affect the quality of the interlaboratory comparison, and document them in the plan.

If necessary, specific requests from clients of the diagnostic laboratories can be considered when developing a plan (e.g. period or frequency of organization of proficiency tests, inclusion of samples at specific level of infestation, etc.).

When developing a plan the organizer can also take into account recommendations or requirements expressed by laboratories involved in plant pest diagnostics (e.g. minimum/maximum number of samples that the laboratory can accept; the period of the year the test(s) should be performed taking into account the seasonal activity of the laboratories, etc.). National regulations may also need to be taken into account.

3.4.1 Elements to be included in a plan for an interlaboratory comparison

(a) A description of the objective of the interlaboratory comparison in particular to clearly identify if the interlaboratory comparison is a proficiency test or a test performance study and to provide relevant details (pest, matrix, test, etc.);
(b) The name and address of the organizer;
(c) When relevant, the name, address and affiliation of the coordinator and other personnel involved in the design (e.g. those in charge of delivery of reference material, of not yet published primer sets);
(d) When relevant, the activities to be subcontracted and the names and addresses of subcontractors involved, if needed, e.g. for statistical analyses of the results. Requirements for proof of competency from the subcontractors should be defined and evidence of competency should be recorded;
(e) A description of the acceptance criteria to be met for participation, e.g. necessary authorizations, use of the methods, technical expertise for the pest/the methods/the equipment to be used, participation in training course. For a test performance study, it is critical that participants can provide evidence that they are competent to undertake the test in question;
(f) The number and type of expected participants in the interlaboratory comparison. The number of participants will depend on e.g. the number of laboratories currently performing the test proposed in the interlaboratory comparison, the availability of the matrix/pest to be tested;
(g) The name and address of the participants where the samples should be sent (e.g. the direct address of the laboratory that will perform the tests);
(h) A description of the information which is to be supplied to participants and the time schedule for the various phases of the interlaboratory comparison; e.g. what will be supplied, when the samples will arrive, how the samples have to be handled during the testing, measures that participants need to undertake after finishing the interlaboratory comparison (e.g. destruction of samples), etc.;
(i) A description of the extent to which participant results, and the conclusions that will be based on the outcome of the interlaboratory comparison, are to be made public;
(j) A description of the target(s) or characteristic(s) of interest, including information on what the participants are to identify, measure, or test for in the interlaboratory comparison (e.g. specific morphological features of the pest, counting of bacteria cells, assessment of severity of signs or symptoms caused by the pest, measurement of DNA, presence/absence of PCR products);
(k) A description of the range of values or characteristics, or both, to be expected from participants for the test samples, e.g. 100% correctness expected above the limit of detection (figure to be defined according to the test) or a defined level of detection;
(l) A list of potential major sources of errors involved in the preparation of interlaboratory comparison, if relevant (e.g. natural infested material with unknown level of infestation, incorrect storage of the sample, time delay in testing procedure, source of ingredients for agar media);
(m) A description of the requirements for the production, quality control, storage and distribution of samples, e.g. special attention is necessary if the tests are based on isolation of living organisms;
(n) A description of the procedure to be used for coding and labeling the samples and randomly assigning the numbered samples to the laboratories;
(o) An identification of requirements related to the delivery of samples to participants that may affect the interlaboratory comparison (e.g. stability, biosafety, specific regulations)
(p) A description of the procedure for coding the participants in order to keep their identity confidential and known only to persons involved in the operation of the interlaboratory comparison, unless the participant waives confidentiality;
(q) A description of the actions to be taken in the case of losing or damaging samples (e.g. during dispatch or in a laboratory);
(r) For continuous proficiency testing schemes[3], a description of the frequency or dates upon which test samples are to be distributed to participants, the deadlines for the return of results by participants and, where appropriate, the dates on which testing or measurement is to be carried out by participants;
(s) Information on methods or procedures which participants need to use to prepare the test material and perform the tests or measurements; e.g. which methods have to be used, which materials have to be provided by the participants themselves (sera, media, plants for bioassay, primer sets etc.);
(t) Description of the test or measurement methods to be used for the homogeneity and stability testing and, where applicable, to determine their biological viability; e.g. time scale for different tests, storage conditions for the materials during the duration of the tests, instructions for counting, measuring, isolation of the organisms and confirmation;
(u) Preparation of any standardized reporting formats to be used by participants, e.g. tables with the dates of arrival of the samples, conditions of the samples at arrival, conditions of storing the samples until testing, dates of starting the tests, dates of finishing the test (e.g. bioassays); sources of consumables (if relevant for the results);
(v) A description of the statistical analysis to be used;
(w) A description of information to be reported by the participants about how they performed the test (ease of use, equipment, deviations to the test under evaluation);

