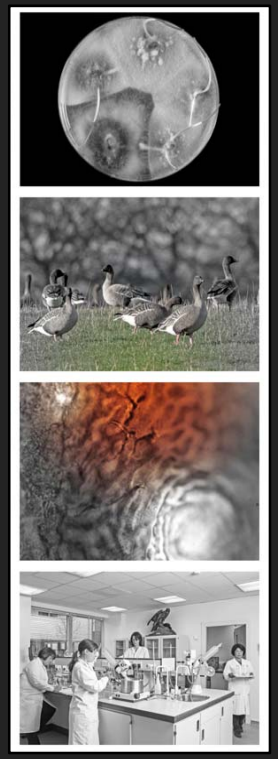
Flexible scope experience in Science and Advice for Scottish Agriculture (SASA), Scotland.

Susan Ross

Quality Manager

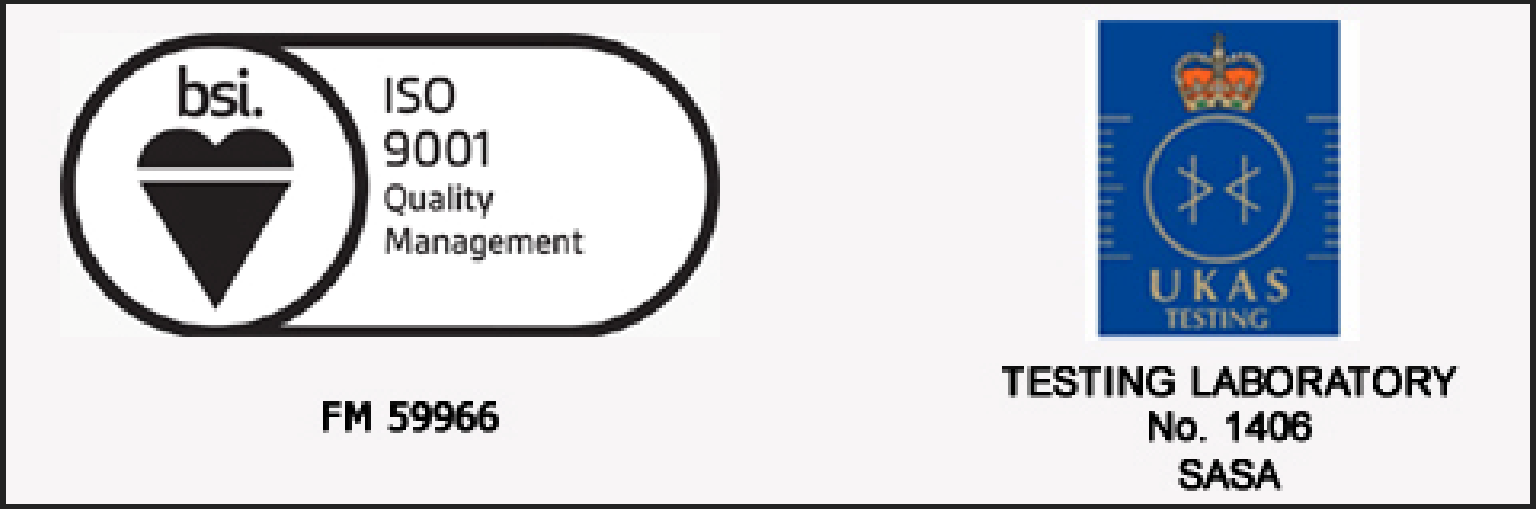
SASA – Who are we and what do we do?



