Quality systems and validation for the safe movement of clonal germplasm

1. Introduction

The acquisition, conservation and distribution of germplasm and planting material is a core function of many agricultural organisations and serves to underpin breeding programs worldwide. Crops are infected by a number of viral, viroid, phytoplasma and bacterial diseases, which can be transmitted through exchange of germplasm and planting material. The aim should be to only distribute material of the sufficiently high health status to prevent the possible movement of pathogens around the world and the consequent damage to food security, livelihoods and economies. Compliance with all National and International phytosanitary treaties, regulations and requirements relating to both phytosanitation and also the Convention on Biological Diversity (CBD) is also a key requirement of this aim.

2. The Quality Standards Landscape

There has been an increasing expectation that organisations delivering critical products and services use quality assurance systems to ensure that their objectives are achieved consistently. Sectors embracing this philosophy includes the health, food and environment sectors and this has been driven by the increased reliance by Regulators on the demonstration of compliance with the various international quality standards.

Advantages shown for the implementation of quality systems include:

- Increased efficiency

- Control of risk

- Access to markets

- Promotion of best practices and knowledge transfer

- Demonstration of due diligence in the event of legal action

The increased interest in the introduction of quality systems has resulted in the production of a number of international quality standards that are now universally used as the basis of quality system design and implementation. The two key standards that potentially impact on the work of the plant health laboratory community are ISO 9001:2008 and ISO/IEC 17025:2005. Both standards are maintained by ISO, the International Organisation for Standardisation and are administered by Accreditation and Certification Bodies. Both these standards developed from national quality standards for specific industries and then European and International guidance documents.

ISO 9001:2008 is a generic quality management system standard that can be applied right across an organisation. Implementation is demonstrated through;

- A set of procedures covering all the key processes

- Monitoring of processes to ensure they are effective

- Adequate record keeping

- Monitoring of non-conforming work and taking corrective action when necessary

- Regular review of the individual processes

- Facilitating continual improvement

ISO/IEC 17025:2005 covers the requirements to demonstrate the competency of a testing laboratory. There are many commonalities with the ISO 9001:2008 standard but ISO/IEC 17025:2005 adds the concept of technical competency.

The standard is divided into two main sections: management requirements and technical requirements. The management requirements relate to the operation of an effective quality management system and the technical requirements address the competency of staff, methodology and control of equipment. Although the standard is aimed at laboratories carrying out testing the standard has also been applied to the management of research as well genetic resource collections such as genebanks.

3. Accreditation and Certification

Third party assessment of ISO 9001:2008 compliance is demonstrated through Certification by a recognised Certification Body. Examples of companies who act as Certification Bodies include British Standards Institute (BSI), Lloyds Register of Quality Assurance (LRQA) and Bureau Veritas Certification.

Gaining accreditation from a national Accreditation Body such as the United Kingdom Accreditation Service (UKAS) demonstrates compliance with ISO/IEC 17025:2005. Accreditation Bodies are nominated nationally in most developed countries.

4. Quality systems for plant health research and diagnostic laboratories

4.1 Options for Quality Management System

ISO 17025 and ISO 9001 have now been used by genebanks as the basis of their QMS. These quality standards were chosen to be compared here since both their requirements have been shown to meet the operational complexities of the genebank process.

The credibility of test results is critical to peer review, successful publication and technology transfer and uptake. In recent years there has been a push internationally for plant diagnostic laboratories to demonstrate this competence through accreditation to ISO/IEC 17025 and this

has now become a mandatory requirement for laboratory approval by some plant health regulatory authorities.

The activities of a laboratory covered by the accreditation are defined in a scope and this will list the range of materials analysed, the range of organisms detected (e.g. virus, phytoplasma, fungi) and the techniques used (e.g. PCR, RT-PCR or ELISA).

4.2 Issues to consider when establishing a Quality Management System

4.2.1 Quality Standard

Considering quality improvement and assurance as well as the routine of regular updates and efficiency, accreditation is the highest quality option due to the fact that accreditation involves the assessment by technical experts in the specific field.

Certification to ISO 9001 is the next level option in terms of quality, since this does not necessarily involve technical assessments, but assures consistency on the methods and procedures over time. Certification still shows a high level of commitment and can add great value to the quality of the service being provided. Documentation of operational procedures is the minimum expected level of quality system implementation with no regulatory bodies to reinforce the routine implementation of predefined procedures and methods.

4.2.2 Costs

All organisations are constantly trying to improve quality while balancing efficiency and cost. The costs involved in Accreditation or Certification are not insignificant so the organisation needs to be clear the cost/benefit balance of the level of quality assurance introduced. Introduction of the quality system should be linked with assessments of the efficiency of each step of the processes involved in the pest screening and diagnosis to assess if efficiency saving can be made. In terms of effort and the staff costs in the implementation of a quality system the production of validation data is probably the most significant factor when considering this issue.

4.2.3 Effectiveness

Quality systems enforced through the Certification or Accreditation routes stand the best chance full and effective implementation. However, with strong management there is no reason why a good system with documented processes in place could be very effective without third party verification but anecdotal evidence would suggest that these cases are

4.2.4 Risk assessment

The risk of failure of the pest screening and diagnostic system implementation in not detecting significant pests needs to assessed and the systems and methodology employed chosen to reflect this risk assessment. Obviously the higher the economic / social risks need to be covered by very effective quality systems with a proven record of consistent high achievement. The risk assessment should also consider sustainability of the systems in terms of the ensuring equipment maintenance and calibration, the availability of trained staff as well as the funds to maintain the third party assessments.