[3]ISO 17043 has defined a proficiency testing scheme as proficiency testing designed and operated in one or more rounds for a specified area of testing, measurement, calibration or inspection.

(x) A description of the procedure to be followed if the same sample is consistently not giving the expected result during the interlaboratory comparison (e.g. investigations to be initiated and actions to be taken, number of participants failing).

3.4.2 Elements only applicable for proficiency testing

When organizing a proficiency test, i.e. where participants can use a method routinely performed in their laboratory, it is strongly recommended that the organizer ensures that:

(a) The organizer has a procedure regarding comparison of results obtained by different tests;
(b) The organizer is aware of which different tests are technically equivalent, and
(c) The organizer will assess participants' results using these tests accordingly.

3.5 Preparation of samples and when relevant equipment and reagents

An important aspect of interlaboratory comparisons is that the sample matches (as closely as possible) the materials encountered in routine testing. This includes the matrix (e.g. host), target (e.g. pest) and concentration (e.g. infection level). For some pest/host combinations this may be a challenge (e.g. phytoplasmas).

The selection of samples is also linked to the statistical requirements needed to evaluate the different performance criteria (e.g. numbers of target and non-target samples, number of replicates, diversity of target and non-target samples, concentrations).

Finally, the selection of samples may also be influenced by knowledge of the pest (survival, detectability) and technical difficulties (availability of reference material).

Examples of types of samples are provided in Table 2.

Sufficient material needs to be prepared in order to allow for:

- The number of participants (as established at the planning phase see point 3.4)
- Performance of homogeneity and stability testing (as established at the planning phase see point 3.4)
- Replacement of materials that are lost or damaged during distribution/handling.

It may also be useful to prepare material to be used after the interlaboratory comparison e.g. as training aids, reference material or controls.

Acquisition, preparation, handling, distribution (see also 3.9) and disposal of quarantine organisms should be carried out in accordance with the relevant regulatory requirements (e.g. specific authorization to move regulated material). Useful information on confinement conditions are provided in the Appendix of PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests* (EPPO, 2006).

3.5.1 Homogeneity and stability

The organizer should take care that the samples prepared and used for the interlaboratory comparison are as homogenous and as stable as possible, because this can affect the evaluation of the participants or the test performance.

The assessment of homogeneity and stability should be performed by the same laboratory (generally the organizer) using the same analytical method and measuring the same characteristic of the samples. The test used for homogeneity and stability testing should be a standardized (or validated) test that can be implemented in the laboratory.

The procedures for the assessment of homogeneity and stability should be documented.

In some cases, it is not feasible for samples to be subjected to homogeneity and stability testing. Such cases would include, for example, when limited material is available to prepare samples.

Samples which are demonstrated to be not sufficiently homogeneous or stable respectively during the homogeneity and the stability testing, should not be used to evaluate the performance of the laboratory (for a PT) or of the performance of the test (for a TPS).

3.5.1.1 Homogeneity testing. The assessment of homogeneity should generally be performed after the samples have been packaged in the final form and before distribution to participants. Homogeneity can be demonstrated prior to packaging where no influence of packaging is reasonably expected.

3.5.1.1.1 Types of samples—In plant pest diagnostics the evaluation of homogeneity depends on the type of pest/matrix to be tested. Examples and exceptions are described below (for numbers of samples see 3.5.1.1.2).

- Slides and mounted specimens of fungal structures

Homogeneity can be ensured by documented verification that each diagnostic feature of the pest is present on each slide before dispatch.

