Eur J Plant Pathol (2008) 121:339–346
DOI 10.1007/s10658-007-9247-0

Quality assurance in plant health diagnostics – the experience of the Danish Plant Directorate

Charlotte Thrane

Received: 26 April 2007 / Accepted: 29 October 2007

Abstract Worldwide, there is increasing focus on implementation of Quality Assurance systems (QA-systems) in plant health diagnostic laboratories. Several laboratories are in the development or implementation phase and some laboratories have gained accreditation through approval by national accreditation boards. To initiate the process of developing and implementing QA-systems, management and staff need a strong motivation factor. First, because it is a time-consuming and demanding process to go through. Second, because plant health testing does not fit very well into the QA-systems that traditionally were developed for chemical or physical testing laboratories. External pressure is often the only way to generate this motivation factor amongst staff and management to initiate the development of QA-systems. The principal motivation factor in our laboratory was a national requirement that official testing laboratories should implement QA-systems. At the Danish Plant Directorate (PD) we have gained experiences with accreditation of plant health diagnostic methods during the past 5 years. The focus of this paper is a presentation of the consequences and the practical approach to comply with the requirements of ISO 17025 in our plant health diagnostic laboratory. This includes the themes: staff competence and responsibilities, documentation and traceability, and continuous assessment and improvement of the QA-system.

C. Thrane (✉)
Laboratory of Diagnostic in Plants, Seed and Feed Stuff,
Danish Plant Directorate, Skovbrynet 20,
2800 Lyngby, Denmark
e-mail: cht@pdir.dk

Definitions

ISO 17025 is a specific standard for testing and calibrating laboratories. It is based on the certification standard *ISO 9001* (ISO/IEC 17025 standard 2005).

A laboratory can gain *accreditation* to do a certain process (method) by approval of an accreditation board after a *third part audit*.

Why are plant health diagnostic laboratories implementing ISO-accredited quality assurance-systems?

For any laboratory or other business, the main driver for implementing ISO-standard QA-systems, is a strong motivation factor. Customer complaints and customer requirements are usually strong motivation factors. Further, enforcing national administrative requirements, governments can place pressure on official testing laboratories to implement QA-systems. This was the case for our laboratory when the Danish government in the late 90s requested that official testing laboratories should implement QA-systems

and possibly gain accreditation to be competitive and to be preferred as a service to the government.

In general, development, implementation, and maintenance of QA-systems can be overwhelming to staff and management. Staff might fear increased bureaucracy, undesirable work routines, and management fear that there will be no added value compensating for the cost in money and time to work under ISO-standard conditions. However, when things go wrong, when the customer complains, or when questions arise during the trade of plant material, the advantages of a QA-system become more obvious.

When working under an ISO accredited quality system, documentation and traceability of the work processes is mandatory which is extremely useful if laboratory results are challenged by the customer. Within plant health in the increasingly enlarging EU and open markets, inspectorates are aware of the importance of international harmonisation of testing of plant products crossing borders. ISO 17025 accreditation together with validated testing methods are effective tools to increase the certainty of laboratory tests and subsequently, the quality of the products.

In our laboratory, the areas selected for accreditation have been tests with a large number of samples for routine screening and testing where findings have a high economical impact on the grower. This is the case for testing for quarantine bacteria of potatoes. Diagnostics of *Clavibacter michiganensis* ssp. *sepedonicus* causing ring rot of potatoes is the case for demonstrating a QA-system.

Impact of implementation of ISO 17025 in the diagnostic laboratory

Several years after gaining accreditation, the advantages of QA-systems have become apparent to laboratory staff and management (Thrane and Scheel 2005). In summary, the standardisation of testing, sample flow, and other processes have increased the quality of laboratory tasks. Because of the detailed descriptions of responsibilities and duties within the QA-system and the focus on training to develop or maintain their expertise, staff have increased confidence in their daily work and express higher satisfaction with their work. In addition, it is a valuable aspect when tests are offered to consumers.