4.2.5 Motivation

The main driver for establishing a quality system is to formalize systems for effective detection of plant pests in light of reducing the risk of distributing infected material. Staff implementing the system from the highest management down to the laboratory support level need to be aware of this driver and be motivated with the desire to do things right.

4.3 The process of Quality Management System introduction

A careful choice of quality standards to be applied is carried out that meets the structure of the system and purpose of the work. If verification of technical competence and validity is deemed to be critical then this should be demonstrated through application of the ISO Standard 17025 with the assessment and accreditation being carried out by expert technical assessors.

Consideration of the management of the documentation through a web site should be considered since this allows easy access for all staff in different areas of organization as well as the potential for remote access for the Accreditation Body. This has been demonstrated as an incredibly efficient way of working as well as having a cost saving benefit.

The introduction of the quality system should build on the already established procedures and works. Workflows and information recording systems need to be formalized and tools such as bar coding assessed to help sample identity verification. The workflows are the flow of activities of different process, that allow:

- To have a map of view of all activities
- Promote scientist/assistants participation in the workflow documentation
- To monitor activities: detecting long path, bottlenecks and select activity to be improved
- This is also useful for new staff, practitioners and visitors

Audit training of staff needs to be introduced and an audit programme introduced. Systems for monitoring the performance of the equipment and environment conditions have to established. Staff training and competence records need to be established and all equipment brought within an equipment records system.

The effectiveness of the pathogen screening process then requires validation with statistical analysis of the results to show the effectiveness of the systems. Infected and clean materials are passed through the screening process and the results recorded at all stages of the screen. Results are statistically analyzed and the probability calculated of infected plant material escaping the screening procedure.

Accreditation against ISO 17025 standards can then be sought and assessment will be carried out by experts in the field.

4.4 Indicators of successful quality system implementation

The key aspects of the success implementation are:

- The systems in place are highly efficient and bureaucratically very lean

- The staff are involved totally with the implementation of the systems and contribute widely to the documentation involved

- The validation of the processes should helped immensely with improving the efficiency of the pathogen screening process

- The final system should complete staff ownership to allow sustainability and the ability to drive forward improvements for the future

Accreditation from a highly respected body will gives the users of the pathogen screening service a level of confidence that the material is clean that has never been possible before.

5. Specific requirements of ISO/IEC 17025:2005 for laboratories

5.1 Validation of the performance of the laboratory to undertake a specified test

The general process for validation is described below:

- Identify the scope of the test e.g. detection and/or identification of organism x in matrix y by method z considering any specific requirement related to the circumstances of use of the test

- Consider the requirements to determine analytical specificity, analytical sensitivity, selectivity, reproducibility and repeatability performance characteristics and then define the type and constitution of samples needed for the validation.

- Plan and perform the validation for individual performance characteristics or in a combined test setup.

- Present the results in a validation report with a conclusion on whether the validated test meets the requirements of fitness for purpose.

The laboratory should confirm that it can properly carry out the selected standard test, for the intended use. This verification should be repeated if the standard test is changed.

Validation is the production of objective evidence that the methodology being used in the tests is fit for its intended purpose. The process of producing validation data for chemical tests is now clearly defined but is not so clear in the area of plant health diagnostics. Key to this process is

the production of data to demonstrate analytical specificity, analytical sensitivity and repeatability / reproducibility.

A good example of how validation data can be used to demonstrate fitness for purpose is taken from the field of immunoassay.

Perfect Immunoassay:
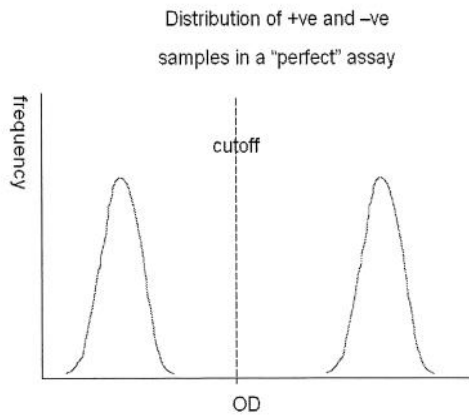
Distribution of +ve and −ve samples in a "perfect" assay

Ideally an assay should separate positive and negative populations completely giving a 100% sensitivity and 100% specificity.

The positive and negative populations should be well removed from the cut-off.
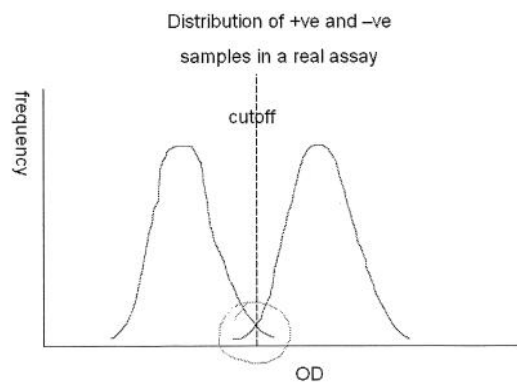
Distribution of real samples

Distribution of +ve and −ve samples in a real assay

In practice the negative and positive distributions of samples will be around the cut-off but may well overlap at the cut-off area. This denotes the possibility of false reactivity of the assay.

The assay needs to be evaluated correctly using a range of different samples with adequate numbers over the whole distribution

Approaches to the validation of plant pest diagnostic testing are being collected in the EPPO guidance document "Specific requirements for laboratories preparing accreditation for a plant health diagnostic activity". This document is in the final stages of preparation and the EPPO website should be consulted to obtain information on availability.

