



# UK Standards for Microbiology Investigations

Evaluations, validations and verifications of diagnostic tests



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

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## Amendment table

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Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	8/16.03.17
Issue number discarded	4.3
Insert issue number	5
Anticipated next review date*	16.03.2020
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Title of the document has been updated. Document has been strengthened to include uncertainty of measurement. The addition of diagram 1 and table 1 have simplified the terminologies used in the document by giving examples.
References.	References updated.

\*Reviews can be extended up to five years subject to resources available.

## UK SMI<sup>#</sup>: scope and purpose

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### Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

### Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

### Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

### Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health

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<sup>#</sup> Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

### **Patient and public involvement**

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

### **Information governance and equality**

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

### **Legal statement**

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested citation for this document**

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## Scope of document

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This guidance note describes each stage in carrying out validations and verifications of diagnostic methods. A method may be a new or modified commercial kit, an in-house method or reagent, or a set of reagents bought separately and used to prepare an in-house method. This document also includes guidance on the principles of in-house evaluations.

For more information on CE marking, refer to the [IVD Directive](#) and for more information on quality assurance, refer to [Q 2 - Quality assurance in the diagnostic virology and serology laboratory](#).

This UK SMI should be used in conjunction with other UK SMIs.

## Introduction

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A key role of the laboratory is to decide which tests should be offered, and to select the most appropriate method. The performance specifications of any new or modified laboratory method are integral to providing a high quality service. Depending on the circumstance it will be necessary for the laboratory to perform either an evaluation, validation or verification of a new or modified method. Each of these terms is described in greater detail below.

Evaluation aims to quantify the performance of a method in relation to an existing method while validation is an evidence-based assessment of how a test performs in the laboratory, and demonstrates suitability for intended purpose. Verification is described as the confirmation of whether or not a product (for example an in-house assay or commercial kit system) complies with a validated method, regulation, requirement, specification, or imposed condition such as environment, computer software upgrade which could affect the performance of the test or initial pre-settings of the product, coupled with other equipment or staff operators.

Validation and verification are both integral requirements for the accreditation of laboratories according to ISO 15189 and ISO 17025; the British Standards Institution (BSI) and the United Kingdom Accreditation Service (UKAS) use the term 'validation' when demonstrating fitness for purpose of a method rather than using the term 'verification' which is used to describe the performance of the test or process in a particular setting<sup>1,2</sup>. These require that all examination procedures should be validated or verified (as appropriate) for their intended use prior to adoption, and the methods and the results obtained recorded<sup>3</sup>.

### Definitions of common terminologies used in diagnostic methods<sup>4</sup>

#### In-house diagnostic tests

In-house tests have been designed and developed in a laboratory and are not distributed or sold to any other laboratories. In-house tests, including molecular diagnostics, are used as the exception, where other tests are not available or due to cost constraints particularly for rare tests which cannot be purchased commercially.



## Off-label diagnostic tests

These are diagnostic commercial tests which comply with the IVD MD Directive 98/79/EU (that is CE-marked) but where the user does not comply with the manufacturer's instructions for use in some significant way for example replacement of a reagent, change of procedure or use of a different sample type. It also includes commercial kits that have been modified for a clinical purpose for which it has not been designated by the manufacturer to suit a laboratory's need. These tests are not in entirety CE-marked and will need to be validated to ensure that they are fit for purpose by the laboratory before putting into routine use.

Other examples include commercial tests which are not CE-marked but rather sold for research use only where the results are used to support clinical decisions or disease management. 'Research use only' tests typically carry manufacturer's limitations and must be used in accordance with the guidelines described by the manufacturer.

## Commercial diagnostic tests

These are diagnostic tests which have been developed and validated by the manufacturer as well as regulated by CE marking. All tests results reported are validated by the manufacturer in compliance with the regulations around the award of CE mark.

For more information, on commercially produced kits, please refer to [IVD Directive](#).

## Evaluation<sup>5</sup>

Evaluation is a generic term used to describe the measurement of the performance capabilities of a system/test method. This is a systematic and extensive process that compares different systems/test methods designed to perform the same or similar functions. Examples of evaluations within microbiology include comparison of different methods designed to detect the same marker/target, comparison of different culture media to isolate the same organism, or comparison of different equipment with the same function. Where two kits have equivalent performance characteristics, the one which is easier to use, cheaper, faster or requires a more easily obtainable sample might be preferred. Examples of evaluation scenarios can be seen in Table 1.

The objectives should be simple and within the capabilities of an evaluation site. Attempting to answer too many questions at the same time can result in practical difficulties, less accuracy in data recording/collection, and a failure to achieve all the objectives set. A checklist of points to consider for equipment and kit evaluations is summarised in Appendix 1.

## Validation<sup>5</sup>

According to ISO 15189:2012, validation is defined as "confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled"<sup>3</sup>. It examines the whole process that is being used to check that results are correct and consistent. To document this ability, each laboratory should produce a Validation File for each method or system which has not been validated formally. The file will include a range of information and will have a different emphasis depending on whether the laboratory is using a modification of commercial systems or has developed an in-house system. Typically the file will include sections such as validation data, tests on known samples, workbooks and relevant publications.

Validation should be performed in the following scenarios before introduction into routine use – non-standard methods, lab designed/developed methods, standard methods used outside intended scope and validated methods subsequently modified.

- when the conditions under which an original validation was done changes (for example use of an instrument with different characteristics or samples within a different carrier matrix)
- when the performance of existing methods has been shown to be unsatisfactory or
- whenever the method is changed or modified beyond the original specification. For example, use of a different sample type or commercial kit that has been modified for a clinical purpose for which it has not been designated by the manufacturer to suit a laboratory's need.

The examples above should be considered significant changes that require revalidation with adequate evidence for equivalent performance before implementation routinely<sup>4</sup>.

Validated test methods or equipment do not need any further validation after it is done once. Ongoing fitness for purpose is monitored through the laboratory's quality assurance which may include personnel competency assessment, quality control, proficiency testing, etc. More validation scenarios can be seen in Table 1.

It should also be noted that validation is performed on in-house diagnostic tests to validate sensitivity, specificity, precision and, in case of a quantitative test, linearity<sup>6</sup>.

The intention of validation is to provide documented evidence that a diagnostic test is performing within required specifications and fit for purpose. This may involve results of experiments to determine its accuracy, sensitivity, specificity, reliability, repeatability reproducibility, and uncertainty of measurement. A validation may be extensive, for example to validate a newly developed in-house method, or narrow in scope, for example to validate a commercial method which is already in use and has had minor modifications. For methods already in use for which no specific existing validation is in place, it is important to provide documentary evidence which supports reasons for their use. It is usually sufficient to prepare a file based on historical evidence, such as results from comparisons or other studies undertaken, copies of published papers, EQA, IQA and IQC results etc. Work book records can be cross referenced if appropriate in the validation report. Refer to Appendix 2 for a summary of what a validation report could contain and Appendix 3, for a checklist of points to consider during kit or reagent validation.

### **Verification<sup>3</sup>**

ISO 15189: 2012 defines “verification as the confirmation, through provision of objective evidence that specified requirements have been fulfilled”. It can also be described as the confirmation of whether or not a product (for example commercial kit system or equipment) complies with a regulation, requirement, specification, or imposed condition.

Verification should be performed in scenarios such as when a laboratory wants to introduce a new validated commercial method/equipment with defined performance (from manufacturer) for routine use or in a case where a previously validated method is modified and then revalidated before use. The laboratory verifies their ability to

achieve acceptable results with the method or system in question. More examples of verification scenarios can be seen in Table 1.