Table 2 Example types of samples

	Virology	Bacteriology	Mycology	Nematology	Entomology	Botany	Phytoplasmology
Plant material	A	A	A	A	A	A	A
Substrate (e.g. soil and water)	A	A	A	A	A	A	NA
Cultures/specimens/vectors	Vectors	Cultures	Cultures	Specimens	Specimens	NA	Vectors
Slides	NA	A	A	A	A	A	NA
DNA/RNA	A	A	A	A	A	A	A

A, applicable; NA, not applicable.

• DNA/RNA

The process for the preparation of RNA/DNA solutions and aliquots thereof ensures homogeneity; a number of aliquots should be tested before dispatch).

• Spiked matrix (e.g. potato extract spiked with known dilutions of bacteria, soil spiked with nematodes, wheat grain spiked with one bunted kernel for *Tilletia indica*)

The process of preparation of samples generally ensures homogeneity; a number of samples should be tested before dispatch whenever technically possible;

• Artificially inoculated matrix (e.g. *Phytophthora ramorum* on leaves, mechanical inoculation of test plants).

A number of samples should be tested before dispatch whenever technically possible.

• Ground or mixed freeze dried material (e.g. plant material infested with viruses/viroids/phytoplasmas)

The process of preparation of samples aims to ensure homogeneity; a number of samples should be tested before dispatch whenever technically possible.

• Naturally infested material

Samples should be taken from a known infested source and randomized. A number of samples should be tested before dispatch whenever technically possible.

• Samples that mimic infested material

In cases where naturally infested material is difficult to obtain or homogeneity difficult to ensure (e.g. *Chalara fraxinea* and ash twigs), laboratories can combine in one sample the matrix and the pest (e.g. a plug of a pure culture associated with the matrix in a tube). A number of samples should be tested before dispatch whenever technically possible.

• Cultures (e.g. on growing media, extracts, suspensions...)

A number of samples should be tested before dispatch whenever technically possible;

• Traps (pheromone traps for insects).

Homogeneity is less an issue for these types of samples because the objective is to determine if the target is present or not. Consequently the only requirement is that the location of the target on each trap should be documented before dispatch.

• Exceptions to testing prior to dispatch

On some occasions, homogeneity testing cannot be carried out prior to dispatch for practical, technical or logistical reasons (e.g. tests that require isolation of viable organisms by incubation, when documented evidence is available from previous homogeneity testing on similar samples prepared by the same procedure). On such occasions parallel testing of extra samples should be conducted.

3.5.1.1.2 Number of samples to be included in homogeneity testing—Current available guidelines recommend to test a minimum of 10 randomly chosen samples (for each pest/matrix/infestation level, including negative samples) in duplicate (e.g. ISO 13528). Some laboratories use the square root (rounded up) of the total number of samples. Based on current experience and depending on the method used it is recognized that this is not always feasible because of the multiple pest/matrix/infestation level combinations. Therefore, number of samples included in the homogeneity testing may be reduced if suitable data are available from previous homogeneity testing on similar samples prepared by the same procedures or according to the expertise of the organizer.

The choice of the number of samples should be documented.

3.5.1.1.3 Statistical analysis of homogeneity testing—When quantitative data is generated (e.g. optical density for ELISA, number of cells for IF, Ct values for real-time PCR, measurements for morphological features), statistical analysis of homogeneity testing is commonly performed by comparing the sample mean and the coefficient of variation obtained during the homogeneity testing to expected levels. Other statistical approaches, such as comparison of the standard deviation of the samples (potentially expanded with F test) to the proficiency testing standard deviation (ISO 13528/IUPAC), or F test if sufficient data is available (Guide ISO 35), may be used depending on the data available and the experience of the laboratory.

When quantitative data is unavailable, the statistical analysis is limited to the qualitative results. In any case (with or without quantitative data), all qualitative results obtained during the homogeneity testing should correspond to the assigned value.