Validation data should be collated in the form of a report. The report should include:

- Purpose of the analysis

- Proposed validation plan

- Validation data

- Discussion on the data produced

Final statement that the method is fit for purpose

5.2 Validation of pathogen testing scheme

Validation is carried out to provide objective evidence that the test is suitable for the circumstances of use. In plant pest diagnosis the test should be fit for routine diagnosis. The minimum test performance criteria to be defined are: analytical sensitivity, analytical specificity, repeatability, reproducibility and if appropriate selectivity. If values for these criteria are not available or accessible (e.g. published sources), the laboratory should produce the missing data or justify why they could not be produced. When values for performance criteria are partly available, the laboratory should verify that it can perform the test according to them. Validation should wherever possible be performed with reference materials including artificially infected/infested samples (spiked samples). When using cultures or isolates for biological tests, care should be taken that they have a proven virulence.

Collected data and results of laboratory-performed validations (in particular related to reproducibility), as well as results of inter-laboratory comparison (evaluation of a test performance), can also provide an indication of the robustness of the test, e.g. to what extent different reagents or altered test conditions affect the established test performance values. These may also provide data on diagnostic sensitivity and diagnostic specificity by comparison to (an) alternative test(s).

Pathogen testing scheme need to be developed and validated to demonstrate a fitness for the purpose of eliminating (within acceptable statistical limits) the risk of spread of economically important pathogens, particularly to developing countries, which often have only poor phytosanitary capacity of their own. The pathogen testing schemes should also support considerable demand for safe planting material for ongoing breeding programs which deliver critical benefits through improved yields, nutritional quality and resistance to diseases, heat and drought to some of the world's poorest farmers. The schemes needs to carefully balance any potential risks of pathogen spread with acceptable delivery times for planting materials and available resources for testing. The scheme needs to also prioritise pathogens of high economic importance as well as those known to be a problem in the specific country. There is also a dichotomy between the relatively well characterized pathogens of crops such as potato and the much less well researched and characterized pathogens of crops such as sweetpotato. The pathogen testing scheme fundamentally should be designed to detect and eliminate the spread of economically significant pathogens rather than their identification per se.

Pathogen testing scheme should be based on the current FAO/IPGRI technical guidelines for the Safe Movement of Germplasm. The scheme for potato was modified in 2006 to include the growing out of a potato plant to at least flowering and to provide two independent methods for the testing of priority pathogens in line with current EPPO guidelines. Additional testing is carried out in line with any importing country special requirements. This document provides evidence for the validation and performance of these methods, particularly the new potato scheme.

Evidence needs to be drawn from as many separate sources of information as possible and reflect the best endeavour but recognises the current lack of any available reference material and systematic proficiency testing in the field of pathogen testing of crops.

Available diagnostic reagents and tests need to be applied to known infected and healthy controls which are serially diluted to demonstrate the end points of detection. Where possible known infected material should be subjected to testing through the entire screening process. Anecdotal evidence where available from colleagues and collaborators in the field and post-entry quarantine testing labs should be assessed to give confirmation of health status and act as additional checks on pathogen detection process. Participation in laboratory ring tests can also give further information on the pathogen detection process performance.

Data from the serial dilutions of infected and healthy sap should indicate that there is a wide margin of safety in relation to test sensitivity. The test pathogens ideally should be detected down to dilutions of approximately 200x less than working dilution. This gives a considerable margin for the detection of low level infections and the potential for sample bulking if required. ELISA values for some viruses are however sometimes low and this is probably a reflection of the difficulty of propagating viruses in specific conditions of the specific growing season or country concerned.

Data from the actual testing of real samples for the detection of plant pathogens needs to shows the expected distribution of a functioning test in terms of the separated populations reflecting healthy and infected plants with a clear separation between the two populations. The effectiveness of the use of different the laboratory tests and bioassays used to complement each other can be assessed again by the testing of real samples and comparing the incidence of positive and negative results. Assessment of the potato testing scheme clearly provided evidence that both an "a" and a "b" test where required and it was also evident that the ELISA tests should be carried out on both in vitro and in vivo material as the two tests clearly complement each other (both detecting samples that the other did not).

The testing validation scheme produced in this manner will provide the best and most direct evidence of the efficacy of the detection procedure for a range of commonly encountered pests.

Work carried out at the International Potato Center (CIP) in Peru demonstrated using infected potato material showed the virus detection system (a combination of ELISA detection (in vitro and in vivo), growing out plants and host ranges plants) had a probability of failure of detection of $p = 0.000039$ or approximately 1 in 25000 chance. Data from the validation experiment was also used to confirm the validity of current threshold levels and also the use of single wells in

ELISA. Anecdotal evidence was also obtained via the feedback from recipients of the CIP in-vitro material of systematic pathogen escapes from detection.

5.3 Staff competency

The demonstration of staff competency is critical to demonstrating ISO/IEC 17025:2005 compliance. When introducing the quality system into long established areas of the laboratory there is normally a general assumption made that the staff performing the testing are competent to carry out the tests. On-going competency of the staff is demonstrated through the day-to-day quality control procedures as well as external proficiency testing where available. When new staff are taken on or when staff are re-deployed to areas where they have no experience then a training procedure will need to be employed and evidence of competence produced.

The training process is normally a four-stage process:

   1. Analyst reads the Standard Operating Procedures

   2. The procedure is demonstrated by a trained analyst to the trainee.

   3. The trainee carries out the procedure under supervision from a trained analyst

   4. The trainee demonstrates competence through the use of reference materials, previously analysed materials and witnessed analysis and is then signed off as competent.

Training records need to be produced for all staff. Records of the competency assessment must be included in or referenced from these training records.

