Introduction to method validation

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Overview

- What is method validation?
- Why is method validation necessary?
- When do you need to validate methods?
- How do you validate methods?
- How do you decide fitness-for-purpose?

The purpose of the introductory lecture is to address five basic questions which cover all aspects of method validation. Other lectures in the series will address in more detail how methods are validated.

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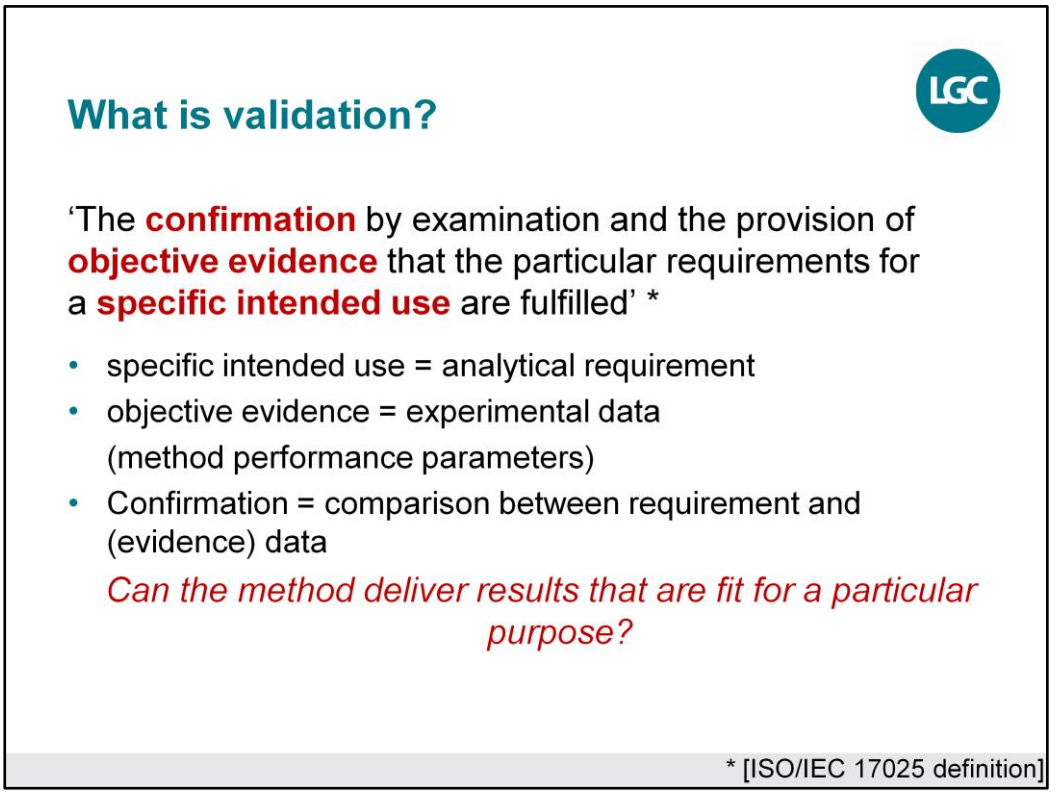

Validation has three important parts and when applied to method validation, these mean:

1. the **specific intended** use or application, is the analytical requirement which derives from the problem that the analysis is intended to solve; this is clarified during the discussions between the laboratory and the customer as part of contract review.
2. the **objective evidence** is usually in the form of data from planned experiments, from which the appropriate method performance parameters are calculated;
3. the **confirmation** is taken as a satisfactory comparison of the performance data with what is required, i.e. demonstrating that the method is fit for purpose.

Advice on how to do method validation is laid out in a number of guides - the actual procedures may vary from sector to sector. It is always worth following any guidance available for your particular sector, so that your validation procedure is compatible with that in peer laboratories. Where particular conventions have been followed these should be stated.

Note that there are a number of different definitions of validation but they are broadly in line with the definition shown in the slide. For example, ISO Guide 99:2007 (International vocabulary of metrology – Basic and general concepts and associated terms (VIM)) defines validation as, 'verification, where the specified requirements are adequate for an intended use' and verification as, 'provision of objective evidence that a given item fulfils specified requirements'. Whichever definition is used, the activities described in the following slides will need to be carried out.