3.5.1.2 Stability testing. Samples should be demonstrated to be sufficiently stable to ensure that they will not undergo any significant change throughout the conduct of the interlaboratory comparison, including storage and transport conditions. When required, stability testing should be conducted in conditions that mimic transport and storage conditions. As an alternative, samples can be sent to the participant with the most challenging environmental or transport conditions and returned unopened for testing. For test performance studies reagents are usually also provided to participants in addition to samples. Stability of those reagents which have an influence on the outcome of the test should be verified following the same procedures.

A stability check may be performed on samples held by the organizer. This should be done after the deadline for performing analyses by the participants, in order to verify that the stability of samples has been maintained throughout the interlaboratory comparison.

Some pest stages are known to be stable over long periods (e.g. *Globodera* spp. cysts, or fungal spores) in such case stability testing is not needed.

3.5.1.2.1 Types of samples—In plant pest diagnostics the evaluation of stability depends on the type of pest/matrix to be tested. Examples are described below.

• Slides and mounted specimens of fungal structures:

Testing for stability is usually not needed for correctly mounted slides except if some staining procedures are used

(e.g. immunofluorescence) and a number of slides should then be examined during or after the interlaboratory comparison. Integrity has to be ensured by appropriate packaging.

- DNA/RNA, spiked matrix, artificially inoculated matrix, freeze drying of infested material followed by grinding/mixing, naturally infested material, samples that mimic infested material, and cultures:

A number of samples should be tested during or after the interlaboratory comparison.

- Traps (pheromone traps for insects)

Stability should be ensured by appropriate packaging to avoid damage to the specimens, and minimizing transport time. A number of traps should be examined during or after the interlaboratory comparison.

3.5.1.2.2 Number of samples to be included in stability testing—Current available guidelines recommend testing a minimum of 3 randomly chosen samples (for each pest/matrix/infestation level including negative samples) in duplicate (e.g. ISO 13528). Based on current experience and depending on the method used, it is recognized that it is not always feasible because of the multiple pest/matrix/infestation level combinations.

Therefore, the number of samples included in the stability testing may be reduced if suitable data are available from previous stability testing on similar samples prepared by the same procedures or according to the expertise of the organizer.

The choice of the number of samples should be documented.

3.5.1.2.3 Statistical analysis of stability testing—When quantitative data is generated (e.g. optical density for ELISA, number of cells for IF, Ct values for real-time PCR, measurements for morphological features), statistical analysis of stability testing is commonly performed by comparing the sample mean and the coefficient of variation obtained during the stability testing to expected levels. Other statistical approaches such as difference between stability and homogeneity means based on size relative to proficiency testing standard deviation (ISO 13528), or t test of stability vs. homogeneity means if sufficient data is available, may also be used depending on the data and the experience of the laboratory.

When quantitative data is unavailable, the statistical analysis is limited to the qualitative results. In any case (with or without quantitative data), all the qualitative results obtained during the stability testing should correspond to the assigned value.

3.6 Assigned values for samples

The organizer has to define/establish assigned values for samples, i.e. value attributed to a particular property of an interlaboratory test sample.

In the plant health field, assigned values correspond to the expected result of the test (pest present or absent, concentration of the pest, morphological characteristics of the specimen, etc.). In some cases the assigned value may be declared as 'undetermined' (e.g. samples yielding an OD between positive and negative threshold in ELISA, specimens presenting overlapping morphological characters).

The organizer should have previously described in a procedure how this value would be assigned e. g.:

- Known values (spiked sample) this is the most common situation, usually qualified as low/medium/high or present/absent.
- Known values (naturally infested plants, samples) in cases where the infestation status of a plant (e. g. nursery tree) is known and the target pest was previously confirmed by an expert, using a validated test considered to be highly accurate and comparable to tests in use, usually qualified as low/medium/high or present/absent
- Reference values obtained from comparison with reference material.
- Consensus values from expert participants/reference laboratories, provided they demonstrated their expertise in diagnosis of the target pest, using a validated test considered to be highly accurate and comparable to tests in use (for a test performance study, this approach may be used)
- Consensus values from participants in proficiency test. Rules for definition of these values from the participants results should be defined: statistical methods, outliers effect (e.g. in virology interlaboratory comparison may assign the values this way).