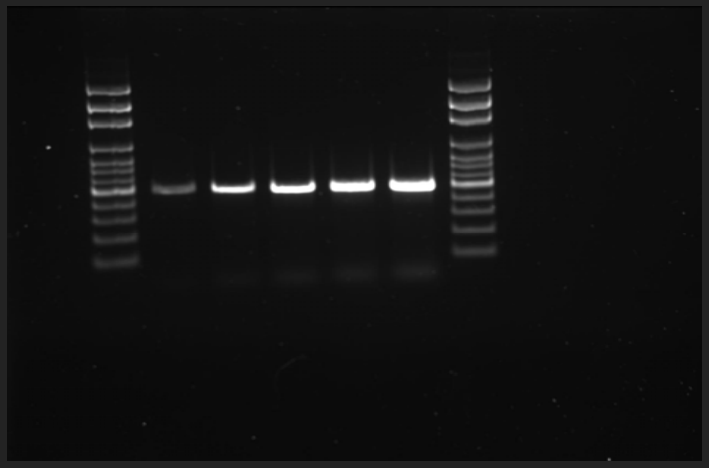
- Pesticides
- Plant Health
- Seed & Ware Potatoes
- Seed Testing & Certification
- Variety Testing
- Wildlife & Environment

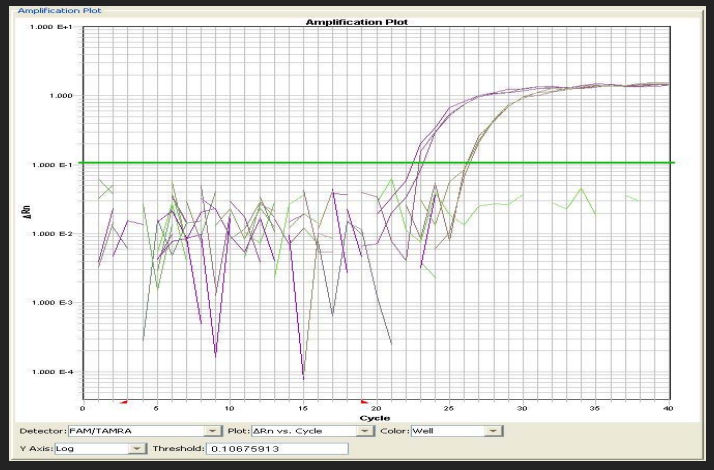
Quality Assurance at SASA

- British Standards Institute (BSI)
- United Kingdom Accreditation Service (UKAS)



