Diagnostics
Diagnostic

Basic requirements for quality management in plant pest diagnosis laboratories

Specific scope

This guideline specifies the general quality management requirements to perform diagnostic methods for plant pests.

Introduction

Development of quality management systems (also referred to as management systems or quality systems) and accreditation have become a concern for many laboratories in the EPPO region. This document describes basic requirements to assist laboratories conducting plant pest diagnosis in designing their management system for quality. Quality management consists of activities that ensure the quality and confidence of a diagnosis performed in a laboratory. It is based on management requirements and technical requirements (see below). Laboratories applying for accreditation should base their systems on the ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration laboratories*.

This document concerns the quality of a diagnosis and is not specifically dealing with health and safety matters. However, laboratory practices should conform to national health and safety regulations.

This document was prepared on the basis of the following standards:

- ISO17025: 2005 *General requirements for the competence of testing and calibration laboratories* (available on www.iso.org)
- EPPO Standard PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests*
- EPPO Standard PM 7/76 *Use of EPPO diagnostic protocols*
- EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

Terms and definitions

Certified reference material: this is reference material derived from a source that certifies the authenticity of the material. Preferably material should come from an internationally recognised source such as a national reference collection. It should go together with a unique identification code allowing traceability and the name of the person who certifies its authenticity. Details of how the material was authenticated should also be supplied. If appropriate, information about its activity (e.g. pathogenicity, antigenic properties) under specified conditions should also be supplied along with any related uncertainty at a stated level of confidence.

Reference material: Live cultures are most commonly used, but other material such as infected plant material, DNA/RNA preparations, images of a diagnostic quality or mounted specimens including insects or fungal spores may be considered. The reference material used should be documented and appropriate to the test and diagnosis being performed. It should be ensured that the material used is producing the features for which it was selected for example expressing a desired antigen for use in serological diagnosis, or display specific physical features (e.g. sporulation) if used for morphological diagnosis.

Quality assurance: part of quality management, focussed on providing confidence that quality requirements will be fulfilled.

Pest: any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products.

Management requirements

The laboratory should establish, implement and maintain a quality management system covering all facilities and activities of the laboratory.

The management system should describe the facilities and activities covered (including details of the customers and tested pests). The quality system should be documented and the quality documents should be archived.

Table 1 Recommendations and suggested frequencies for external calibration and calibration checks for equipment

Type of equipment	Recommendation	Suggested frequency
Reference thermometers and reference thermocouples	a) full traceable re-calibration	a) every 7 years
	b) single point (at working temperature)	b) annually
Calibration weight(s)	full traceable calibration	every 7 years
Microscopes	traceable calibration of stage micrometer	initially
Pipettes	calibration	annually
Autoclaves (for media preparation)	calibration	annually

The management system of the laboratory should ensure that:
- Appropriate resources are available to conduct the diagnosis, for example personnel, facilities and consumables (see also the section 'Technical requirements')
- Purchased supplies, reagents and consumable material are appropriate for the intended use
- Responsibilities and tasks of personnel are clearly defined (e.g. by organizational flow charts) and appropriately assigned
- Possible conflicts of interest between personnel and activities performed are identified and prevented
- Training is documented and assessed (see also the section 'Technical requirements')
- Procedures and instructions are available to and implemented by the personnel. This includes Standard Operating Procedures (SOPs)
- The client is informed upon request of the relevant data regarding the diagnosis of its sample
- Any subcontracted work is performed by a laboratory that is following the requirements of this standard, which should be checked by the laboratory (the minimum being a written declaration of compliance)
- Confidentiality of diagnosis results to customer is guaranteed[1]
- A mechanism is in place to deal with complaints
- Mechanisms are in place to record, analyse and correct any deviation from procedures or requirements of the customer
- Documentation is maintained and archived.

The quality management system should be reviewed periodically by the top management. This implies a periodical assessment of all the components of the system and routine recording of deviations in the system and subsequent corrective actions that have been taken.

Technical requirements

General

Many factors determine the reliability of diagnosis performed by a laboratory. These factors include:
- personnel
- accommodation and environmental conditions
- diagnostic methods
- equipment
- reference materials/cultures
- sampling
- sample handling.

Personnel

The laboratory management should define and ensure the competence of those who perform each specific stage of diagnosis, and their competence to use the equipment.

Personnel performing specific tasks should be qualified on the basis of appropriate education, training, experience and/or demonstrated skills. Staff undergoing training should be appropriately supervised and monitored. Staff records should be maintained including records concerning the date on which authorization and/or competence to perform a specific task is confirmed and training records.