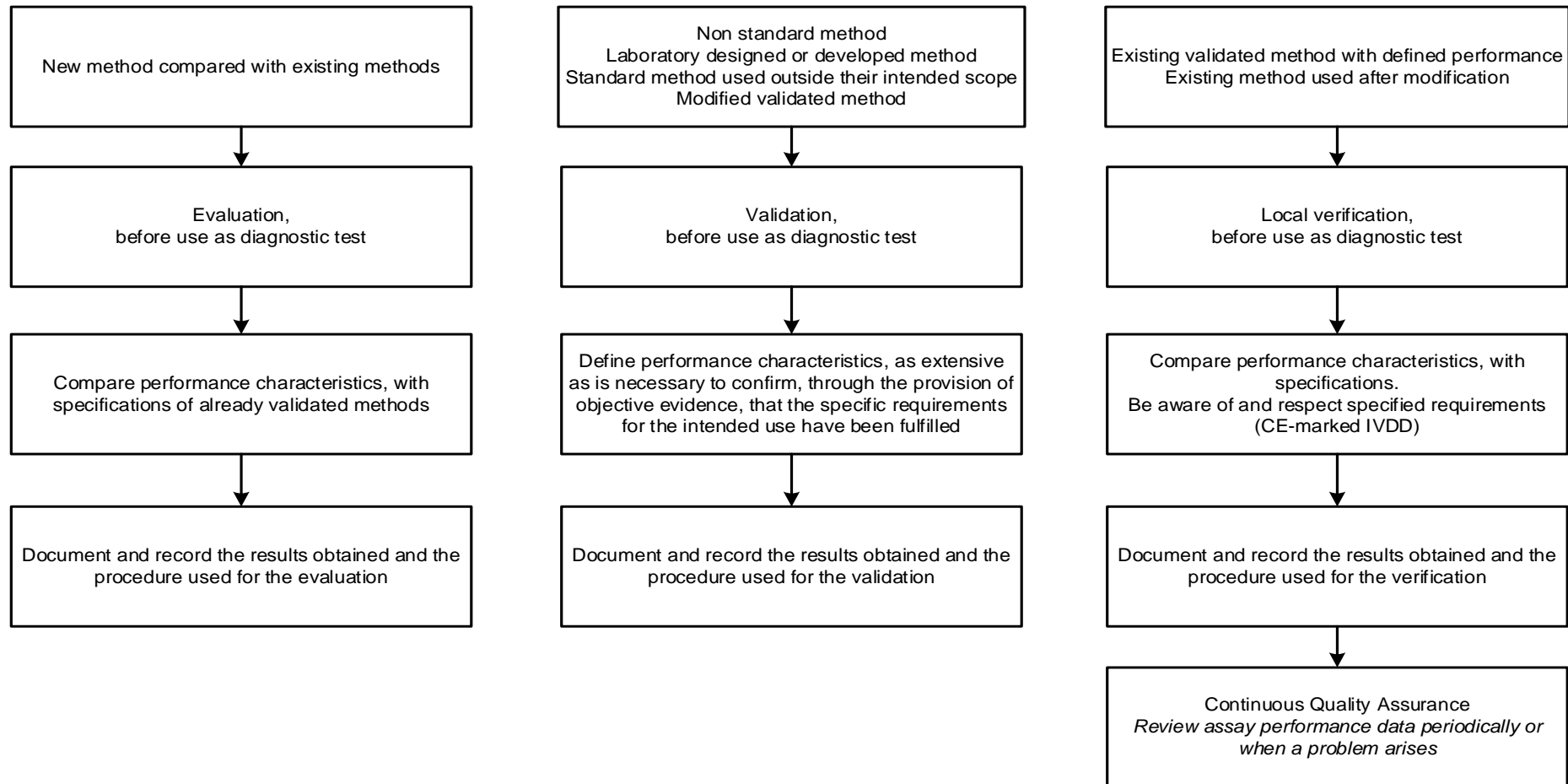
It should be noted that verification is done through performance parameters and it varies considerably with the type of test method/equipment being verified. It is an ongoing process.

The purpose of verification is to confirm whether or not a product (for example a validated method, commercial kit system or equipment) is complying with a regulation, requirement, specification, or imposed condition. The minimum tested attributes in verification may include accuracy, precision and linearity but are not limited to these. Refer to Appendix 4 for a summary of what a verification report should contain.

**Note:**

Validation of commercial assay kits as well as the equipment used should be performed by the manufacturer to ensure that they achieve the stated performance. The user should obtain this information from the manufacturer. However, verification should be performed by the user, confirming through review (published and unpublished evaluations, EQA data, etc.) and testing that the equipment and the commercial kits meet the written specification requirements<sup>1,6</sup>.

## Diagram 1: Evaluation, validation and verification of diagnostic methods flowchart



This flowchart is for guidance only.

**Table 1: Table showing examples of evaluation, validation and verification of diagnostic methods**

Examples	Evaluation	Validation	Verification
<b>Example 1</b>	New method compared with existing methods: Evaluating the results of using a new chromogenic commercial media for isolation of ESBL producing Enterobacteriaceae and then comparing with MacConkey agar currently used. This is done to compare their specificity and sensitivity.	<b>Revalidation of modified assays:</b> an example is where the use of ethidium bromide in gel electrophoresis for visualisation of DNA in agarose or acrylamide gels is replaced with the use of SYBR Green which is not carcinogenic or toxic. This should be revalidated to ensure that it works well in the assay.	<b>Existing method with defined performance:</b> If a laboratory is considering the use of a validated commercial kit for an assay based on published literature with validation data, some form of verification will be need to be done by the laboratory to determine whether the assay complies with either the manufacturer's kit or the author's assertions.
<b>Example 2</b>	Identifying <i>Clostridium perfringens</i> genes using real time PCR and then comparing these PCR results to the conventional PCR methods (gel electrophoresis) used.	<b>Standard method used outside their intended scope:</b> Acridine orange stain is used for staining of <i>Trichomonas vaginalis</i> smears but where used outside its scope for example in the staining of clue cells, laboratories should ensure that this is validated.	<b>Existing method used after modification:</b> using the first example above in the validation column, after revalidation of a modified assay is done; it should still be verified by the user to ensure that it gives the desired results.
<b>Example 3</b>	Evaluation of PCR results for <i>Clostridium perfringens</i> genes using two different commercial PCR kits.	<b>Laboratory designed methods/assays:</b> An example is where a laboratory develops a method for purifying a heavily contaminated plate using the alcohol shock. The concentrations used for this procedure should be tested, validated and formally introduced into the relevant SOPs.	<b>Acquiring of new equipment/machinery:</b> Validation and verification of new equipment used should be performed by the manufacturer to ensure that they achieve the stated performance; however, verification should also be performed by the user, confirming through review and testing that the equipment meets the written specification requirements.