5.4 Internal Quality Control (IQC)

IQC procedures are used to check the performance of the methodology in use. Wherever possible each batch of samples analysed should include some from of IQC monitoring to give feedback on both the method and analyst performance. Obviously there needs to be a balance between the risk associated with things going wrong with the analysis and the level of the IQC included with each set of samples. The key to an effective IQC regime is the availability of quality control (QC) samples that can be taken through the whole analytical process and this can be problematic for some areas of plant health diagnostics where positive control material is difficult to obtain consistently. Where available the quality control material should be included with each batch of samples and the results assessed against the determined acceptable range of the material. This range should be established where possible by the analysis of 20 samples. Data collected from the QC samples should where possible be graphed to identify shifts or trends in the results. Where QC samples have failed the decision making process of acceptance or rejection of the samples associated with the QC sample should be clearly documented, particularly if the decision is made to accept their results and not re-analyse the samples.

5.5 Proficiency Testing

Proficiency testing is an independent and unbiased assessment of the performance of all aspects of the laboratory, both human and hardware. Proficiency testing should not be confused with collaborative trials where the method rather than the laboratory is being tested.

Laboratory proficiency testing is a critical element of laboratory quality assurance. With the increasing demands for independent proof of competence from regulatory bodies and customers, proficiency testing has the potential to be relevant to all laboratories testing plant health in every country.

In proficiency tests the laboratory is encouraged to use its usual method therefore simulating the testing of a routine laboratory sample as closely as possible. Whilst the outcome of the analysis may be dependent upon the choice of method it could also be affected by the performance of the laboratory equipment or the competence of the analyst.

Each participant receives a report which reveals their own laboratory number allowing them to identify their performance assessment. Anonymous results and assessments are also listed for all other participants in the proficiency test allowing a laboratory to compare its performance with other laboratories. Reports also contain information on the methods used by participants.

Laboratories who do not perform satisfactorily in a proficiency test need to take remedial action to identify the cause of the poor performance.

Proficiency test schemes covering the detection and/or enumeration of bacterial, fungal, insect, nematode and viral plant pests and pathogens are in the process of development at the present time. Qualitative proficiency testing will eventually cover bacteria, viruses, nematodes, fungi and insect samples in a range of matrices such as cultures, plant material, DNA/RNA and slides. The proficiency test scheme chosen should be run according to the requirements of the international proficiency tests standard, the ISO/IEC Guide 43-1:1997, through assessment against ILAC G13:2000.

5.6 Validation of Real-time PCR Techniques

Real-time PCR technology (also commonly known as TaqMan® PCR) is based on the theory that there is a quantitative relationship between amount of starting target sequence and amount of PCR product at any given cycle. The kinetics of the reaction are measured via the emission of fluorescence from a labelled probe and this provides distinct advantages over traditional PCR detection: precision, sensitivity, automation and results that are expressed as numbers (rather than gel images).

Real-time PCR is a robust and powerful diagnostic tool that can provide both quantitative and qualitative results. In addition, it allows a generic approach to the way assays are validated, plates are set up and results analysed.

The process of method development and validation includes the following: establishment of the assay/method, reference standard preparation, establishing the limitations of the method and application of the method/assay to routine use.

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5.7 General method validation

A flow diagram summarising the whole process is included in Figure 1

An assay should be reliable and fit for purpose and the validation should be reflected in this. The process of method development and validation includes the following: establishment of the assay/method, reference standard preparation and application of the method/assay to routine use. There are three main areas where data is required (see Appendix 1), performance reliability and specific limitations. The specific limitations of an assay are defined as matrix/analyte (or host/pathogen) combinations where for whatever reason the assay does not perform as well as it should, if at all.
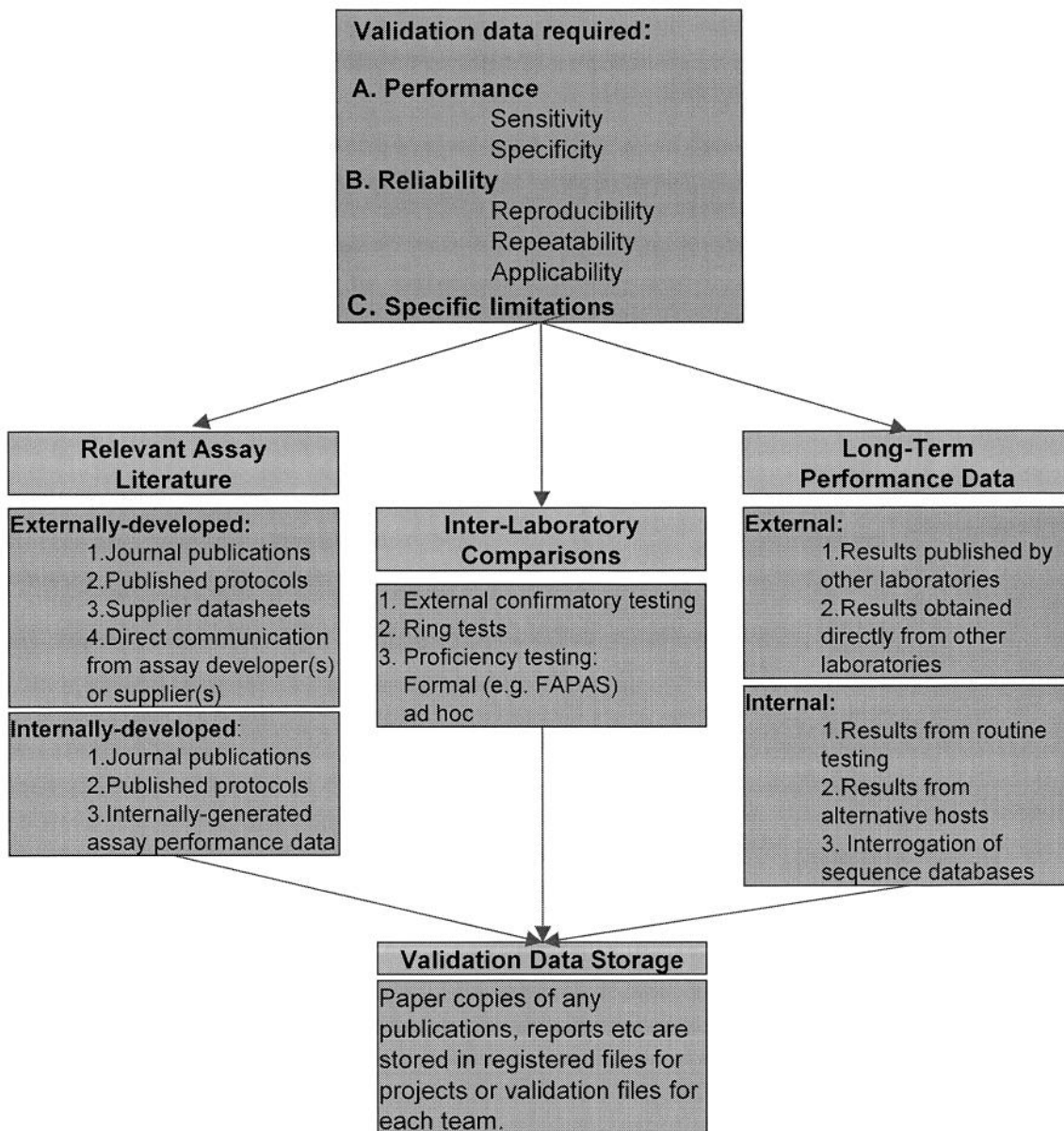
The fundamental parameters for validation include a) sensitivity, b) specificity, c) repeatability/reproducibility and e) applicability (robustness). In addition a limit of detection (LoD) should be established. The LoD is defined as the lowest amount or concentration of analyte in a sample that can be reliably detected. It is important to include this figure when reporting results as it helps to define the limitations of the test.