Accommodation and environmental conditions

Laboratory facilities should be such as to enable a correct performance of the diagnosis. Depending on the type of testing being carried out, different steps of a diagnosis may also be combined in a working area, provided that necessary precautions are taken to avoid cross-contamination resulting from samples, reference materials and facilities (see Appendix 1). Specific guidance on handling quarantine organisms have been developed (see Table 1 *Confinement conditions* in EPPO Standard PM 3/64 *Intentional import of live organisms that are plant pests or potential plant pests* and EU Directive 95/44/EC).

A typical laboratory is comprised of testing facilities and ancillary facilities (entrances, corridors, storage rooms, toilets, archives, etc.). Separate locations or clearly separated/designated working areas are recommended for the following:
- reception of samples
- preparation of samples (segregate location for samples likely to be highly contaminated or powdery, e.g. soil samples, plants infected by fungi, insects or mites, tubers with soil)
- diagnosis of samples (with a separation between greenhouses and laboratory rooms)
- storage of samples
- appropriate disposal of material
- maintenance of reference materials/cultures
- preparation of media, reagents.

Different activities can be separated by time. The work area should be appropriately decontaminated between different

[1]Nevertheless, laboratories are encouraged to develop a procedure to report findings of regulated pests to the NPPO when the NPPO is not the customer.

samples and/or activities. Specific requirements are mentioned in Appendix 1.

The laboratory should be appropriately equipped to ensure proper storage, diagnosis and containment of samples.

Access to the laboratory should be restricted to authorized personnel who should be aware of the intended use of a particular area and restrictions imposed on working in such areas.

The laboratory should monitor, control and record environmental conditions where they may influence the quality and reliability of the diagnosis. Failures resulting from deviating environmental conditions should be documented and corrective actions recorded (see Appendix 1).

Measures should be taken to ensure good housekeeping in the laboratory. Space should be sufficient to allow work areas to be kept clean and tidy. Clothing appropriate to the testing being performed should be worn, especially when working in the microbiological and molecular biological laboratory.

Diagnostic methods

General The laboratory should use appropriate methods and procedures for all tests within its scope. These include sampling where relevant, handling, transport, storage, preparation and testing of samples. It is expected that diagnostic laboratories will have an understanding of the biology of organisms and take this into account when sub-sampling and/or when preparing the sample for analysis. Supplies, reagents and consumable material should be appropriate for the intended use.

All instructions, standards, technical manuals and reference data relevant to the work of the laboratory should be kept up-to-date and made readily available to personnel. Deviation from methods should occur only if documented, technically justified and authorized by an appropriate person.

Selection of methods The laboratory should use diagnostic methods that are suitable according to the circumstances of use (see EPPO Standard PM 7/76 *Use of EPPO diagnostic protocols*). Methods published as international, regional or national standards should preferably be used. When not available, laboratory-developed or adapted methods could be considered.

The laboratory should ensure that it uses the latest valid edition of a method unless it is not appropriate or possible to do so. When necessary, the method should be supplemented with additional details to ensure consistent application.

Performance of the laboratory The laboratory should confirm that it can properly carry out the selected diagnostic methods (see 'Ensuring the quality of diagnosis' section). This confirmation should be repeated when the diagnostic method changes.

Equipment

The laboratory should have the equipment required for correct diagnosis and these should be operated by qualified personnel. Equipment should be listed and a programme should be documented and implemented for maintenance, calibration and corrective action of key equipment, which significantly affects diagnostic results.

Equipment that has either been subjected to overloading or mishandling, or gives suspect results, or has been shown to be defective or outside specified limits, should be taken out of service, clearly labelled or marked, and appropriately stored until it has been repaired and shown to perform correctly.

Calibration and verification programmes Specific calibration and verification requirements are given in Appendix 2. Only qualified personnel should perform calibration and verification programmes, using procedures appropriate to the intended use. Calibration may be performed internally or externally (by specialized companies).

Documents on external and internal calibration and verification of performance (including when the next calibration is due) should be maintained and made available within the laboratory. Equipment should be appropriately labelled (see Appendix 3).

Maintenance of equipment Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer of the equipment) should be readily available for use by the appropriate laboratory personnel. Maintenance of essential equipment should be carried out at specified intervals as determined by factors such as the rate of use and age/complexity of the equipment, and this maintenance documented (see Appendix 4 for guidance on maintenance of equipment).