United Kingdom Potato Quarantine Unit (UKPQU) , ISO 17025 and fixed scope

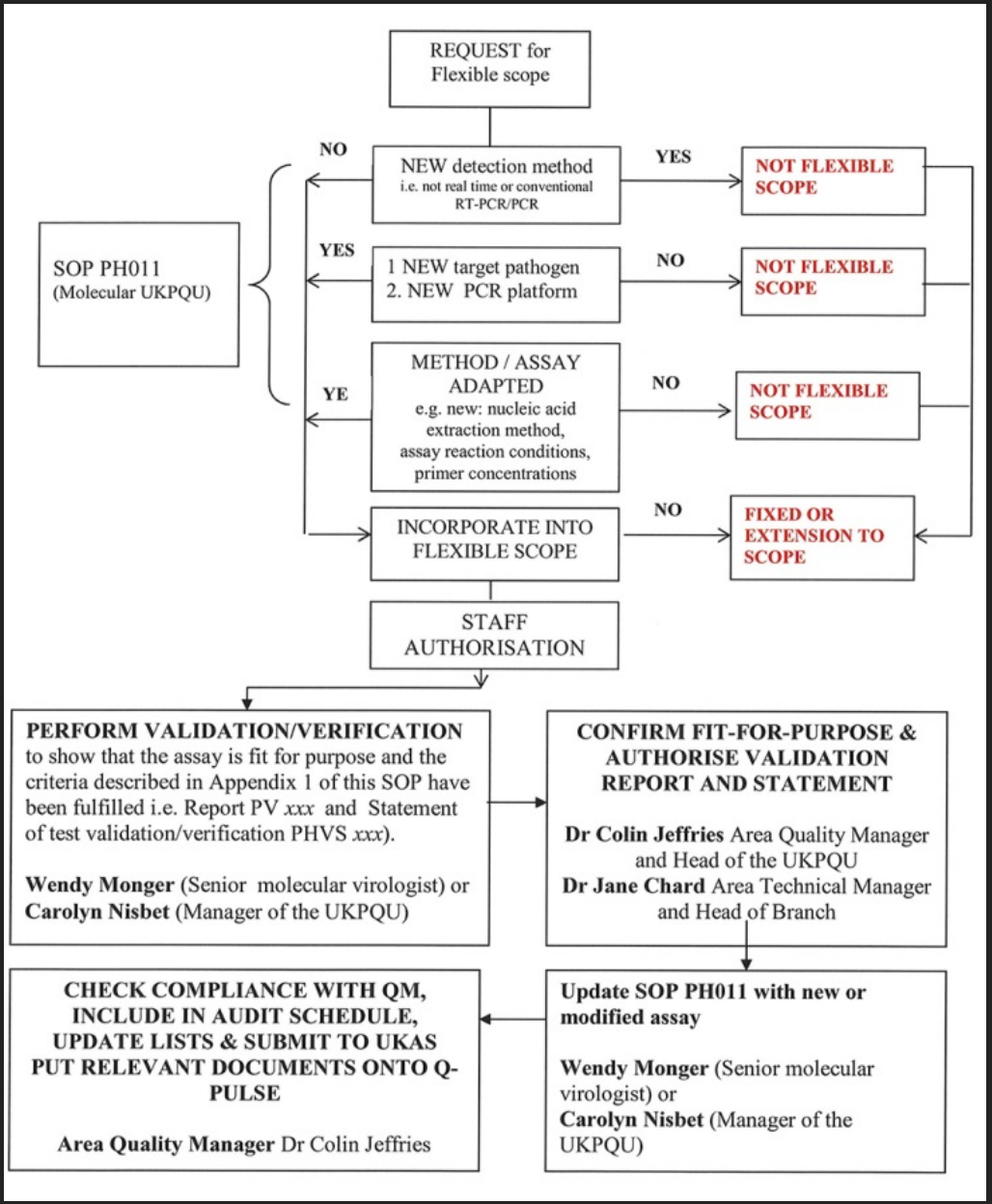




Accredited molecular assays under ISO 17025 fixed scope

Molecular tests for RNA viruses multiplexed with the internal control nad5	
Carlavirus (CarF2b/Not1pdt)	Conventional one-step RT-PCR
Potexvirus (Potex-5/Potex-1RC)	Conventional one-step RT-PCR
Potyvirus (PV2/POT1)	Conventional one-step RT-PCR
Potato yellowing virus (Rd-4F/Rd-4R)	Conventional one-step RT-PCR
Potato yellow vein virus	Real time one-step RT-PCR
Tomato chlorosis virus	Real time one-step RT-PCR
Tomato infectious chlorosis virus	Real time one-step RT-PCR
Tobacco rattle virus	**Real time one-step RT-PCR**
Molecular tests for DNA viruses multiplexed with the internal control (COX)	
Begomovirus (AV494/AC1048)	Conventional one-step PCR
Curtovirus (BCTV2F/BCTV2R) (specifically *Beet curly top virus*)	Conventional one-step PCR

UK PQU Flexible scope: Management flowchart



UKPQU Flexible Scope - Validating Flexible Scope

- Validation or verification of molecular tests: conventional and real time RT-PCR (PCR) as fit for purpose by the UKPQU follows (with deviations, see Table 1) the processes described in the EPPO standard *PM 7/98 (2): Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity.*

- Flexible scope validation data and statement was presented to UKAS for *Tobacco rattle virus* (TRV) and accreditation gained in 2016.

How do we carry out validation?