The techniques used to determine the performance of a method should be one or a combination of the following:

- calibration using reference standards or reference materials;

- comparison of results achieved with other methods;

- interlaboratory comparisons;

- systematic assessment of the factors influencing the result (e.g. host effects);

- assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

Validated methods can be obtained from external sources as well as being developed 'in-house'. Relevant literature on externally developed assays can be obtained from peer-reviewed publications, supplier datasheets and direct communication from the assay developers or suppliers.

Once a method has been validated and is in routine use it is important to record long-term performance data. This data will provide an indication as to whether the assay is still performing to the same standard. If the results appear to be deteriorating then action can be taken to investigate why e.g. reduced quality of reagents or a change in the characteristic of the pathogen. Plant pathogens will evolve and mutate and an important aspect of long term monitoring of PCR performance is the investigation of published sequence data to ensure that the primer/probe sets will detect the necessary range of isolates. This can be done through a combination of ENTREZ and BLAST searches using the NCBI database. Although there are no specific rules about this, it would be prudent to check for sequence misses every 2-3 years or when new information is published e.g. new pathogen isolates.

Validation data required:

A. Performance
- Sensitivity
- Specificity

B. Reliability
- Reproducibility
- Repeatability
- Applicability

C. Specific limitations

Relevant Assay Literature

Externally-developed:
1. Journal publications
2. Published protocols
3. Supplier datasheets
4. Direct communication from assay developer(s) or supplier(s)

Internally-developed:
1. Journal publications
2. Published protocols
3. Internally-generated assay performance data

Inter-Laboratory Comparisons

1. External confirmatory testing
2. Ring tests
3. Proficiency testing:
   Formal (e.g. FAPAS)
   ad hoc

Long-Term Performance Data

External:
1. Results published by other laboratories
2. Results obtained directly from other laboratories

Internal:
1. Results from routine testing
2. Results from alternative hosts
3. Interrogation of sequence databases

Validation Data Storage

Paper copies of any publications, reports etc are stored in registered files for projects or validation files for each team.

5.8 Documenting validation data

A validation sheet has been provided in Figure 2. The purpose of this document is to enable the method developer to gather together the appropriate validation information so that it is easily accessible for end users. In addition to the criteria mentioned above (e.g. sensitivity, specificity), the form also asks for details to help users interpret the data e.g. cut-off points. This information can provide guidance to the end-user in deciding whether an assay has worked well or not.

Validation sheet for PCR-based diagnostic methods

|	
Target Organism:	
Publication details Enter details of publication	
SOP number(s)	
ISO accredited? (Yes/No)	
Intellectual property Are there any restrictions on use?	
Nucleic acid extraction method Procedure for DNA/RNA extraction (or reference to SOP).	
Sensitivity (units) What is the limit of detection?	
Specificity Do these primers cross react with other species? What organisms were the primers tested against?	
Reproducibility Has someone other than yourself got the same results from the same DNA extractions or used this method?	

Repeatability Are the reps close? Do you get the same results from the same extraction?	
Matrices tested What host material was tested (e.g. root, leaves, petioles or soil)?	
Methods for different matrices Fill in if different matrices need different extraction methods.	
Ring tested Has this primer set been used in a ring test? Include brief details of the ring test.	
Proficiency tested Has this primer set been used in a formal proficiency testing scheme?	
Standards/ controls Detail any Certified reference standards or known reference controls needed for the tests.	
Is it Quantitative? Yes or no answer: Is there validation data to support this?	
Statistical analysis on batch testing and/or reporting results. Reference to relevant documentation or spreadsheet.	
Long term monitoring for sequence misses. Describe the frequency and method of checking.	