**Note:**

Examples of modification in diagnostic tests/equipment as mentioned in Table 1 are;

- change in specimen handling, incubation time, temperature
- change in specimen or reagent dilution
- replacement of a critical component of an equipment or reagent used
- using a different calibration material (or changing the manufacturer's set-points)
- change or elimination of a procedural step
- change in the cut-off or method of calculating the cut-off for semi-quantitative assays
- Any change in intended use such as
  - different sample matrix (for example serum vs CSF)
  - using test for another purpose (for example screening vs diagnostic)
  - changing the type of analysis used (for example qualitative results reported as quantitative)

## Parameters used in evaluations, validations or verifications of diagnostic methods<sup>7,8</sup>

The parameters that should be taken into account during evaluations, verifications or validations of diagnostic methods are as follows:

### Sensitivity<sup>1</sup>

Sensitivity is the ability of an assay under evaluation to identify correctly true positive (reference assays positive) samples. Therefore, sensitivity is the number of true positive samples (a) correctly identified by the assay under evaluation divided by the total number of true positive samples (for example those positive by the reference assays) (a+c), expressed as a percentage as indicated in the table below. It is expressed as:

$$\text{Sensitivity} = a/(a+c)$$

### Specificity<sup>1</sup>

Specificity is the ability of an assay to identify correctly true negative (reference assays negative) samples. Therefore, specificity is the number of true negative samples (d) correctly identified by the assay under evaluation, divided by the total number of true negative samples (for example those negative by the reference assays) (b+d), expressed as a percentage. It is expressed as:

$$\text{Specificity} = d/(b+d)$$

### Positive predictive value (PPV)

PPV is the probability that when a test is positive, the specimen does contain the designated pathogen. It is expressed as:

$$\text{Positive predictive value (PPV)} = a/(a+b)$$

### Negative predictive value (NPV)

NPV is the probability that when a test is negative, the specimen does not have the designated pathogen. It is expressed as:

$$\text{Negative predictive value (NPV)} = d/(c+d)$$

### Calculation of sensitivity and specificity<sup>9</sup>:

		True positive specimens	True negative specimens	Total
Results of assay under evaluation	positive	a True-positives	b False-positives	a+b
	negative	c False-negatives	d True-negatives	c+d
	Total	a+c	b+d	a+b+c+d

**Note:** these parameters are highly population dependent and influenced by the prevalence of disease. Predictive values are always affected by prevalence while the terms 'sensitivity or specificity' are better considered as inherent to the assay and will only be affected if the population is qualitatively rather than quantitatively different. For

example, if two populations have the same prevalence but with a different proportion of people in the very early phase of infection (with a low microbial load), the sensitivity but not specificity of the assay will be different. In contrast in a situation where populations have different prevalences but similar microbial loads in the infected cases, then the sensitivity and specificity are likely to be the same. Predictive values will be different in this case.

## Uncertainty of measurement (UM)

Uncertainty of measurement can be defined as “a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand”. It is essential for the correct interpretation of a result and matters when results are close to a specified limit. The ISO 17025:2005 which covers the accreditation of calibration and testing laboratories outlines specific requirements for laboratories to evaluate and report uncertainty of measurement. This has also been introduced into the ISO 15189 standard to which UKAS assesses.

For microbiology tests, the uncertainty of measurement will apply to measured results, in particular zone sizes or MICs, microscopy of fluids, including cerebrospinal fluids (CSFs) and urines, quantitative and semiquantitative organism counts, for example cultures in Continuous Ambulatory Peritoneal Dialysis (CAPD) fluids and urines. There are other variables that could affect a result which does not entail an actual measure such as specimen collection, specimen preparation, transport time and conditions, media inoculation, incubation temperature, etc<sup>2</sup>. Non-numeric tests such as subjective plate reading are also important in UM.

For more information on uncertainty of measurement, see [Q 2 - Quality assurance in the diagnostic virology and serology laboratory](#).

## Precision<sup>6</sup>

Precision is “closeness of agreement between results of replicate measurements”. It could also be defined as level of concordance of the individual test results within a single run (intra-assay precision) and from one run to another (inter-assay precision). Precision is usually characterised in terms of the standard deviation of the measurements and relative standard variation (coefficient of variation). It is often expressed as the percent coefficient of variation (%CV), where:

$$\%CV = (\text{standard deviation of measurements} / \text{mean}) \times 100$$

## Linearity<sup>6</sup>

Linearity is defined as the determination of the linear range of quantification for a test or test system. Using laboratory equipment as an example, linearity in simple terms defines how well the device's actual performance across a specified operating range approximates a straight line. However, in terms of a test, linearity is achieved when measured results are directly proportional to the concentration of the analyte (microorganisms or nucleic acid) in the test sample, within a given range. Linearity is usually measured in terms of a deviation, or non-linearity, from an ideal straight line and it is typically expressed in terms of percent of full scale, or in ppm (parts per million) of full scale.

Linearity testing challenges the entire equipment calibration range, including the extremes, and can detect problems such as reagent or equipment deterioration earlier than quality control or proficiency testing failures. It should also be noted that it is



good laboratory practice to periodically demonstrate linearity to detect reagent deterioration, monitor equipment performance, or re-confirm linearity after a major servicing of equipment. Refer to Appendix 5 for a worked example of linearity.

### **Accuracy<sup>10</sup>**

Accuracy is the closeness of agreement between the value obtained from a large series of test results and an accepted reference value. In microbiology, the factors that determine the degree of accuracy are as follows:

- the uniformity of microbial load in the sample
- the accuracy of equipment
- the volume of sample/ reagents used for testing
- the media used and the incubation conditions
- the reading and interpretation of results by staff performing the test
- operator error

The last two factors both require training and competency of staff.

### **Reproducibility<sup>11</sup>**

Reproducibility is the ability to produce essentially the same diagnostic result, under different conditions (different operators, test batch, different apparatus - laboratory or validated ancillary equipment, different laboratories and/or after different intervals of time).

**Note:** The higher the number of variables the more robust a test must be in order to achieve this.

### **Repeatability<sup>11</sup>**

Repeatability is defined as the closeness of agreement between the results of successive measurements of the measurand (quantity intended to be measured) carried out under the same conditions of measurement by the same operator in the same laboratory over a short period of time, over which the underlying value can be considered to be constant. This may be expressed quantitatively in terms of the dispersion characteristics of the results.

### **Reliability<sup>12</sup>**

Reliability is the ability of a system or component to maintain performance within the manufacturer's stated specifications over time. The level of downtime considered acceptable is likely to vary between systems.

### **Analytical sensitivity<sup>10,13</sup>**

The analytical sensitivity of an assay is defined as the ability of the assay to detect very low concentrations of a given substance in a biological specimen. It is also known as the "limit of detection".

### **Analytical specificity<sup>10,13</sup>**

Analytical specificity is the ability of an assay to detect only the intended target (for example antibody, organism or genomic sequence) and that the quantification of the

target is not affected by cross-reactivity from related or potentially interfering nucleic acids/components or specimen-related conditions. The two aspects of analytical specificity are cross-reactivity and interference. This assessment is qualitative in that the choice and sources of sample types, organisms and sequences reflects the test purpose and assay type.

### **Limit of quantification<sup>12</sup>**

This is the smallest measured content from which it is possible to quantitate the analyte with an acceptable level of accuracy and precision.

### **Limit of detection<sup>8,10</sup>**

The limit of detection is a measure of the analytical sensitivity of an assay and an important characteristic that must be determined for both quantitative and qualitative tests. It is the lowest actual concentration of analyte in a specimen that can be consistently detected (for example, in 95% of specimens tested) with acceptable precision, but not necessarily quantified, under routine laboratory conditions and in a defined type of specimen.