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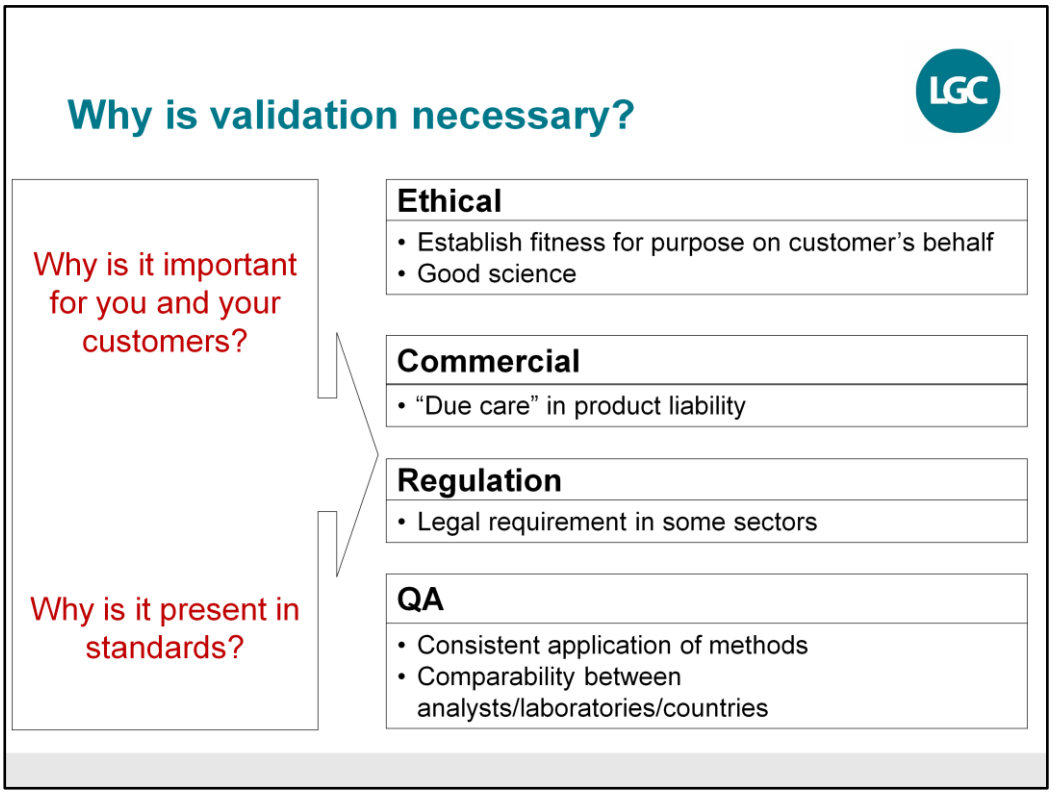

From an ethical point of view, method validation is important because the customer employs the expertise of the laboratory to do an analysis it could not do itself. It trusts the laboratory to use a fit for purpose method. The laboratory in turn should apply all aspects of good science to the problem - this includes appropriate validation of the methods used.

It is good commercial sense to have some assurance that a measurement will be correct before it is carried out. Validation helps to provide that assurance. The unsatisfactory alternative is to carry the measurement out, detect errors and have to repeat the measurement. (It is cheaper/better to prevent problems from happening rather than have to correct them afterwards.) In a production environment the producer has a duty to have taken all reasonable care to ensure the quality of a product before releasing it to the consumer. Validation provides part of the minimum liability.

In some areas, the validation of methods is a regulatory requirement. Compliance with Good Laboratory Practice (GLP), a legal requirement for certain types of study, requires technically valid operating procedures. Likewise methods accredited to the ISO/IEC 17025 standard must be validated.

Evaluation of method performance parameters during the validation process yields data that show which parts of the method are stable and which can cause problems in overall performance. Thus validation helps in the design and implementation of suitable quality control procedures. Method validation data provide information which enables the comparability of results from samples analysed in different laboratories and using different methods to be assessed.