Records Records should be maintained for equipment significant to the diagnosis performed. Depending on the type and sensitivity of equipment, and the conditions required by the manufacturer to ensure failure-free running, the records should include:

- identity of the item of equipment
- manufacturer's name, type identification
- the manufacturer's instructions
- dates, results and copies of reports and certificates of all calibrations, adjustments, date of next calibration where appropriate
- maintenance carried out to date, the maintenance plan where appropriate
- damage, malfunction, repair to the equipment.

Reference materials

Reference materials provide essential traceability in diagnosis and are used, for example:

- for identification
- to demonstrate the accuracy of results
- to calibrate equipment
- to monitor laboratory performance
- to validate methods
- to enable comparison of methods.

If possible, certified reference material should be used, from which reference material, and subsequently working material

can be produced but it is not always available. Laboratories may also produce their own reference material from which working material is produced.

Biological reference materials In order to maintain confidence in the status of biological reference materials verification should be carried out according to defined procedures and schedules (e.g. morphology, pathogenicity, antigenic properties, molecular properties, etc.). The laboratory should have procedures for safe handling, transport, storage and use of biological reference materials in order to prevent contamination or deterioration and in order to protect their integrity (see Appendix 5).

Working material deriving from biological reference materials (e.g. reference cultures) from an international collection should be made and kept separate from the original material (See Appendix 5).

Other sources These could include books, pictures, slides collections, morphological identification keys, scientific literature, sequence databases that can be used to support diagnosis.

Sampling

Sampling is a procedure in which material is taken to perform a diagnosis. A sample should be representative of the material under test and the type of sample should be selected based on knowledge of the distribution of the pest to be detected. Such a representative sample may not always be available: if so, this should be documented.

Correct sampling is an operation that requires careful attention. Not all laboratories are involved in sampling. When this is the case, the laboratory should have a sampling process (both plan and procedure) for collecting samples to be followed whenever practicable. This process should address the factors to be controlled and be based on appropriate statistical methods to ensure the validity of the diagnosis.

The laboratory should have procedures for recording relevant data relating to sampling whether the process is performed by the laboratory staff or by the customer.

Deviations, additions or exclusions from the documented sampling procedure should be recorded in detail and communicated to the appropriate personnel.

Sample handling

The laboratory should have procedures for safe transportation, receipt, handling, protection, storage, retention and/or disposal of samples, including all provisions necessary to protect the integrity of the sample.

Sub-sampling by the laboratory prior to testing is considered to be part of the test method. Sub-sampling should be designed to take into account uneven distribution of pests.

The laboratory should have a system for identifying samples. The system should be designed and operated so as to ensure that samples cannot be confused physically or when referred to in records or other documents. The system should, if appropriate, accommodate a sub-division of groups of samples and the transfer of samples within and from the laboratory. The identification of a sample should be retained as long as this sample is in the laboratory. Suggested content for a form to identify a sample is presented in Appendix 6.

Plant pests may be sensitive to factors such as temperature or duration of storage and transport, so it is important to check and record the condition of the sample on receipt by the laboratory. If there is insufficient material in the sample or the sample is in poor condition due to physical deterioration, incorrect temperature, torn packaging or deficient labelling, when a sample does not conform to the description provided, or if the test method required is not described in sufficient detail, the laboratory should consult with the customer before deciding whether to test or refuse the sample. In any case, the facts and the results of discussion should be recorded.

Samples awaiting testing should be stored under suitable conditions to minimise changes to any pest populations present and to protect it from cross-contamination. Storage conditions should be defined, and recorded when necessary. Where samples have to be returned to the customer after diagnosis, care is required to ensure that they are not damaged or injured during handling, testing or storage.

A procedure for the retention and disposal of samples should be written. Samples should be stored until the test results are obtained, or longer if required. Reasons for keeping a sample include for potential complementary analysis.

A laboratory should have procedures to treat contaminated samples after testing to conform with national or international regulations for quarantine and other plant pests. The procedures should also be designed in order to minimize the possibility of contaminating the test environment or materials. Further details on confinement conditions may be found in EPPO Standard PM 3/64 *Intentional import of live organisms that are plant pests or potential plant pests*.

Ensuring the quality of diagnosis

Assuring the quality of analyses should be made at different levels: for each diagnosis as well as for global quality control of the laboratory.