# 1 General considerations when carrying out evaluation, validation or verification

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Some basic guidance to describe core requirements for validation and verification (and maybe evaluations) for routine laboratories are stated below. If multiple laboratory sites are involved, the requirements may vary. For more information, refer to Appendix 6 which is generic and all may not apply to every organisation. Bearing in mind that studies/projects may be either small, big or even collaborations between different organisations, it may be used as it applies to the individual organisations' projects. The core requirements are as follows:

- Identifying the **personnel** that will be responsible for the project. This will include the project manager and the key staff in the laboratory that will be involved in the project. The responsibilities of all personnel involved should be defined in the protocol.
- Identify **computing requirements** such as software requirements, transfer or manipulation of data
- Preparing a **project design plan** and defining the purpose and objectives of the project. This plan will include any training requirements, risk assessments and COSHH assessments, standard or reference materials, etc
- Identify any **potential commercial companies** whose products that may be used in the project
- Identifying any **potential problems** that may be encountered in the project and addressing these
- Measuring and comparing all aspects of **costs** associated with implementation of the test
- Determining a suitable **time scale for the project** in advance before it commences. This depends on a number of factors such as statistical sample size, time needed to optimise test procedures to the time it takes to becoming approved, etc

## 2 Statistical analysis

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### Statistical advice

Although statistical analysis is not usually required until the end of a study, it is essential to obtain advice at the outset of study design from a statistician on sample size and the data collection system and documentation. The main statistical methods to be used in analysis should be identified at the planning stage. This will help ensure that the study has sufficient power to meet all its objectives and that the data are collected in the most appropriate form for an efficient and timely analysis. It is advisable to identify a statistician to provide any necessary advice during the study.

Preliminary analysis of results should not be undertaken except by the project statistician. Any premature communication of results could lead to bias being introduced into the study, thus undermining the reliability of the conclusions.

## Statistical sample size

For the study to have sufficient power to meet all its objectives, that is to detect the smallest important differences or estimate test characteristics sufficiently precisely, it must use an appropriate number of samples for microbiological testing. This is the study sample size in statistical terms.

The study sample size and the assumptions made to obtain it should be part of the evaluation protocol. The study sample size will identify the number of specimens or tests to examine and how it is calculated will depend on the magnitude of the test characteristics to be estimated or compared, that is sensitivity and on the precision required or the smallest important difference to be detected.

Whereas information on likely outcomes may exist, there may not be enough information at the planning stage on which to base a calculation of the size of study needed. In these cases, a pilot study may be used or alternatively a deferred estimate by the project statistician could be made once the study is underway using the results from the early specimens. The statistician should advise on how many results are required for this estimate.

Where it becomes apparent that very large numbers would be required to show that a small but important difference is statistically significant (as may be the case when isolation rates are very low), the benefits of continuing the study need to be addressed carefully.

Rabenau et al reported that a representative number of specimens (positives, low positives, and negatives) must be tested in parallel. Results of the new test or test system and those obtained from the existing gold standard test at the time are compared to assess their performance<sup>6,10</sup>. The actual number of specimens needed for each part of the evaluation, verification or validation study may vary depending on the test system and the laboratory's testing volume. More importantly, the impact of the test result needs to be taken into account when determining an appropriate sample size for the verification of an externally-validated test. For example, a diagnostic test for an infection with known modest sensitivity or specificity (regardless of test type or platform) may require a small sample size in each category of positive, negative and equivocal/borderline; on the other hand, a new diagnostic test for a critical test, for which other high-performing tests are available and in use (for example tests for diagnosis of most blood-borne viruses) will require a robust verification process, particularly for equivocal/borderline results.

It is key that laboratories consider testing challenging borderline or difficult organisms from samples in different scenarios as well as rare pathogens when performing evaluation/validation/verification work<sup>10</sup>. Laboratories may explore collaboration with other laboratories when performing validation or verification of a novel new test/technology, which should be performed on a large scale in order to provide suitable confidence in the predictive value of results obtained. This will enable collection of a suitable number of samples to attain the required power for diagnostic validation as well as a much more comprehensive investigation of the critical parameters relevant to the specific technology to provide the highest chance of detecting sources of variation and interference<sup>14</sup>.

**Note:** It is not the responsibility of the UK Standards for Microbiology Investigations to stipulate the number of samples to be tested by laboratories as the requirement is dependent on a wide range of factors, the size of the project by the intending

laboratory, which will in turn include the nature and performance of the test, the critical parameters, the way in which the test will be used in practice and the confidence level required for clinical use.

This applies to evaluation, validation and verification.

## Data collection

The more complex data collection is, the less likely it is to be accurate. Data recorded at the bench should be entered to record sheets either manually or electronically.

Record sheets should be simple and, whenever possible, be reduced to a series of “tick-boxes” or simple key strokes. Some form of data entry audit is necessary.

Results should be read and recorded independently, without influence of one method on another (the identity of specimens should be anonymised at the time of reading so the reader is “blind”).

Entry of data to the main study database should be the responsibility of the local study co-ordinator. Although spreadsheets are often simple to set up and use, a database should be designed which limits the options available for entry and thereby reduces the likelihood of incorrect or incomplete entries. The type of database used should be determined based on the size of the study involved.

This applies to both evaluation and validation.

## Analysis of results

The main statistical analysis planned should be specified as part of the study protocol.

Results should be assessed statistically against the criteria specified in the protocol which may include:

- all or specific aspects of the methods, kits or equipment
- available product information
- relative workload
- sensitivity/specificity
- measuring of uncertainty during calculation and interpretation of results where relevant
- user acceptability
- clinical relevance
- cost-benefit analysis
- health economics

The design of the study, the statistical analysis and the conclusions of evaluations should be peer reviewed.

Where two methods give similar isolation rates, one might have significant benefits over the other. Benefits include fewer false-positive/false-negative results, lower costs associated with the method or test, and less labour involved in performing the method or test.

This applies to evaluation, validation and verification.

## 3 Documentation of evaluations

### Documents required for evaluations

The main body of the report should cover all details as outlined in the protocol design. The format and level of detail will vary with the study. Broadly, a report will include title, authors, location, summary, introduction, materials and methods, results, conclusions, discussion, bibliography, and appendices. Other documents that may be required or are essential for evaluations are:

- evaluation checklist
- correspondence with manufacturers/suppliers (for example agreed loan of equipment, modifications to 'Instructions For Use', comments on evaluation report)
- agreement/contract with manufacturer/supplier
- agreements with collaborating laboratories
- published and unpublished papers and reports
- protocol
- specimen panel details
- statistical advice/analyses
- manufacturer's product 'Instructions For Use' (IFU)
- invoices (where applicable)
- equipment details, service and maintenance
- workbooks (evaluation results and supplementary/confirmatory tests)
- results
- adverse incident forms (where applicable)
- report edits/reviews/final version. These reports cannot be used as the only evidence in a file since evaluations establish kit performance only at a particular point in time and within a particular evaluation setting
- review meeting minutes

Complete the appropriate summary report form, the accompanying checklist (see Appendix 1) and if the key information is already documented, it is not necessary to transcribe it to the form, it is sufficient to cross-reference.

### Availability of the evaluation report

The availability of the report will depend on the nature of the evaluation and any contractual agreement with manufacturers/suppliers.

A commissioned evaluation of equipment, assay kit or media not yet marketed may be made available only to the commissioning supplier. If the supplier uses the evaluation to develop pre-production equipment further it would not be appropriate to publish the data widely. If the equipment is to be marketed as evaluated, or if it is already being

marketed, it is unacceptable for the report to remain confidential. The supplier should be allowed to comment on the report before it is published.

### **Publication of evaluation**

Results of any new, well performed evaluation may merit publication in a peer-reviewed journal. The work is thereby given further credibility by peer-review, duplication of effort is avoided and laboratories are given valuable information on which to assess best practice. Publication of results should be sought without delay.