6. Example of diagnostic test validation - Potato In-vitro Diagnostic Test Validation

Andean Potato Mottle Virus APMoV

Occurrence: S.America

Economic importance: May be significant

Method of detection   DAS-ELISA

                              Host range
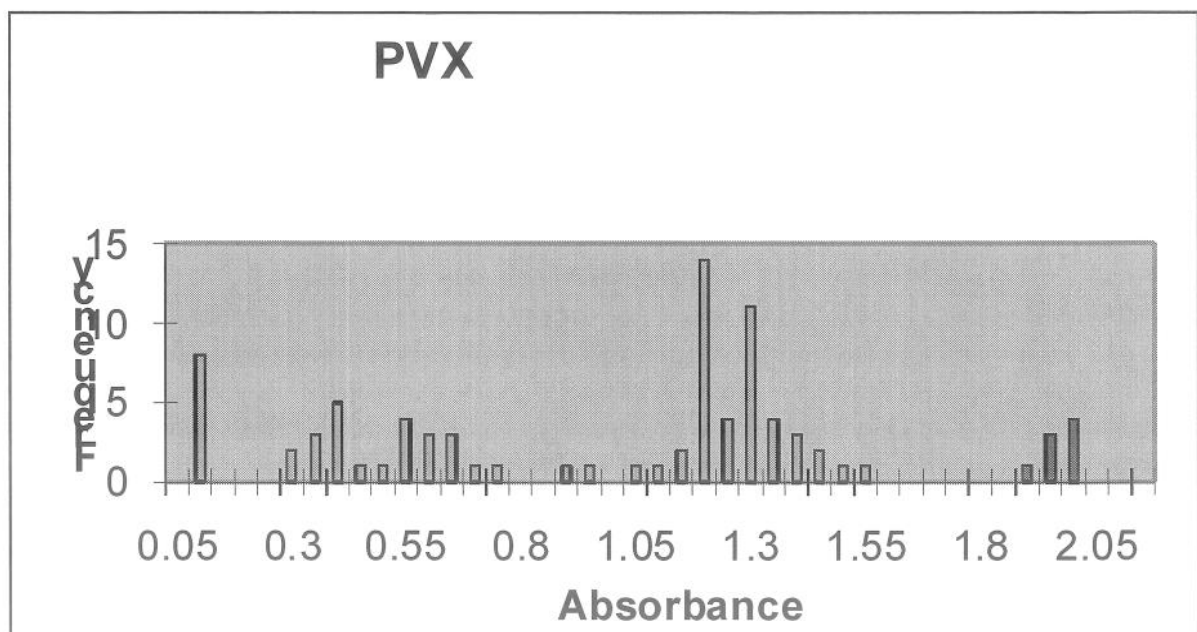
                              Symptoms on grown on plant

Result distribution – In-vivo validation samples infected material
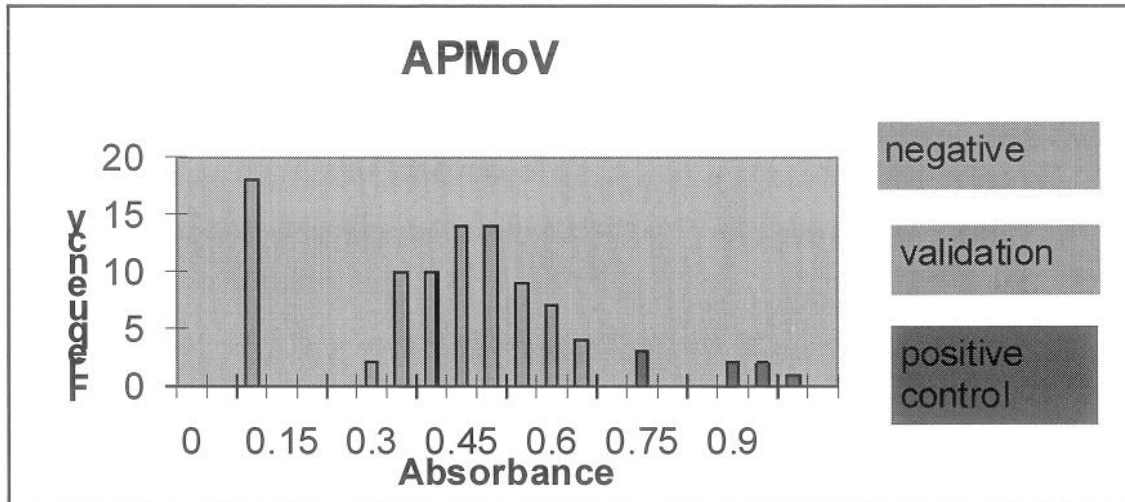
Potato Virus X PVX