The work in the diagnostic laboratory should be looked at as processes. The laboratory does certain processes from receiving samples to issuing the final test result to the customer (Fig. 1). The management of the quality is documented through process control at critical and measurable steps along the process. Such control steps can be calibration of equipment, use of controls (reference material) as part of the testing, blind samples, documenting the staff member that did the step etc. The in-built requirement to continuously improve the QA-system is one of the most important advantages of the ISO 17025 requirements (Fig. 2). The most important tools for the continual assessment and possibly improvement of the system are internal control (e.g. blind testing), recording of errors, internal and external audits, participation in proficiency testing, and finally, the yearly management review. The general quality of the diagnostic work has increased in the laboratory. But it is also obvious that the full implementation of the quality system depends on continual assessment of the system through internal and external audits.

Quality assurance in the diagnostic laboratory – examples on the practical approach to comply with ISO 17025

In all diagnostic laboratories, a certain level of quality assurance of the testing procedures and laboratory management is implemented. However, for accredited laboratories, the quality assurance has to be systematically approached and all points of the ISO 17025 Standard should be addressed and implemented accordingly. DANAK is the national accreditation board in Denmark.

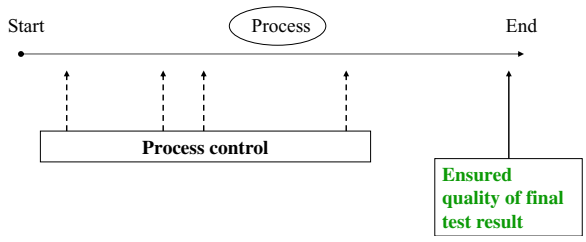

Fig. 1 All parts of a quality system should be subjected to continuous improvement. By actively responding to shortcomings of the system (errors, complaints etc), the quality system will improve

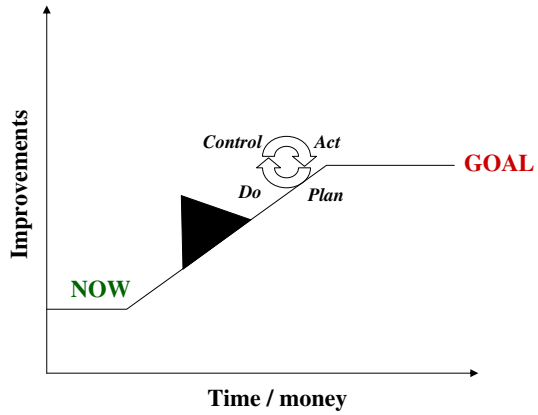

Fig. 2 Management of quality in laboratory testing through control checks at critical points of the process

Development and implementation

The resources required to develop and implement a QA-system depends on the scope for accreditation. We initiated our accreditation process for a limited part of the total laboratory activities. This served as a pilot exercise in assessing the process of implementing the various steps of an accredited QA system, thereby gaining valuable experience for a later scale-up. This initial exercise resulted in the following lesson being learned: rather than starting in a corner of the laboratory, it would have been valuable to involve all staff from the start and building up QA-systems for selected areas of testing that presented the different areas of diagnostics (virology, bacteriology, mycology, entomology, nematology). Now, 6 years after we started the process, all staff are involved with the QA-system at some level. Before, staff working under the ISO 17025 standard felt treated unfairly and isolated from the other staff whereas now all staff show a mutual perception of the QA-system. This development has increased the awareness and understanding of quality in their daily work and staff show an increased share of responsibilities for maintenance of the system.