Uncertainty of assigned values should be defined. For known values uncertainty is low or almost = 0 (depending on homogeneity and stability), but for consensus values from participants, the uncertainty might be high depending of the competence of the participating laboratories.

The organizer should have a policy regarding the disclosure of assigned values. The policy should ensure that participants cannot gain advantage from early disclosure. Disclosure should happen as late as possible.

3.7 Instructions for participants

Announcement

To announce an invitation for participation in an interlaboratory comparison test, the organizer may consult the EPPO database on diagnostic expertise in order to target relevant laboratories.

Test information required in the invitation

The organizer should make possible applicants fully aware of all conditions and demands for participation in the interlaboratory comparison test offered. This includes information on:

- Whether the interlaboratory comparison is organized for proficiency testing or a test performance study.
- Test organism.
- Matrix.

- Description of the test(s) to be used in the case of a test performance study.
- Full protocol (or link to the reference) with contact details for clarification if needed and the policy on possible changes to any part of the protocol during the test period.
- Possible phytosanitary requirements such as official documents required and facilities required to avoid unintended spread of test organism.
- A time schedule including documents and agreements to be signed and returned, approximate timing of sample dispatch, mode of dispatch, estimated time for testing, date for receiving results from participants as well as an estimation of time needed for reporting.
- Possible selection criteria between applicants, and any geographic limitation for participation or any pre-qualification requirements involved (see 3.4.1. e).
- Equipment and other facilities required.
- Chemicals involved (for evaluation of national/local safety and transport regulations). It should be possible to judge whether the samples may be legally sent to participants and are not listed in the export control list.
- Any other item (e.g. thermocycler) which may be sent with the samples with essential details.
- It should also be explained if any of the items/samples need to be returned to the organizer and who will pay for this.
- Any fees and other expenses involved as well as costly chemicals to be purchased by the participants and any excessive delay to be expected for the receipt of specialized equipment or chemicals required for the study.
- Information about whether the report/results will be made public and to which extent.
- Information about how to apply for participation in the test.
- The consent to the participation in the interlaboratory comparison (including the requirement to provide the direct address of the laboratory that will perform the tests).
- Confidentiality agreement, if necessary.

3.8 Packaging, labelling and distribution of interlaboratory comparison test samples

When distributing the samples for the interlaboratory comparison precautions should be taken to maintain the quality and integrity of the material.

For example:

- Care should be taken that there is no contamination or mixing of samples.
- When distributing biological organisms such as cysts or bacteria or infested material (naturally or artificially), extremes of hot and cold temperatures should be avoided if these were shown to be detrimental during the stability testing. In such situations appropriate distribution means should be selected.
- The time of the year should be taken into account e.g. summer or winter, public holidays in order to make allowances for extremes of temperature and packages waiting for long periods before being unpacked.

There is specific packaging material for sending out live quarantine material (bio hazard – symbol) or DNA/RNA solutions.

A parcel service that guarantees the delivery of the samples to the participants in a similar timeframe should be used.

In addition the organizer should ensure that:

- The interlaboratory comparison samples are clearly labeled in such a way that the label will remain legible throughout the interlaboratory comparison;
- The package contains all the relevant paperwork e.g. instructions, plant health license, unique laboratory identifier and samples identifiers;
- The package and its content conform to international safety and transport requirements

A procedure should be followed to enable confirmation of delivery of interlaboratory comparison samples.

3.9 Data analysis and evaluation of the results of the interlaboratory comparison

Data analysis methods should be appropriate to the nature of data (see 1).

The standard way of analyzing qualitative results is based for each participant on the numbers of positive agreements (PA), negative agreements (NA), positives deviations (PD) and negatives deviations (ND), as presented in Table 3:

Participant(s) submitting results that fall outside expected ranges should be identified; this should be documented. Consideration should be given to exclude this/these participant(s) from the analysis of the interlaboratory comparison depending on the reasons for the difference from expected results and the objective of the interlaboratory comparison (i.e. PT or TPS).

The values from Table 3 may be used to make an evaluation of the interlaboratory comparison based on calculations as described in Table 4.

From these data, an evaluation of the performance of the laboratory (for PT) or of the performance of the test (for TPS) can be made.