Internal quality management consists of compliance to all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results day-to-day and their conformity with defined criteria. The interval between internal quality checks will be influenced by the number of actual tests performed. Monitoring of test quality should be planned, reviewed and registered. Wherever possible positive/negative controls should be used: this should be the minimum level for internal quality control. An internal quality control programme may also consist of:

- The use of reference material (for example closely related organisms or collection material, non-target organisms which might be naturally present in a composite material)
- The use of artificially contaminated samples
- Replicate testing using the same method
- Comparative testing between analysts of the same sample

- Comparison of results of different methods identifying different characteristics of a plant pest
- Retesting of retained plant material or extracts thereof, water or soil samples and insects traps (within a predetermined suitable storage time and condition of the material before retesting)
- Inter- or intra-laboratory evaluation of documentation of the specific determinants on which diagnosis are based (in particular for visual determination of insects, nematodes and fungi)
- Blind testing by processing samples with known levels of pests between routine samples.

In special instances, a laboratory may develop a quality assurance system for a diagnosis that it is rarely called on to do. It is recognized that in such cases an ongoing internal quality control programme may be inappropriate and that a scheme for demonstrating satisfactory performance, which is carried out in parallel with the testing, may be more suitable. The use of an external quality assessment is recommended if an external proficiency programme or *ad hoc* proficiency tests are available. The validity of test results is influenced by both technical performance and assay performance characteristics. If the validity of test results is called into question, it is important to be able to distinguish between the two. A test may demonstrate appropriate process control but poor diagnostic performance or vice versa.

Reporting the results

See EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

Appendix 1

Environmental monitoring and avoiding of contamination

The laboratory should ensure that environmental conditions, laboratory arrangements and working procedures are such as to minimize risk of cross-contamination through air, surfaces, equipments, personnel, etc. Contaminations can be minimized or avoided in the following ways:

- Laboratory equipment should not routinely be moved between different areas inside the laboratory
- Where relevant a documented vector control programme should be in place
- Reference materials/cultures should be stored in a separate location in the laboratory
- Housekeeping and cleaning procedures should be defined, implemented and documented
- Hygienic working procedures (e.g. use of gloves, disinfectants, filter tips for pipettes, disposable plastic ware) should be defined and implemented.

Specific requirements for molecular biology laboratories:

- Dedicated PCR work areas should be organized following the 'forward flow' principle for a) DNA and RNA extraction and purification, b) preparation of mastermix, c) addition of sample to the mastermix and d) analysis of amplification products. It is highly recommended to have at least 3 distinct rooms (only activities a and c, or with extreme care b and c, might be performed in the same room but spatially separated or conducted under a laminar flow cabinet)
- Dedicated equipment (including pipettes) should be used in each work area. Dedicated laboratory coats should preferably be used in each work area (at least a specific coat for mastermix preparation) and gloves should be worn
- Tubes containing amplification reaction products should not be opened within work areas used for nucleic acid extraction or mastermix/reaction mixture preparation.

The laboratory should monitor the quality of laboratory air and surfaces of relevant areas at regular intervals. The monitoring can be done by using air settlement plates (e.g. plate count agar or other appropriate non-selective plates), contact plates (for even surfaces) or swabbing (for other surfaces and equipments), and insect traps. Buffers exposed to air or surfaces can also be tested. For laboratories working on nematodes, the normal hygienic procedures ensure that contamination is avoided.

Specific guidelines for monitoring contamination with bacteria and fungi

Air settlement plates, preferably 3 in each area to be monitored, should be exposed to air contaminants for a definite time (30 min recommended), closed and incubated for 3 days (at 30°C) to 5 days (at room temperature). Contact plates should be exposed on the surfaces to be monitored for 15 seconds (recommended), closed and incubated as above.

The number of colony-forming units (cfu) per plate should be recorded and remain under a defined level, e.g. < 15 cfu/plate in standard laboratory environment. The acceptable level of cfu/plate/area (background counts) should be defined by the laboratory according to the testing being carried out and according to the special requirements of the environment (e.g. clean rooms). Environmental monitoring should be documented, corrective actions described, performed if needed and recorded. Cleaning should be intensified if needed and new samples taken after corrective actions have been performed.

Appendix 2

Calibration and verification

1. External calibration and calibration checks

The information in Table 1 is provided for guidance purposes and the frequency will be based on the need, type and previous performance (in particular in relation to the drift observed between calibrations) of the equipment.

2. Verification of performance

The information in Table 2 is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment. Monitoring frequency should be adapted to the conditions of the laboratory with a frequency being higher at the beginning and adapted later based on identified risk.