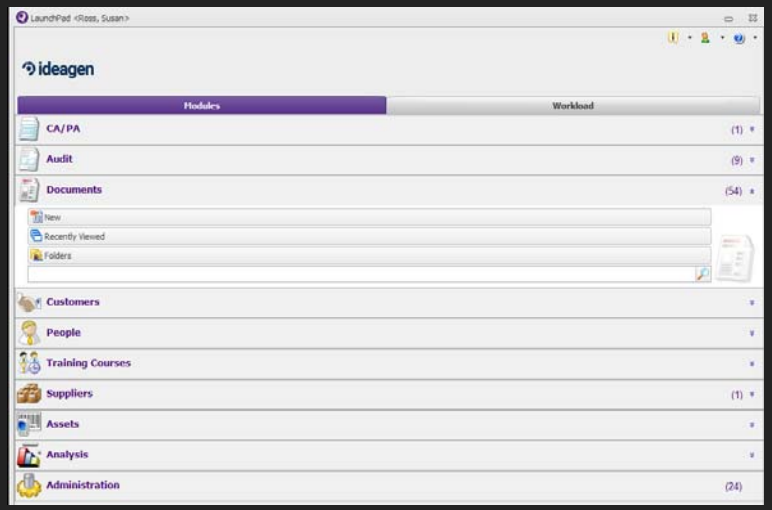
Criteria	UKPQU SASA	EPPO	UKPQU deviation from EPPO and reason for deviation or comment
Analytical sensitivity **(Relative sensitivity)**	Because the concentations of viroids, viruses, 'Candidatus Liberibacter solanacearum'and phytoplasmas are not known determine the maximum dilution of RNA/DNA detected. This is a relative sensitivity.		
	For a minimum of 2 different nucleic acid extractions prepare 5 serial dilutions of target RNA/DNA in potato nucleic acid 1:10, 1:100, 1:1000, 1:10,000, 1:100,0000. (The number of serial dilutions may be reduced or increased if previous experiments have indicated that other dilutions are more appropriate).	Perform at least three experiments with serial dilutions. If consistent results are not obtained after three series, additional series should be prepared and tested. Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardised,e.g. brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions.	It is not clear what is meant by experiment therefore this has been defined as different nucleic acid extractions. It is also not clear what is meant by consistent results since in practice there may be a considerable difference in sensitivity between experiments because virus concentration in each sample is not known and may vary between samples. Due to this variability, in practice 2 different nucleic acid extactions are sufficient for assessing sensitivity. Sensitivity is of more relevance when comparing assays e.g with and without nad5 as the internal control.
Analytical specificity	Carry out in silico comparisons of primer/probe sequences to sequencs of target on the NCBI database to determine whether there are mismatches at critical positions that may affect detection. Support by testing against several different isolates of target and if indication that primers may detect non target from in silco studies test against these non targets (if obtainable) if likely to be present in the sample.	Analyse (i) a range of targets and (ii) relevant non-targets, covering genetic diversity, different geographic origin and hosts, in particular those that might be present in the sample material. For non-targets, the concentration of nucleic acid should be high enough to maximize the possibility of cross reaction but remain realistic. In addition, the test results can be supported by 'in silico' comparison of probe/primer sequences to sequences in genomic libraries.	We have put the emphasis on the non detection of isolates of the target using in silico studies Detecting non target that is likely to be in the sample is less of an issue. The primers (or at least one) are designed to target conserved regions and will therefore only detect specific target or targets. Positive samples will always be confirmed by sequencing therefore cross reactions will not lead to misdiagnosis.
Selectivity	To determine the whether potato variety affects the sensitivity prepare serial dilutions of target RNA/DNA in potato nucleic acid from 3 to 5 potato varieties (e.g. serial dilution 1:10, 1:100, 1:1000, 1:10,000, 1:100,0000 or with only a medium or low level of target	Determine whether variations of the sample material (e.g. by using different cultivars of the host plant) affect the test performance.	We use nucleic acid rather than sap because this is easier to manage. It is unlikely to affect the result. We use a serial dilution since this provides additional evidence for selectivity. However a medium or low level of target may sometimes be used