Occurrence :   Worldwide

Economic importance : Moderate, losses reach 15 –20%

Method of detection :   DAS-ELISA

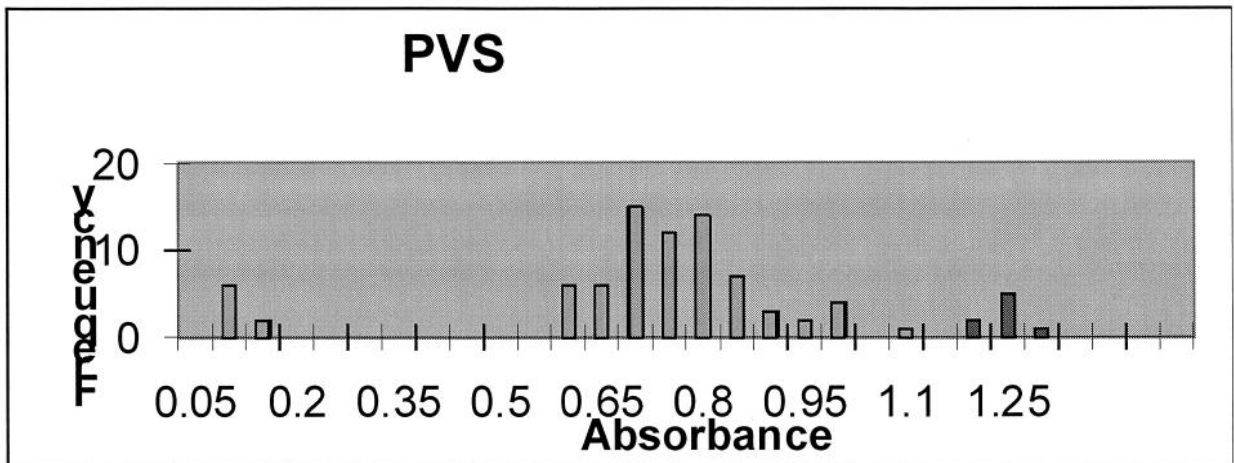   Host range

                         Symptoms on grown on plant

Result distribution – In-vivo validation samples infected material

Potato Virus S PVS

Occurrence :	Worldwide
Economic importance :	Moderate, losses at worst reach 15 –20%
Method of detection :	DAS-ELISA
	Host range
	No symptoms on grown on plant
Source of Antibodies	CIP polyclonal antibody

Result distribution – In-vivo validation samples infected material

PVS

Frequency vs Absorbance (x-axis: 0.05, 0.2, 0.35, 0.5, 0.65, 0.8, 0.95, 1.1, 1.25; y-axis: 0, 10, 20)

Potato Yellowing Virus PYV

Occurrence :	S.America
Economic importance :	Unknown
Method of detection :	DAS-ELISA
	Host range
	Symptoms on grown on plant
Source of Antibodies	CIP polyclonal antibody

Result distribution – validation samples infected material

PYV

Frequency vs Absorbance (x-axis: 0.05, 0.25, 0.45, 0.65, 0.85, 1.05, 1.25, 1.45, 1.65, 1.85; y-axis: 0, 8)
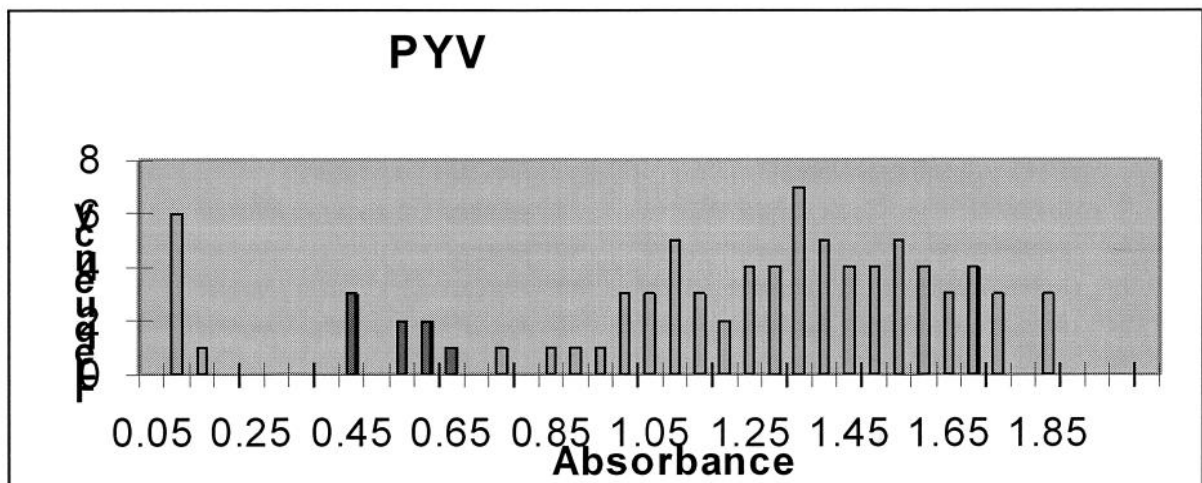
Variation of signal across the ELISA wells

Virus	% CV for ELISA plate well variation											Mean CV
PYV	8.8	22.4	24.5	16.2	18.5	25.9	19.6	18.7	10.8	12.8	15.3	17.6
PVS	14.5	10.1	11.5	13.9	15.3	16.1	14.5	5.7	10.8	11.2	2.7	11.5
PVX	12.7	24.4	9.8	10.4	16.7	30.5	39.0	48.6	40.4	36.8	17.8	26
APMV	18.3	25.6	17.5	13.4	23.0	11.1	8.01	14.4	5.5	12.7		15
APLV	10.8	7.5	8.2	8.8	7.4	12.5	9.1	8.8	7.0	16.5		9.7
PLRV	34.8	14.5	30.1	33.0	27.5	19.4	21.1	15.5	19.5	11.7		22

Threshold Limits and the distribution of positive results

Virus	Threshold Limits x Healthy Control Absorbance		Number of positives within limits %	
PYV	x 1.5	0.14	< 1.5	0.00
	x 2	0.18	1.5-2	0.00
	x3	0.28	2-3	0.71
			> 3	99.29
PVS	x 1.5	0.14	< 1.5	0.00
	x 2	0.19	1.5-2	0.00
	x3	0.28	2-3	0.00
			> 3	100.00
PVX	x 1.5	0.13	< 1.5	0.00
	x 2	0.18	1.5-2	0.00
	x3	0.26	2-3	3.57
			> 3	96.43

APMoV	x 1.5	0.12	< 1.5	0.00
	x 2	0.17	1.5-2	1.59
	x3	0.25	2-3	3.97
			> 3	94.44
PLRV	x 1.5	0.15	< 1.5	0.00
	x 2	0.2	1.5-2	0.00
	x3	0.3	2-3	7
			> 3	93

Assessment of DAS-ELISA Method Sensitivity

Sap from positive control material was extracted and diluted to different levels of concentration. The response of the DAS-ELISA was measured for the different dilutions.