## **4 Documentation of validations and verifications**

### **Documents required for validations**

A file should be produced for all existing as well as new methods, and may include a summary of and reference to existing data which are likely to be recorded in workbooks, papers and reports. A file is also required for modifications to existing methods.

All documents relating to validation must be filed in a retrievable and auditable manner. There should be a file where all related documents are either stored or cross-referenced. Some of the documents used for validation may apply for verification.

Documentation includes review meeting minutes, workbooks, worksheets, methods used, published and unpublished papers and reports, manufacturers' instructions, previous test results, and any other supporting information. This file may contain some or all of the following information, as appropriate:

- Internal Quality Control (IQC) data
- Internal Quality Assessment (IQA) data
- for in-house methods, research and development carried out during the development of the procedures
- results of testing known positives, known negatives, low and high positives and samples which are known or likely to be problematic (possibly carried out before the introduction of the test to the department)
- published and unpublished papers and reports
- work books (especially applying to in-house testing)
- work carried out with collaborating laboratories
- comparisons with alternative methods
- comparisons with previously used test methods
- evaluation reports. These reports cannot be used as the only evidence in a file since evaluations establish kit performance only at a particular point in time and within a particular evaluation setting
- manufacturer's instructions
- manufacturer's product specification
- health and safety information (data sheets, COSHH, Risk Assessments etc)

- relevant SOPs

Complete the appropriate summary report form and the accompanying checklist (see Appendix 2 and 3). If key information is already documented, it is not necessary to transcribe it to the form. It is sufficient to cross-reference.

The Project Manager must review the data, complete the checklist, and sign the section to authorise release of a new or modified kit or reagent or to assure that sufficient information has been provided to confirm that a method already in use is performing within the manufacturer's specifications.

All SOPs relating to modified or new kits, reagents or equipment must be reviewed to verify that processes are in line with current procedures. It may be necessary to maintain new or revised SOPs as working drafts while their contents are being validated. SOPs should be authorised as fully controlled documents when the study has been completed.

### **Documents required for verifications**

Documentation includes review meeting minutes, workbooks, worksheets, methods used, published and unpublished papers and reports, manufacturers' instructions, previous test results, and any other supporting information. This file may contain some or all of the following information, as appropriate:

- External Quality Assessment (EQA) data (several years, as available)
- Internal Quality Control (IQC) data
- Internal Quality Assessment (IQA) data
- in-house methods, research and development carried out during the development of the procedures
- results of testing: known positives, known negatives, low and high positives and samples which are known or likely to be problematic (possibly carried out before the introduction of the test to the department)
- published and unpublished papers and reports
- work books (especially applying to in-house testing)
- work carried out with collaborating laboratories
- comparisons with alternative methods
- comparisons with previously used test methods
- evaluation reports
- review meeting minutes
- manufacturer's instructions
- manufacturer's product specification
- summary of the raw results/evidence/ as well as known and added limitations
- health and safety information (data sheets, COSHH, Risk Assessments etc)
- relevant SOPs



Complete the appropriate summary report form and the accompanying checklist (see Appendix 4). If key information is already documented, it is not necessary to transcribe it to the form, it is sufficient to cross-reference.

## Appendix 1: Evaluation report summary for the evaluation of a new or modified diagnostic equipment, kit or reagent

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Due to the diversity of what can constitute an evaluation, it is impossible to be prescriptive about the information required. The following section gives some sample forms, plus more detailed suggestions of things to consider.

### EVALUATION PROCESS FORM

(1) Title of evaluation:

#### (2) Project team

Role	Name	Laboratory	Area of expertise*
Project Manager			
List other key individuals			

\* for example statistician, molecular scientist, HIV serology

(3) Background and purpose of evaluation:

(4) Brief details of evaluation design, including “gold standard” method:

#### (5) Target completion

Evaluation phase	Target date
Preparation/Setup	
Technical	
Report	

#### (5) Summary of specimen panel composition:

Specimen category/type	Number

#### (6) Relevant SOPs

SOP number	Title

#### (7) Relevant COSHH and Risk assessments

Number	Title


**(8) Cross-reference all other related documents associated with this study**

(list can be added to and deleted from as appropriate)

For example

- Evaluation checklist
- Correspondence with manufacturers/suppliers (for example agreed loan of equipment, modifications to 'Instructions For Use', comments on evaluation report)
- Agreement/contract with manufacturer/supplier
- Agreements with collaborating laboratories
- Published and unpublished papers and reports
- Protocol
- Specimen panel details
- Statistical advice/analyses
- Manufacturer's product 'Instructions For Use' (IFU)
- Invoices (where applicable)
- Equipment details, service and maintenance
- Workbooks (evaluation results and supplementary/confirmatory tests)
- Results
- Adverse incident forms (where applicable)
- Report edits/reviews/final version
- Review meeting minutes

**(9) Diary** (include dates of all important events, such as review meetings)

Event	Date
Project start	
Project end	

**(10) Conclusions** (include brief summary and lessons learnt)

Complete evaluation checklist before completing the authorisation section below

**EVALUATION AUTHORISATION SECTION**

**The assessment/evaluation has fulfilled its aims as stated in section 3**

**Comments:**

**Signed (Project Manager)**

**Date** \_\_\_\_\_

**Post-evaluation: A change in practice is desirable which will be instigated following validation/ no further action required at this time\***

**\*Delete as appropriate.**

**Comments:**

**Signed (Project Manager)**

**Date** \_\_\_\_\_

## EVALUATION CHECKLIST

### 1) Evaluation planning and setup:

- |  |   |
|--|---|
| a) Proposal prepared                                   | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |
| b) Key kit/system information obtained                 | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |
| c) Specimen panel obtained                             | Yes <input type="checkbox"/> No <input type="checkbox"/> ongoing <input type="checkbox"/> |
| d) Collaborators/stakeholders identified and recruited | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/>     |
| e) Protocol written and approved                       | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |
| f) Target dates defined                                | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |
| g) Costings prepared and funding agreed                | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/>     |
| h) Risk and COSHH assessments completed                | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |
| i) Training date(s) arranged                           | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/>     |
| j) Kits/reagents and equipment ordered/access arranged | Yes <input type="checkbox"/> No <input type="checkbox"/>                                  |

### 2) Technical assessment:

- |                                     |   |
|-------------------------------------|---|
| a) Acceptance testing <sup>1</sup>  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| b) Performance testing <sup>2</sup> | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| c) Retests/confirmatory testing     | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/> |
| d) Report any Adverse incidents     | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/> |
| e) Usability comments               | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |

### 3) Data analysis, report & archive

- |  |   |
|--|---|
| a. Data checks   | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| b. Results analysis  |   |
| o Sensitivity  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| o Specificity  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| o Reproducibility  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| c. Write 1st draft report                                  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| d. Vertical audit  | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| e. Review and revise as appropriate                        | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/> |
| f. Sign off by project leader/manager                      | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| g. Manufacturer comments                                   | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |
| h. Final evaluation report (publish, distribute, web link) | Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input type="checkbox"/> |
| i. Archive data, emails, report copies etc                 | Yes <input type="checkbox"/> No <input type="checkbox"/>                              |

1: Acceptance testing – does the kit/component/equipment perform as described in the manufacturer's literature in your laboratory? (a 'trial run')

2: Performance testing – testing specimens as described in the agreed protocol

**Next step: validate any new or modified practices**

**Comments:**

**Signed:**

**Date:** \_\_\_\_\_

## AREAS FOR CONSIDERATION

## **Details of equipment**

### **Equipment details including:**

- make, model, manufacturer (is this a trial model, or are any modifications planned?)
- manufacturer and supplier name and address
- purpose of equipment
- principle of operation
- technical operation of the system
- physical specifications – dimensions, weight, electrical requirements, additional features
- recognition by any official international regulatory body, for example FDA, CE mark
- availability of COSSH and risk assessments available for the system, reagents and any aerosols produced by the system

### **Ownership and acquisition of equipment:**

- who owns the equipment?
- how has the equipment been purchased or leased?
- is there a contractual agreement for the purchase of consumables?
- is there an arrangement to assess the machine?