Table 2 Guidance on verification of performance of equipment

Type of equipment	Recommendation	Suggested frequency
Temperature-controlled equipment (incubators, baths, refrigerators, freezers, Berlese funnels, slide drying benches, etc.)	a) establish stability and uniformity of temperature b) monitor temperature	a) initially, and after repair, modification b) daily/each use
Thermocyclers	verification of efficiency	annually
Working thermometers & working thermocouples	check against reference thermometer at ice-point and/or working temperature range	annually
Sterilizing ovens	a) establish stability and uniformity of temperature b) monitor temperature	a) initially, and after repair/modification b) each use
Autoclaves (for destruction)	a) establish characteristics for typical loads/cycles b) monitor temperature/time	a) initially, and after repair/modification b) each use
Safety cabinets	a) establish performance b) microbiological monitoring c) air flow monitoring	a) initially, and after repair/modification b) weekly c) yearly (with a service contract)
Laminar air flow cabinets	a) establish performance b) check with sterility plates or swabbing	a) initially, and after repair/modification b) weekly
Growth chambers	a) monitor temperature, humidity and light b) monitor for pests using sticky plates	a) each use b) weekly
pH meters	adjust check using at least two buffers	daily/each use
Balances	check zero, and reading against check weight	daily/each use
Check weight(s)	check against calibrated weight or check on balance immediately following traceable calibration	annually
Stills, de-ionisers and reverse osmosis units	a) check conductivity b) check for microbial contamination	a) daily b) monthly if the treated water or the end-use product containing the treated water are not sterilized by autoclaving or filtration before use.
Gravimetric diluters	a) check weight volume (weight) dispensed b) check dilution ratio	a) daily b) monthly
Automatic media preparators	check sterility using chemical and biological indicators	as recommended by manufacturer
Pipettors/pipettes	check accuracy, fidelity and precision of volume dispensed	regularly (to be defined by taking account of the frequency and nature of use, and depending on the drift observed)
Spiral platers	a) establish performance against conventional method b) check stylus condition and the start and end points c) check volume dispensed	a) initially and annually b) daily/each use c) monthly
Colony counters	check against number counted manually	annually
Anaerobic jars/incubators	check with anaerobic indicator	each use
Laboratory environment (microbial)	monitor for airborne and surface microbial contamination using, e.g. air samplers, settle plates, contact plates or swabbing	weekly
Laboratory environment (entomological)	monitor for pests using sticky plates	every two weeks

Appendix 3

Equipment – identification and labeling procedures

This example document suggests the information sufficient to clearly identify equipment.

Identification procedure

Each piece of equipment should be identified by a unique code, all of which should be recorded in a specific register. Different methods and codes can be used. These 2 following methods may be used:

- The identification code is composed of 5 alphanumeric characters: 3 letters referring to the equipment type, and 2 numbers indicating the number in a series.
  Example: BAL02 represents the second (02) balance (BAL) in the lab.
  The main advantage of this coding method is that the code indicates the type of equipment to which it refers.
- The material is identified by a unique specific serial number.
  Example: material n°250, whatever it may be, is the 250th material registered and identified in the lab.

Although this system is very easy to apply, it is impossible to have an idea of the type of equipment concerned from its number.

Labelling procedure

Each piece of equipment should be permanently labelled with its unique code. This label should not be modified or removed.

Therefore, it is often suggested to etch the equipment with its unique code. The code should be positioned to be easily read without needing to handle the equipment. Care should be taken when etching equipment to avoid damaging it.

A temporary label may also mention the date when next calibration or maintenance is due.

Appendix 4

Guidance on Maintenance of equipment and environment

Table 3 is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Table 3 Guidance on Maintenance of equipment and environment