The quality manual and document management

The quality manual is digital. A digital quality manual is less laborious to update than a paper-based manual. Thus, there is in general reduced bureaucracy in the document management of a digital quality manual than of a paper-based quality manual. Therefore there is less risk of using outdated documents as long as staff make sure that they only use the current digital version. All staff have access to computers. Staff can make hard copies of the documents they need but it is the responsibility of the staff to make sure that the printed copy they use is the most recent version. Document control of the digital documents should be controlled by an employee with this designated responsibility to ensure that the documents cannot be changed without authorization. The current quality manual is an internet-based 'Qualiware'-system (Fig. 3). This system has many advanced applications and one of them is the extended use of flow charts to demonstrate the work processes and the decision trees. This is combined with a possibility to link to relevant documents. This facilitates the understanding of the processes and is especially useful when introducing new staff and for auditors. In all aspects of documentation it is important to minimize and restrict written text to only one or a few documents to prevent discrepancies and missing documentation when changes are made. In general, the staff doing a certain process are involved in developing the work flow and the necessary documents.

Staff competence

There are strong requirements in ISO 17025 that staff should be competent to do the tasks they are supposed to do. This includes specific training in different diagnostic tests and general training in sample flow, trouble shooting, and QA-requirements and principles. The competence of staff is ensured by training, evaluation of the acquired training, followed up by continual assessment of staff to perform their assigned duties. In general, internal training for a flexible period of time is required depending on the experience of the person and the complexity of the task they are going to do. Most frequently the most efficient way of training new staff is to let the responsible technician train the new staff in the specific task. If the effect of training can be evaluated objectively, this is done before approval of the person for the specific task (blind testing of samples or comparative testing between the new and the experienced staff). Sometimes specific courses (external) are needed later to increase the depth of knowledge enabling staff to trouble shoot in their work, to introduce new techniques etc.

The internal training for Ring rot testing includes training in all aspects of the testing including handling