### **Equipment maintenance / service continuity**

- is maintenance contract required?
- cost of maintenance contract
- level of user maintenance required
- records of down time from a laboratory which has been using the system for at least 1 year? (where applicable)
- annual service down time, and routine servicing and maintenance to be undertaken by trained laboratory staff
- response times of the company service engineers
- time to replace the system in the event of a catastrophic failure? Can the tests be run manually if necessary? Will another local laboratory be able to take on the testing for a short time?

### **Level of automation provided**

- throughput of the machine (for example expressed as specimens or tests/hour)
- number of samples/plates run at any one time
- range of incubation temperatures available
- reusable or disposable parts (for example tips for reagent additions)

- closed system or operator programmable
- bar-code reading ability (for example for data entry or reagent batch details)
- does the machine check that sufficient volumes of the reagents etc are available
- method of data capture for example wavelength of spectrophotometric reader

### **Computing information**

- operating system and software
- can the software be interfaced with the laboratory diagnostic software to enable exchange of worksheets and results?
- specimen entry by hand and/or bar-code
- is there on-line help?
- can the user modify the software?
- specify the walk-away time associated with a test run; will the machine run to conclusion from the start of the walk-away time?
- can the equipment run safely overnight?
- total method time
- can the software analyse results?
- does the system maintain a dated audit trail at any level?
- is access to the system password controlled with tiered levels of access?

**The method of equipment installation (by the supplier or the user) should be recorded. Any problems encountered during installation should be recorded.**

### **Details of kit/reagent**

#### **Kit/reagent details including:**

- name, description and purpose
- manufacturer/supplier name and address
- format (for example number of tests per kit/per run, number of controls per kit)
- lot/batch number; expiry date
- principle of kit/reagent
- technical details of the system
- associated automated equipment requirements
- additional requirements
- incubation times and conditions
- time to availability (for example currently available or under development)
- suitability for automation

**Kit/reagent presentation and packaging:**

- usefulness of product insert
- adequate safety information provided
- container and reagents clearly labelled
- Are lot numbers, expiry dates and other important details easily located?
- is the manual clear and unambiguous – can it be used as an SOP?
- does it fulfil requirements for COSHH assessment?

**Manufacturer/supplier support**

- has the level of company support been satisfactory?

**Safety considerations**

- did any additional hazards arise throughout the course of the evaluation which were not identified during the initial risk assessment?
- has the manufacturer done everything possible to control the risks?

**Documentation, training and support**

These should be assessed in terms of:

- quality of documentation provided - is it comprehensive, accurate, unambiguous and easy to follow?
- ease of learning to use the equipment/kit
- need to refer back to manuals or the supplier because it is unclear what should be done at any stage
- ease of user modifications (if appropriate)
- possibility of modifications by the manufacturer and likely costs to the user
- need for interpretation by the user
- training provided by the manufacturer's representative
- grades of staff required to operate the equipment. The staff involved in the evaluation should include those expected to perform the tests routinely, under the supervision of senior staff
- other support required: was it adequate, timely and satisfactory?

**Results and data analyses**

Raw data should be appended to the report (if appropriate). The results should be presented in detail, and analysis of results presented.

**The following points should be addressed:**

- identify the positive and negative features of the equipment/kit/media
- has the equipment/kit/reagent performed satisfactorily?

- any changes in performance compared to the previous/current equipment/kit/method used
- improvements in consistency
- improvements in turnaround times
- evidence that the kit/equipment is inadequately sensitive or specific
- reproducibility of results
- stability of reagents
- ease of result interpretation
- would you recommend the use of this equipment/kit/reagent or method to other laboratories?

**Problem log (equipment/kit)**

- detailed records should be made of any problems, faults or supplier interventions
- observations on any aspect of the system should be recorded in a log book to be completed daily by anyone using the equipment

**Faults may be recorded in terms of:**

- nature of fault
- time/date observed
- time/date reported to supplier
- response time of supplier
- outcome



## Appendix 2: Validation report summary for the introduction of a new or modified diagnostic kit or reagent

1) Brief description of the method:

### 2) Project team

Role, for example statistician, lab. worker	Name	Laboratory	Area of expertise for example, statistician, molecular scientist, HIV serology
Project Manager			
List other key individuals			

3) Purpose of method and background, including reason for introduction:

4) Brief details of method validation plan:

### 5) Relevant SOPs

Number	Title

### 6) Relevant COSHH and other risk assessments

Number	Title

### 7) Cross-reference all other related documents associated with this study

(list can be added to and deleted from as appropriate)

EQA data  
 IQC data  
 IQA data  
 In-house R&D records  
 Results of testing:  
   known positives  
   known negatives  
   low positives  
   high positives  
   problem samples

## Evaluations, validations and verifications of diagnostic tests

Published and unpublished papers and reports  
Work books (especially applying to in-house testing)  
Work carried out with collaborating laboratories  
Comparisons with alternative methods  
Comparisons with previously used test methods  
Evaluation reports  
Review meeting minutes  
Manufacturer's instructions  
Manufacturer's product specification

### 8) Diary (include dates of all important events, such as review meetings)

Event	Date
Project start	

### 9) Conclusions (include brief summary)

**Complete validation checklist before completing the authorisation section below**

### VALIDATION AUTHORISATION SECTION

**This method is suitable for diagnostic use**

**Signed (Project Leader)**

**Date** \_\_\_\_\_

**Introduction of method authorised**

**Signed (Project Manager)**

**Date** \_\_\_\_\_

## Appendix 3: Diagnostic kit or reagent validation checklist

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### 1) Validation of method:

- i. Has a comparison with existing methods (currently or previously in use) taken place? Yes  No
- ii. Has the performance in EQA and/or IQC schemes been evaluated? Yes  No
- iii. Has the method been assessed by an IQA scheme? Yes  No
- iv. Has all or part of this work been published in a peer-reviewed journal? Yes  No
- v. Are there any other reports related to the method available, for example project reports, manufacturers literature? Yes  No
- vi. Are test results available for samples which challenge the performance of the method? Yes  No
- vii. Are work books cross referenced? Yes  No
- viii. Has the test been validated by collaborating laboratories? Yes  No
- ix. Are comparisons with alternative methods available? Yes  No
- x. Are evaluation reports available for this test? Yes  No
- xi. Has the assay been costed? Yes  No
- xii. Is the assay to be distributed outside the organisation that created it? Yes  No   
 If YES, has CE marking been affixed to the reagent? Yes  No

### 2) Have the following method characteristics been evaluated:

- i. Sensitivity Yes  No
- ii. Specificity Yes  No
- iii. Reproducibility Yes  No
- iv. COSHH assessment of method Yes  No
- v. Risk assessment of the new procedures, equipment etc. Yes  No

### 3) Have customers been informed of significant changes in

**method performance:** for example sensitivity, specificity, turn-around times Yes  No

Has the User Manual been updated? Yes  No  N/A

Comments:

## Appendix 4: Verification report summary for the introduction of a previously validated test method or procedure

<b>Name of test or procedure verified:</b>	
<b>What kind of work is this report describing?</b> Is it Prospective, Retrospective, Concurrent or Re-validation / verification?	
<b>Report Author (Name and Role):</b>	
<b>Date of Report:</b>	

**1) Brief description of the method:**

### 2) Project team

Role for example statistician, lab worker	Name	Laboratory	Area of expertise for example statistics, molecular science,
Project Manager			

**3) Purpose of method and background, including reason for introduction:**

**4) Brief details of method verification plan:**

### 5) Relevant SOPs, COSHH and other risk assessments

Number	Title

### 6) Aims of Verification

Mention acceptable performance criteria for the test/assay/procedure

### 7) Cross-reference all other related documents associated with this study

(list can be added to and deleted from as appropriate)

EQA data  
IQC data  
IQA data  
In-house R&D records  
Results of testing:

<p>known positives and known negatives                  low positives and high positives                  problem samples                  Published and unpublished papers and reports                  Work books (especially applying to in-house testing)                  Work carried out with collaborating laboratories                  Comparisons with alternative methods                  Comparisons with previously used test methods                  Evaluation reports                  Review meeting minutes                  Manufacturer's instructions                  Manufacturer's product specification</p>
<p><b>8) Results</b>                  Give summary of the results and give the location of the raw results/evidence</p>
<p><b>9) Limitations</b>                  Summarise the known limitations of the verified test or procedure (keeping it specific to the test/assay/procedure in question; there is no need to include general limitations that could happen to any test like sample labelling mix ups).</p> <ul style="list-style-type: none"> <li>- for verifications this can be done by reference to the existing SOP/ literature from manufacturer/developer if it stated there. State any additional limitations noted during the verification that are not covered by other documents</li> <li>- include uncertainty of measurement if relevant</li> </ul>

<b>10) Diary</b> (include dates of all important events, such as review meetings)	
<b>Event</b>	<b>Date</b>
Project start	

<b>11) Conclusions</b> (include brief summary)	
<b>12) Project Sign off</b>	
Has the verification demonstrated that the new method/procedure meets the set criteria required for the change?	Yes / No
If No, what action will be taken?	
Is the new method fit for purpose?	Yes / No
<b>If the project sign off is yes, the validation is deemed to have passed. Therefore the appropriate managers should complete their local verification checklist before completing the authorisation section below.</b>	

**VERIFICATION AUTHORISATION SECTION**

<b>This method is suitable for diagnostic use</b>	
<b>Signed (Project Leader)</b>	<b>Date</b> _____

<b>Introduction of method authorised</b>	
<b>Signed (Project Manager)</b>	<b>Date</b> _____

## Appendix 5: Linearity example<sup>10</sup>

An example of linearity can be seen in the graph plotted below for a laboratory developed test where seven concentrations of the organism tested is prepared by dilution of a high concentration standard were tested in triplicate. This example has been adapted from Burd et al<sup>10</sup>.

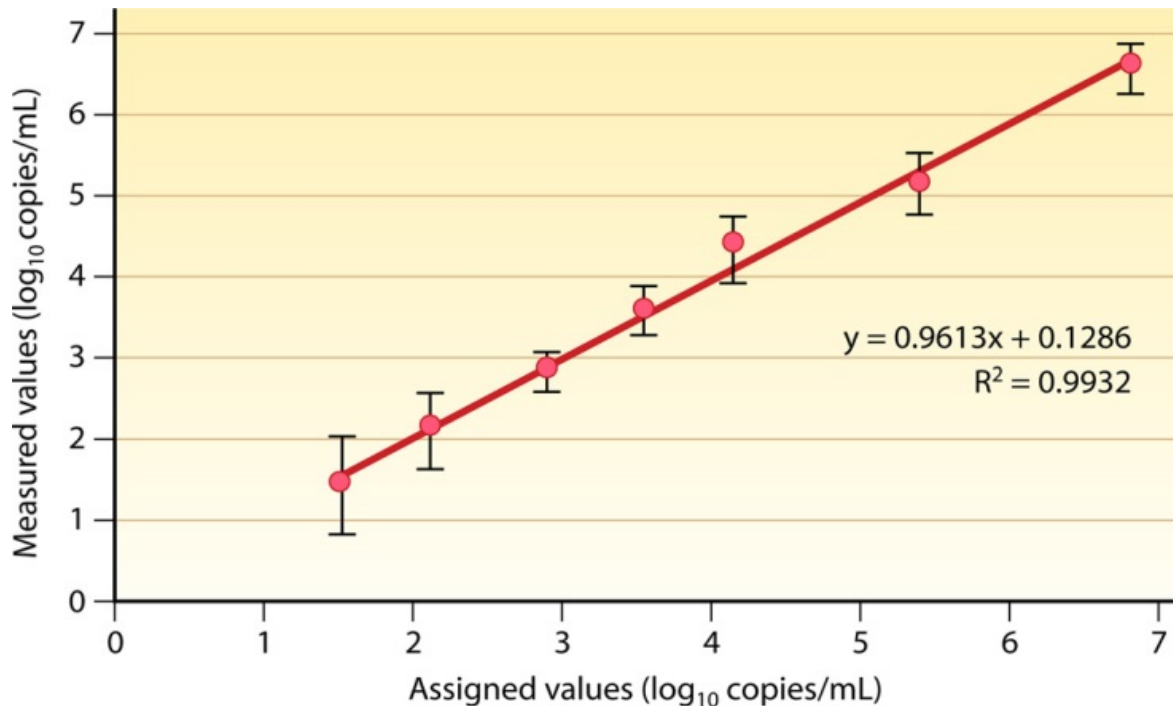


Figure 1: Plot of results from a linearity experiment to determine reportable range. Assigned values, (converted to log<sub>10</sub>) were plotted on the x axis versus measured values (converted to log<sub>10</sub>) on the y axis using Microsoft Excel. The reportable range in this example translates to 30 copies/mL (lower limit of quantification) through 3,000,000 copies/mL (upper limit of quantification).

## Appendix 6: Detailed considerations when carrying out an evaluation, validation or verification

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### Personnel

#### Project manager

The Project Manager is the person with overall responsibility for the successful completion of the project and the organisational aspects of the work including production of the protocol, data collection and analysis, writing the report and ensuring that there is peer group assessment of the protocol and report. The Project Manager should document any conclusions made based on the results analysis as well as sign a formal declaration that the method is suitable for diagnostic use. The report should have the support of stakeholders identified in the protocol design.

#### Project group

The Project Group includes staff in the laboratory that are competent and experienced and who have defined responsibilities (that is people with sufficient expertise) to cover all aspects of the method involved. The size of the project group can range from as little as one person, that is the Project Manager (if the individual performing the evaluation/validation has sufficient expertise in all aspects of the project area) to many, and will depend on the complexity of the project. All project personnel should have clearly defined lines of accountability. It may be necessary to include people from other laboratories to ensure sufficient expertise is available for a successful evaluation/validation to be conducted. Consideration should be given to the inclusion of a statistician (refer to Section 2).

**Note:** If multiple evaluation sites are involved, each site will also need a named local co-ordinator to manage the study.