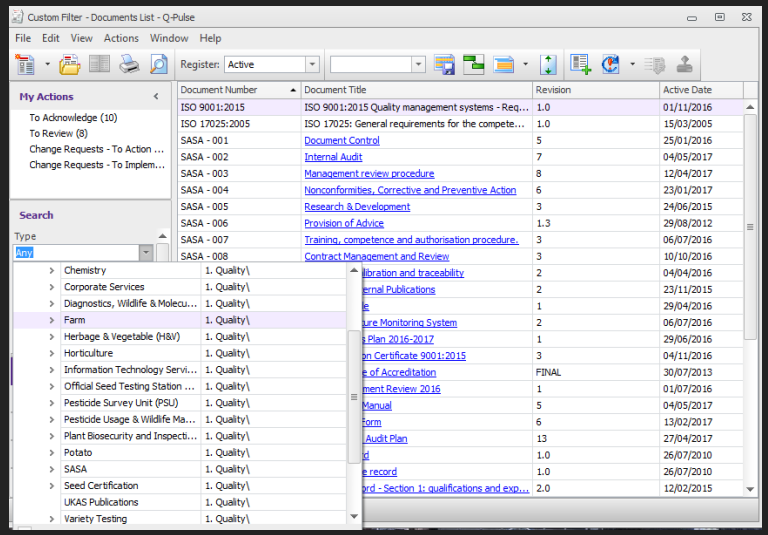
How do we carry out validation continued

Criteria	UKPQU SASA	EPPO	UKPQU deviation from EPPO and reason for deviation or comment
Repeatability	**Conventional RT-PCR (PCR) For one of the serial dilutions above repeat twice (or repeat twice using a dilution with only a medium or low level of target as judged by band brightness.) The repeats should be done simultaneously and within a short time frame i.e 24 h of the original sample dilution. Occassionally a longer time frame may be used but this runs the risk that if there has been sample degradation repeatability will not be achieved and the experiment would have to be done again and 3 replicates tested simultaneously.** **Real time RT-PCR (PCR) For the serial dilutions (or dilutions with only a medium or low level of target organism) the triplicate wells used per sample are considered replicates for repeatability.** **For repeatability the spread of Ct values should be no more than 3**	**Analyse at least three replicates of sample extracts with a low (relative) concentration. If consistent results are not obtained, additional replicates should be prepared and tested.** **(Perform at least three simultaneous tests on the same material with low levels of target).. Artificial subsamples created from one sample can be used.**	**Normally serial dilutions are used rather than only a medium or low level of target organism. This gives additional information on repeatability**
Reproducibility	For one of the serial dilutions repeat (or repeat using only a medium or low level of target organism) but with a different operator. This should be done on the same day as that done by the first operator (if nucleic acid degeneration is likely to affect the result) or within a time frame dependant on nucleic acid stabilit. For conventional RT-PCR (PCR) all dilutions should be detected by all operators and for real time the Ct values should be within 3 Cts	As for repeatability but with different operator(s) if possible, on different days and with different equipment when relevant	As for repeatability but no replication is done for conventional RT-PCR/PCR. For real time the mean of the triplicate wells is used to assess reproducibility.We also have more flexibility when the sample is tested since this is dependant on sample degradation.

Implementing Quality Assurance at SASA

- Q-Pulse software for document control
- Standard operating procedures
- Internal Audits including Systems Overview
- External assessments via BSI and UKAS
- Management Review Meetings
- In house quality control





How does SASA demonstrate expertise?

- Proficiency tests – Viroids and viruses
- Annual in-house competency checks
- Technical experience within specific areas and involvement in R&D projects – EUPHRESCO Virus Collect and Virus Collect 2
- Publications on subject area

Summary – Thoughts on Flexible Scope

PROS

- Allows reporting of a result from a test as accredited in advance of a scheduled extension to scope visit.

CONS

- Limited by boundaries as set out in LAB 39.
- Does not take into account reduction in uncertainty of detection if using several different methods for testing.

Thank you

- Colin Jeffries - Chief potato quarantine and plant health consultant (SASA)

- Wendy Monger - Senior Plant Pathologist (UK PQU, SASA)