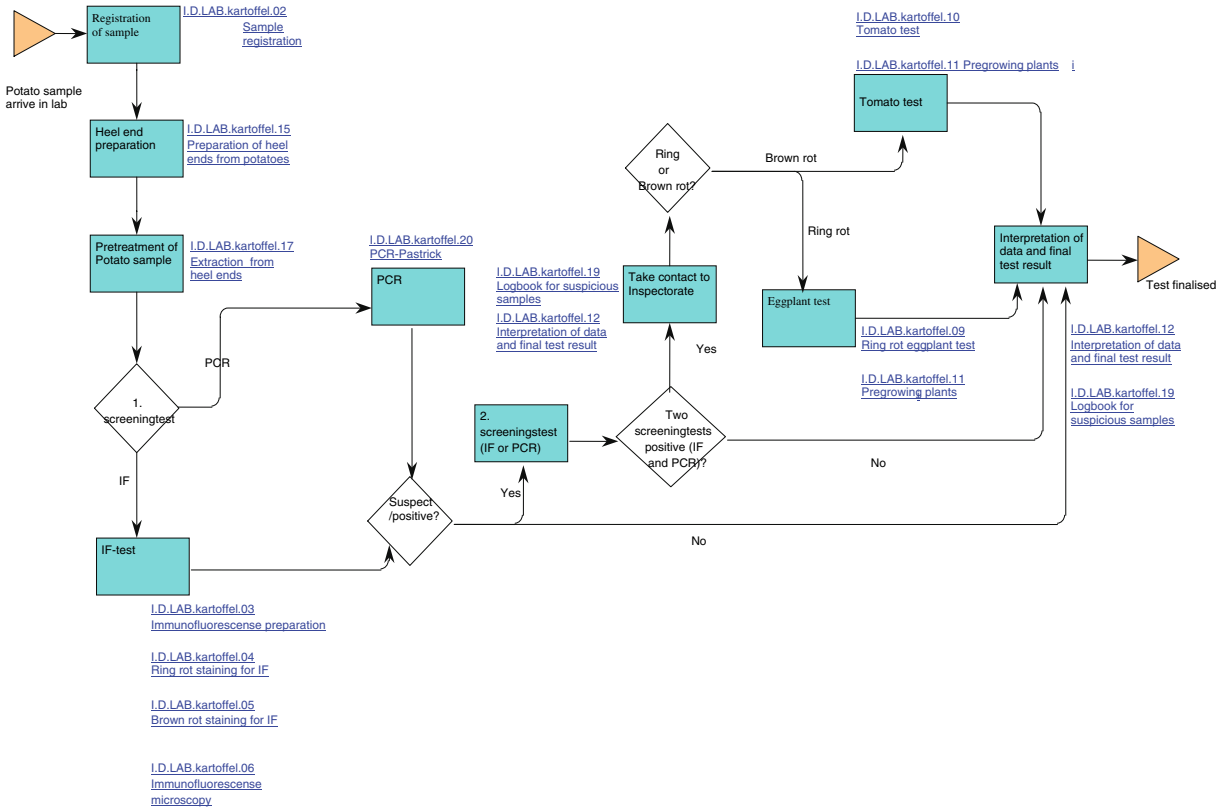

Fig. 3 Workflow of the potato testing scheme. **a** *Yellow triangles* start and end of process. **b** *Green boxes* activities. **c** *White diamonds* decision pathways. **d** *Blue underlined text* direct links to the written instructions of that part of the process (*I.D.LAB.kartoffel* Instruction Diagnostic Laboratory – potato.)

of samples as well as in special tasks as PCR, IF, egg plant testing. We have broken down the special tasks into separate training parts. Thus, one person can be approved for some parts of the testing only, or for the whole testing procedure. For the general handling of samples staff are trained under supervision for at least a month. This training is hard to assess objectively but in this case the responsible technician makes an evaluation where e.g. errors are recorded. For specific tasks as IF-microscopy and PCR, training might be longer and is always finalised with a test to ensure the competence of the trainee. No results can be issued by the trainee during the training period before the trainee has consulted the staff responsible.

Responsibilities and tasks

The QA-management at PD is an umbrella structure including three different laboratories. The overall responsibility of the quality management is the Controller-Unit (Office of Planning, Quality and International Projects) which is associated with the senior management of the organisation. The Controller-Unit prepares and issues the general procedures, approves laboratory specific procedures, and is responsible for the execution of internal and external audits as well as management reviews. In accredited laboratories, responsibilities and tasks should be clearly defined. This parameter is very important for the confidence of staff and subsequently for the quality of the work they do and the decisions they make. In the diagnostic laboratory, a database was developed for the staff to give an overview of the responsibilities and duties of all staff specified for the various tasks. One of the advantages of this database is to be able to foresee shortage of staff in specific areas of the laboratory tasks. For most tasks it is necessary that more than one person can do the work to make sure that the laboratory will be able to continuously meet customer requirements.

Documentation and traceability

Documentation is an essential part of QA-systems. Examples of documentation are the steps in the diagnostic work, evaluation of results from proficiency testing and ring tests, audit reports, records of staff training, records of equipment maintenance etc. Further, the documentation is necessary to keep track of the history of events in the laboratory. It is essential to do an initial assessment of which steps and parameters are necessary to document to ensure traceability in the work. And further, by doing this assessment it is possible to avoid non-essential documentation to reduce work load in order to be able to focus on the important parts of the QA-system. In general all documents are kept for six years. The long term storage of documents facilitates back-tracing in case errors or non-compliances are later identified. Samples are uniquely labelled by barcodes (Fig. 4). This ensures impartiality during testing, easy handling of samples, full traceability of all steps in the process, and thus fewer errors in the final diagnostic results.

Testing of equipment is a very important part of the process. Documentation of the characteristics and history of all equipment has a large focus in the ISO 17025 to reduce errors that otherwise could originate from malfunctional equipment (Fig. 5).

Non-compliances

The ISO 17025 standard determines that the laboratory should have a system for registration of errors and non-compliances. Specific forms are used for this purpose. When an employee identifies a problem, he or she describes it on the form. Then the impact of the non-compliances on the work is assessed. Together with the technical or quality-responsible staff, it is decided which corrective measures should be undertaken. The individual forms are evaluated on a regular basis to determine whether non-compliances appears to be systematic or only occurring accidentally, and whether the non-compliance is serious or of minor importance. It has been continuously necessary to emphasize the importance of identifying and registering non-compliances to staff and to assure staff that there is nobody personally to blame for errors occurring. The registration of non-compliances and implementation of corrective measures are very effective sources for improvements of testing quality.