Type of equipment	Recommendation	Suggested frequency
Incubators (for microbiological purpose)	clean and disinfect internal surfaces	monthly
Incubators (for other than microbiological purpose)	clean and disinfect internal surfaces	every 3 months
Refrigerators, freezers, ovens	clean and disinfect internal surfaces	annually
Centrifuges	a) service	a) annually
	b) clean and disinfect	b) each use
Autoclaves	a) make visual checks of gasket, clean/drain chamber	a) regularly as recommended by manufacturer
	b) full service	b) annually
	c) safety check of pressure vessel	c) annually
Safety cabinets	full service and mechanical check	annually
Laminar flow cabinets	service and mechanical check	as recommended by manufacturer
Microscopes	a) clean, and full maintenance service	a) annually
	b) check eye-piece graticule	b) every 6 months
pH meters	clean electrode	each use
Balances, gravimetric diluters	a) clean	a) each use
	b) service	b) annually
Stills	clean and de-scale	as required (e.g. every 3 months)
De-ionisers, reverse osmosis units	replace cartridge/membrane	as recommended by manufacturer
Anaerobic jars	clean/disinfect	after each use
Media dispensers, volumetric equipment, pipettes, and general service equipment	decontaminate, clean and sterilize as appropriate	each use
Spiral platers	a) service	a) annually
	b) decontaminate, clean and sterilize	b) each use
Mixers/blenders	clean	each use
Thermocyclers	general service	annually
Growth chamber	clean	after each use
Berlese funnels	clean	each use
Slide drying benches	clean	weekly
Laboratory	a) clean and disinfect working surfaces	a) daily, and during use
	b) clean and disinfect floors, sinks, and basins	b) weekly
	c) clean and disinfect other surfaces	c) every 3 months

Appendix 5

Maintenance of reference cultures (bacteria, fungi, etc.) derived from certified reference collection

- All parts of the process presented in Fig. 1 should be fully documented and detailed records of all stages should be maintained
- It is not permitted to use reference material produced in the laboratory to provide an international/certified reference collection.

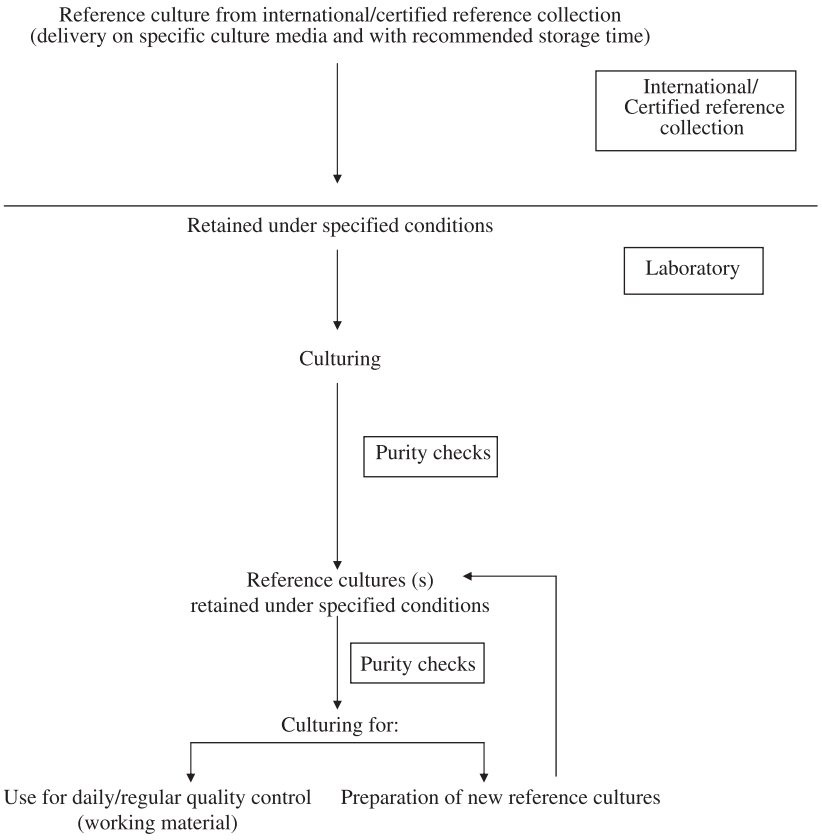

Fig. 1

Appendix 6

Suggested form for sample identification

Sample record form

This example form enables anonymous tracing of samples or batches of samples within a laboratory. A group of samples may be recorded as 1 batch when they arrive from the same client, are all of the same plant or plant part, and will receive the same analysis.

Batch identification code : (if appropriate)	
Plant species: Analysis requested by the client:	Nature of the submitted product to analyse (e.g. plant part, isolated pest):
Name of the person receiving/recording the sample:	Date of reception/recording:

Comments (e.g. urgent....)

Sample identification codes	
Laboratory identification code *(code given by the laboratory, unique to each sample)*	Client's identification code *(identification code given by the client, unique to each sample)*

Analysis undertaken	
Analysis protocols *(used by the laboratory)*	Date and signature *(of the operator responsible for choosing the relevant analysis protocol)*

Report of the analysis sent	Date and signature *(of the operator in charge)*