In proficiency testing, the performance of the laboratory is evaluated in terms of the minimum levels required for the performance criteria (e.g.: 100% for accuracy). The minimum levels are defined independently of the proficiency test results.

In test performance studies, the performance of the test is evaluated either by the simple characterization of the performance criteria of the test, or by comparison to other tests, or by comparison to the performance criteria obtained for the test during the single laboratory validation. Test performance studies require a minimum number of participating laboratories (ideally a minimum of 10 valid laboratory data sets),

Table 3 Definition of positive agreement (PA), negative deviation (NA), positive deviation (PD) and negative deviation (ND) adapted from ISO 16140

Reference participant	Assigned value = Positive	Assigned value = Negative	Assigned value = Undetermined*
Result obtained is positive	PA = Positive Agreement	PD = Positive Deviation	PA = Positive Agreement
Result obtained is negative	ND = Negative Deviation	NA = Negative Agreement	ND = Negative Deviation
Result obtained is undetermined	ND = Negative Deviation	PD = Positive Deviation	PA = Positive Agreement

*only in specific cases (see 3.6) and should be avoided.

Table 4 Evaluation of results from ISO 16140

Performance values	Calculation
Accuracy	$(\sum PA + \sum NA)/N\ 100\%$
Rate of true positives	$\sum PA/N^{+} \times 100\%$ Note: the rate of false negative results obtained by the laboratory can be calculated as follows: $1-(\sum PA/N^{+} \times 100\%)$
Rate of true negatives	$\sum NA/N^{-} \times 100\%$ Note: the rate of false positive results obtained by the laboratory can be calculated as follows: $1-(\sum NA/N^{-} \times 100\%)$

N^{+} number of samples with a positive assigned value $\sum PA + \sum ND$.
N^{-} number of samples with a negative assigned value = $\sum NA + \sum PD$.
N total number of samples = $(N^{+} + N^{-})$.

however, it is recognized that this may be a constraint in plant pest diagnostics.

The organizer can also calculate the probability of obtaining the same result (positive, negative or undetermined) from replicate samples analyzed in different laboratories. For test performance studies this will provide information on reproducibility.

The organizer can also calculate the probability of obtaining the same result (positive, negative or undetermined) from replicate samples analyzed in the same laboratory. For test performance studies this will provide information on repeatability.

Other data analysis methods based on calculations of POD (probability of detection) can be used, in particular for test performance studies (Wehling *et al.*, 2011).

3.10 Reports

The reports should be clear and comprehensive and include data covering the aggregated results of all participants as well individual results. The data are anonymized but each laboratory should receive information allowing identification of its results.

Reports should include the following:

- the name and contact details of the organizer
- the name of staff involved and respective responsibility
- identification of person(s) authorizing the report
- the date of issue and status (e.g. preliminary, interim, or final) of the report
- page numbers and total number of pages of the report
- a statement of the extent to which results are confidential
- unique identification of the report and the interlaboratory comparison
- a clear description of the test samples used, including necessary details of the sample preparation and homogeneity and stability assessment
- the participants' results especially for proficiency tests (when possible, otherwise a summary of the results, e.g. in tabulated or graphical form, can be supplied)
- when relevant statistical data and summaries, including assigned values and range of acceptable results and graphical displays
- procedures used to establish any assigned value
- when relevant details of the metrological traceability and measurement uncertainty of any assigned value, e.g. for quantitative analysis or qualitative analysis including measurements
- procedures used to establish the standard deviation for proficiency assessment, or other criteria for evaluation
- assigned values and summary statistics for test/method evaluated (test performance study) for test methods/procedures used by each group of participants (if different methods are used by different groups of participants) (proficiency test), when relevant
- comments on participants' performance by the organizer and technical advisers
- information about the design and implementation of the interlaboratory comparison
- procedures used to statistically analyse the data
- advice on the interpretation of the statistical analysis and
- comments or recommendations, based on the outcomes of the interlaboratory comparison.

Reports should be made available to participants within planned timescales. The organizer should have a policy for the use of reports by individuals and organizations (for test performance study especially, results might be for example the property of a research project).

References

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