Test methods

The European Plant Protection Organisation (EPPO) is one of the most important sources for improvements and standardisation of plant health diagnostic methods. For ring rot of potatoes the only official method accepted within EU, is stated in the ring rot control Directive (EU-Directive 2006/56/EEC). However, for most plant diagnostics, EU does not set the method for testing and laboratories need to find the best suitable method (preferably EPPO-methods or other validated or recognised methods). Unlike strict quantitative tests as in chemistry and physics, plant health diagnostics frequently implies interpretation of data from one or more tests before it is possible to provide a reliable test result to the customer. The more traditional methods in plant health diagnostics involve microscopy or morphological studies which require that staff are trained and experienced in the interpretation of results.

If the laboratory strictly follows official testing methods (validated methods), only verification of testing performance should be shown to ensure the quality of testing in the laboratory. It is useful to consult the guideline EA 4/10 for evaluation of testing quality of qualitative microbiological tests (European co-operation for Accreditation 2002). In case an official method is outdated or because the laboratory has another reason to deviate from the method, verification or even validation should be performed.

For morphological diagnostic methods, it is necessary to include some flexibility in the working instruction for the interpretation of results. Flexibility is necessary because of the biological variation between specimens of the same species and the similarity between different species (life stage of the pathogen, host plant, genetic variability, natural range of morphological variation between individuals etc). Because data can be hard to interpret it is:

- very important to standardise the process as much as possible,
- critical to develop decision trees of the diagnostic process,

Fig. 4 The use of bar coding for sample registration (**a**), sample processing (**b**), and entering the final test results (**c**) ensures a high degree of sample integrity and quality of the test result

- necessary that staff are familiar with relevant literature and other sources of information,
- mandatory to document the findings on which the final judgment is made.

The use of molecular methods in plant health diagnostics has increased in recent years. First, technology has made this development possible. Second, molecular testing is often a fast way of testing samples and customers in general want results as quickly as possible. Third, younger staff prefer to work with molecular methods rather than traditional methods. Fourth, it is easier to train new staff in molecular methods than in classical diagnostic methods. And fifth, despite the requirements for technical skills, advanced equipment and facilities, the advan-

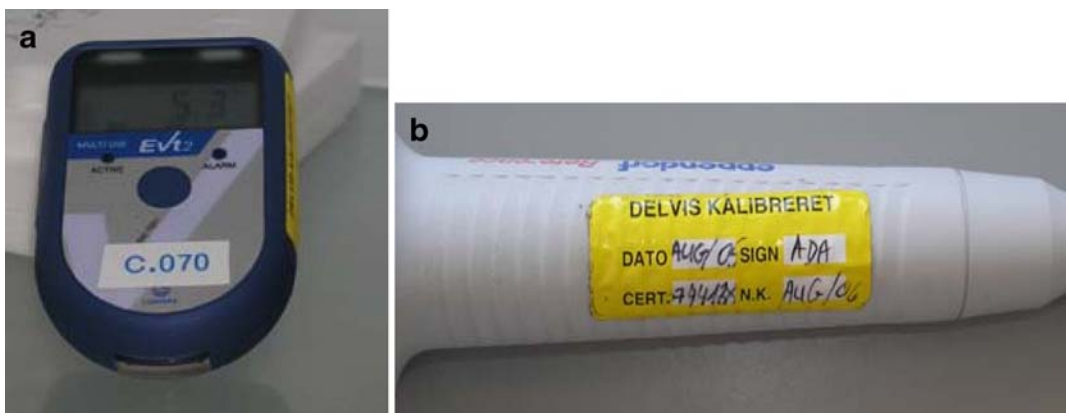

Fig. 5 Documentation and traceability of equipment history by unique labelling (**a**, thermo logger) and with calibration labels including certificate number to the reference material used for the calibration (**b**, pipette)

tage of molecular methods is that data interpretation does not depend as much on the skills of the analyst compared to morphological identification. Molecular methods can be standardised and the correct use of the methods can relatively easily be verified. Thus, it has in general been easier to become accredited for molecular tests than for classical morphological plant diagnostic tests. In particular, it can be difficult in morphological identification to:

- create standardised working instructions, and to
- demonstrate the competence of staff and laboratory.

Ring rot diagnostics

For ring rot testing, the method used is an official, validated, and robust method primarily developed for latent testing of the bacteria in tubers. EU member states have to follow the same standardised methods prescribed in the ring rot control EU-Directive. Frequently, new and improved methods are developed for diagnosis of plant pests. Such improvements of methods in general occur faster than updating of official methods. This is especially true when the official method is prescribed in an EU-Directive as it is the case for ring rot diagnostics. Thus, it is crucial in the preparation of official test methods that some flexibility is included, and that non-essential constraints on testing procedures are eliminated to make it possible to use new improved methods before they are published in the official document.

Assessment of testing quality

The ring rot test is a qualitative test and no strict statistical uncertainty measurement is possible and appropriate to evaluate the validity of test results. Thus, the components that can be easily evaluated should be under control to eliminate the uncertainties that could otherwise arise from the use of equipment etc. that is not well-maintained. Thus, uncertainties from the use of equipment should be regarded as minor in comparison with the uncertainties contributed by the subjectivity of analyst interpretation and the non-homogenous nature of the potato samples. The best way to assess the quality of ring rot testing is a top-down approach by some of the following initiatives; participation in proficiency testing, analysis of blind samples, comparative testing between analysts, and comparison of results obtained with different methods. External assessment of laboratory competence by proficiency schemes is currently being developed for plant health diagnostics by for example, the UK company, FAPAS.

Awareness of the risk of obtaining false positive and false negative results should be addressed in the choice of method for each test This is specifically important when testing non-symptomatic samples such as the ring rot survey. The use of controls during all testing is imperative and adds an essential component in verification of test results obtained. Threshold levels of detection should be addressed for all methods used. In ring rot testing, all suspicious samples are confirmed or refuted by alternative testing methods based on different biological principles according to the EU-directive. Based on positive screening tests, a preliminary result is issued to the customer. A positive finding in the screening tests for ring rot is further confirmed by Koch's postulates. This thorough testing before releasing a result of a positive finding, ensures a high quality of the issued test result.

Audits

DANAK performs external third part audits every 15 months on technical matters and on general quality management. Every 5 years DANAK performs a substantial audit and decides whether the laboratory can continue to be approved for accreditation. Internal audits are performed routinely and more frequently with assessment of equipment, test methods, and the general quality of management, respectively. There is no doubt that updating of the quality manual, organisational structure and general improvements of the QA-system largely depend on the accomplishment of internal and external audits.

Approval for accreditation

In many cases the national accreditation boards have only limited experience in auditing qualitative micro-

biology tests and especially in auditing plant health diagnostics. Thus, in general, the only feasible way to proceed is to use international experts in the specific fields. This can be expensive to the laboratory. However, it is crucial that the auditor knows the critical control points and possible problems in specific testing procedures in order to obtain a qualified assessment of the testing quality of the specific applicant.

References

EU-Directive (2006/56/EEC) for the control of potato ring rot.

European co-operation for Accreditation (2002). Accreditation for Microbiological Laboratories. EA - 4/10.

ISO/IEC 17025 standard (2005). General requirements for the competence of testing and calibration laboratories.

Thrane, C., & Scheel, C. (2005). Organization of quality assurance in diagnostics at the Danish NPPO. *EPPO Bulletin 35*(1).