Results of the responses of different viruses and virus strains are given below.
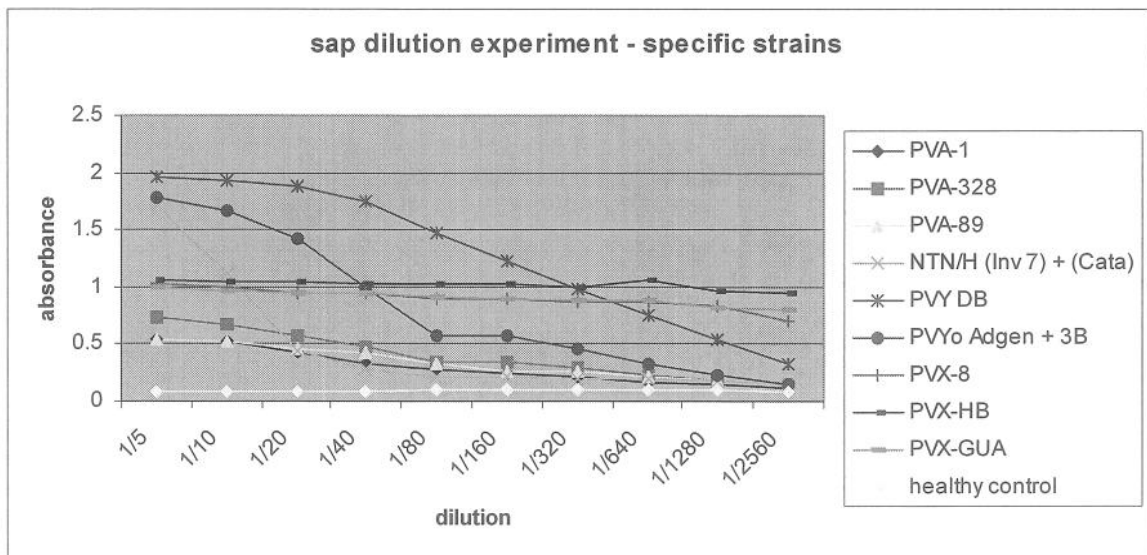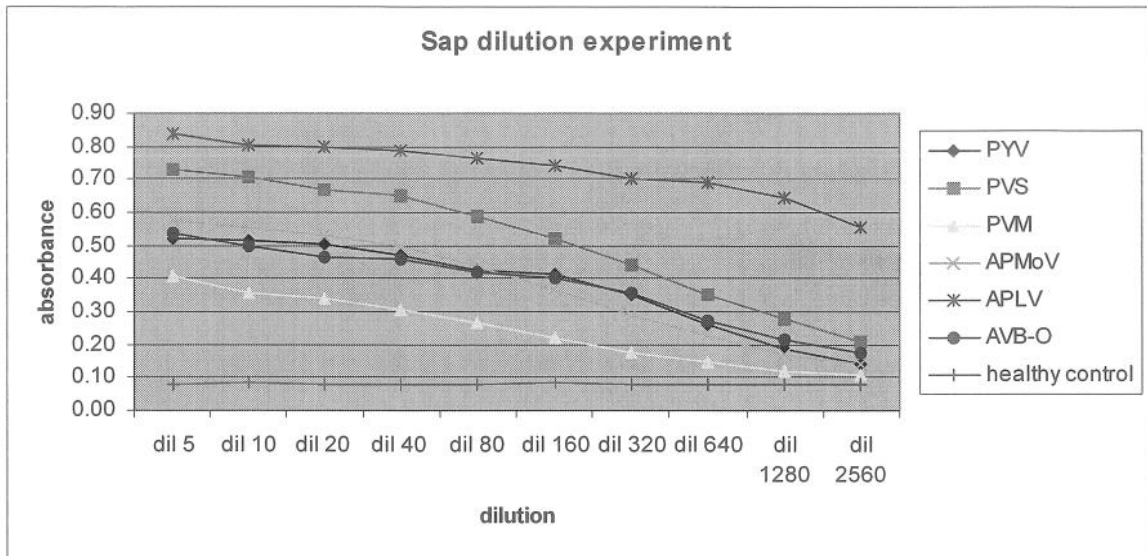
References / Links

ISO/IEC 17025:2005 see http://www.iso.org/iso/Catalogue_detail?csnumber=39883

ISO 9001:2008see http://www.iso.org/iso/catalogue_detail?csnumber=46486

ISO 14001:2004 see http://www.iso.org/iso/catalogue_detail?csnumber=31807

EPPO Technical requirements for laboratories see http://www.eppo.org/ for document availability

ISO/IEC Guide 43 see http://www.iso.org/iso/catalogue_detail.htm?csnumber=27216

ILAC G13 see http://www.ilac.org/documents/ILAC_G13_2000_guidelines_for_the_requirements_for_the_competence_of_providers_of_proficiency_testing_schemes.pdf

Proficiency test providers : FAPAS see http://www.fapas.com/index.cfm