### Computing requirements

Requirements should be assessed prior to starting the evaluation or validation or verification including:

- laboratory information management system (LIMS) available
- requirement for manual input, transfer or manipulation of data
- whether equipment can be interfaced to the laboratory computer system to download and/or upload data as appropriate
- whether software can be tailored to individual laboratories by the user, versus that requiring intervention by the manufacturer (and any cost implications of this)
- compatibility of systems between laboratories should also be considered
- statistical/data analysis software availability

### Project design<sup>4,15</sup>

The complexity of the project will depend on the circumstances. Projects involving new in-house methods and/or considerable changes to a method already in use will require a more thorough investigation than minor changes to existing methods or performance

verification. Likewise evaluations involving multiple sites will also require a more complex design. Whereas multi-centre evaluations will allow more samples or tests to be examined within a specified time-frame, care is needed to ensure data are rigorously derived and comparable.

During preparation of the protocol, it is important to ensure that assays / kits being evaluated are the same version as those currently marketed or about to be purchased. Assay kits changed at any time may render assessments invalid. Under the current licensing requirements, the dates of changes, or acknowledgement that they have occurred at all, may not be recorded other than by manufacturers.

The Project Manager must prepare a plan, considering the following as appropriate:

- define the purpose and objectives of the investigation. For example, is the project designed to define differences or similarities between assays / kits?
- identify any training requirements where necessary to ensure everyone involved in the project has suitable levels of competency. Ensure training records are up to date for procedures being carried out. Where assays / kits are involved, the supplier should be given the opportunity to ensure that users are competent and the training provided by the supplier should be assessed
- identify any risk assessments and COSHH assessments which need to be reviewed or written
- identify standard or reference materials where available to allow the method to be standardised, to facilitate method comparison, and to permit test stability over time to be determined. If reference materials are used it is assumed that commutability between the reference material and the patient material has been demonstrated and documented by the manufacturer<sup>13</sup>
- identify a method to be used as a “gold standard” for comparison of the method undergoing testing. If a gold standard is not available, it may be appropriate to use the consensus result approach. On occasion, there may be no true or widely recognised “gold standard” against which to compare a particular method, in which case pre-existing assessed methods for example, UK Standards for Microbiology Investigations, should be used, and justification for their use included in the project proposal. It is not appropriate to compare two non-validated processes
- identify the types and numbers of samples to be tested (refer to section 2). Consider the need to include known positives (low and high), known negatives and samples which are known or likely to be problematic or representative of a particular population whose values are known. Samples for analysis should be selected carefully to reflect the objectives of the study which in turn will relate to the ultimate use of the kit/equipment for example target population. These may include stored organisms with known characteristics and should ensure adequate representation of all known variants
- where applicable, material should be sourced from a wide geographic area including the area where use is intended. Test samples should be split wherever feasible so that the same material is used to compare different methods. Samples should be transported to the laboratory under defined conditions and examined within a stated period of time. Stored sera, giving a predetermined range of results form the basis of most serology



evaluations/validations. All samples containing those interfering substances identified by the manufacturer for example haemolysis should be excluded. If the manufacturer's instructions and the stability of the measurand allow, refrigeration is the preferred method of storage to avoid artefacts introduced in the freeze thaw cycle<sup>13</sup>

- approval from the relevant ethics committee must be sought if samples used originate from patients. However, ethics approval is not required for the use of residual sera in kit validation or evaluation. This should be done in accordance with "The Use of Human Organs and Tissue act"<sup>16</sup>
- as far as possible all methods used in a project should be subjected to full quality control procedures. Quality control samples should be processed during the study period, to assess the quality of the data collected and possible differences between sites. For further information, refer to UKAS publication [TPS 47: UKAS policy on participation in proficiency testing](#) for the expectations with regards proficiency testing participation
- methods of data collection and analysis of results should be determined consulting a statistician where necessary
- consider the need to hold reviews of project progress and who needs to attend those reviews. Reviews may be set to take place after a period of time or when a particular stage of the project has been reached
- carry out the project as determined by protocol design and record results

### **Involvement of commercial companies**

Confidentiality agreements may be sought by companies where prototypes are tested or where developmental work is undertaken.

Although commercial influences must not compromise scientific integrity, the manufacturer should be included in the study design where possible and must be given the opportunity to ensure that the protocol describes the correct use of the product and that the equipment is used correctly.

The commercial company should be given the opportunity to comment on the report and any manuscripts to be submitted for publication.

### **Avoiding bias**

Great care should be taken to avoid bias at all stages. The possibility of bias might be introduced at almost every stage of an evaluation/validation/verification and this may skew the results of the study in a particular direction. Potential problems should be considered and addressed before the study begins.

Areas where bias may be introduced include:

- failure to standardise the procedures fully, for example sampling, method, media and reagents
- failure to read results independently (results from one method may influence the interpretation of those from another)
- inappropriate use of panel of specimens, for example selected on the basis of results from an assay involved in the evaluation/validation or that have been

pre-screened by a kit that is the same as that tested within the evaluation/validation. This can be avoided by for example including specimens from different ages, risk or sex mix from expected test population. Interim analysis should only be conducted where pre-planned e.g. to ensure panel is appropriate.

- premature discussion or analysis of results (except statistical analysis)
- where multi-site evaluations are concerned, failure to perform the study simultaneously, thereby introducing potential differences due to seasonal differences in isolation or detection rates
- failure to give full training in the techniques, protocol, use of kit or equipment involved in the study before commencement

### Cost benefit analysis

All aspects of costs need to be measured and compared with the specified “gold standard”.

Cost approaches to be considered include:

- comparison with standard in terms of consumables, labour and overheads
- equipment costs including capital/lease/reagent rental, maintenance, service costs, spare parts (availability), consumables, ancillary equipment required, staff costs and overheads for the instrument as used in a routine situation
- comparison with specified manual method
- cost of isolating specific organism
- cost of isolating specific extra organism
- costs of work done to prove a negative or confirm a positive result
- staff time constraints are very substantial as this makes it impossible for staff to do everything within timeframe allocated

A study of how the project will impact on the running of the laboratory should be considered as a way of objectively assessing all aspects of costs. The cost-benefit balance should be assessed in terms of the clinical value of the result and the effect on turn-around times. To this end, a health economists’ involvement may also be appropriate.

### Time scale

A suitable time-scale should be agreed in advance of the start of the project. This will depend on the statistical sample size, the rate of acquisition of suitable specimens, and the time needed for the test procedures to be approved. Studies should be carried out in centres where the samples or organisms to be tested are sufficiently common to achieve results in a reasonable time, or in laboratories which hold a repository of characterised samples. Isolation and detection of organisms are subject to seasonal differences for example respiratory pathogens; where feasible, the study should be conducted at times of high incidence to optimise the use of resources.

When preparing a study time table any phasing of the trial should be taken into consideration. For example, with equipment the following three phases might be indicated:

- familiarisation, ensuring that the equipment is ready for evaluation, and preliminary testing with reference material
- extended testing with reference material and routine samples
- routine use for all specimens in parallel with current routine method

## References

### Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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4. World Assembly of Delegates of the OIE. Development and Optimisation of Nucleic acid detection assays. *OIE Validation Guidelines*; 2014. p. 1-11. **B, V**
5. European Committee for Standardization. BS EN 13612:2002 *Incorporating Corrigendum No.1* - Performance evaluation of in vitro diagnostic medical devices. BSI 2002. **A, V**
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14. Mattocks CJ, Morris MA, Matthijs G, Swinnen E, Corveleyn A, Dequeker E et al. A standardized framework for the validation and verification of clinical molecular genetic tests. Eur J Hum Genet 2010;18:1276-88. **B, III**
15. World Assembly of Delegates of the OIE. Principles and Methods of Validation of diagnostic assays for infectious diseases. OIE Terrestrial Manual; 2013. p. 1-17. **B, V**
16. UK Legislation. Human Tissue Act. 2004. **A, V**