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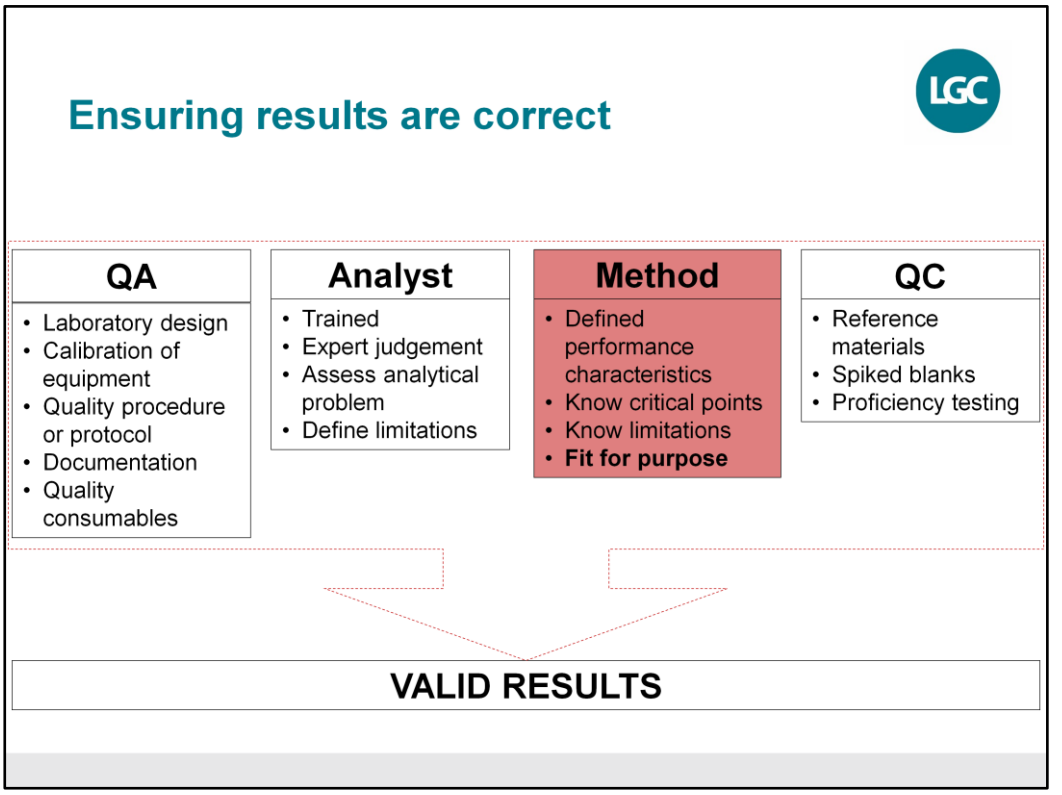

Method validation is an essential part of the process of ensuring that measurement results reported to customers are correct. However, it is important to have other aspects under control such as satisfactory laboratory design, stable environmental conditions, suitable quality control (QC) procedures (all of these fall under the general heading of quality assurance (QA)).

Well trained analysts are also essential to assess the analytical problem (understand the customer needs) and consequently determine the required method performance parameters.

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When do you validate a method?

- During method development
  - is the proposed method likely to be fit for purpose
- Before using any method for samples
  - including standard/published methods which have been pre-validated by others
  - verify own ability to match published data
  - verify suitability for analytical requirement
- Change of application/working environment/analyst
- Following period of non-use

Validation usually begins during the method development stage when some performance parameters are evaluated approximately to determine whether the method capabilities are in line with the levels required. Once the method is deemed good enough the development phase finishes, giving way to more formal validation studies.

Published methods may not necessarily be properly validated. The analyst is always advised to check the level of validation against that required and add further validation as needed.

The analyst who uses the method routinely will not necessarily be the same one who has carried out the validation. Methods are sometimes validated in one part of a laboratory and then transferred to other parts for routine use.

Whether the validated method is published or has been developed in-house, the analyst who will actually use it to analyse samples should first confirm that the validation data and subsequent fitness for purpose applies to the method when they are using it. This is sometimes known as verification.

A change of use of the method, or use after a period of non-use, requires the validation to be checked. Extending the use of the method to different sample types, or analyte levels, will require the performance to be checked using the new type of samples. The effect of changes to other parameters such as analyst, instrument, or laboratory environment should also be checked.

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Who validates a method?

- The analyst
  - in-house development and validation of new methods
  - verification of the performance of pre-validated methods
- The laboratory
  - method development and validation section
- Peer laboratories
  - groups of laboratories working in the same sector
- Sectoral/professional/standardisation body
  - validation of methods via interlaboratory study

It is principally the analyst who validates methods although they may work to a standard laboratory protocol to do so.

It should be recognised that methods may also be validated by groups of laboratories co-operating in a collaborative trial. Methods so validated may be published by sectoral, professional or standardisation bodies.

Large laboratories may have a central development section which develops and validates methods before passing them on to other sections for routine use.

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How you validate a method

- Decide analytical requirements
- Identify suitable method
- Plan and carry out experiments to evaluate performance
- Use data to assess fitness-for-purpose
- Statement of validation

Method validation is not an accidental activity, it should be both deliberate and planned.

The first stage is to examine the problem presented by the customer. Look at the reasons behind carrying out the analysis and find out what it is that the customer hopes to establish from having the work carried out. From this it should be possible to decide which method performance parameters are relevant to the work and what sort of target values are required. From this a suite of experiments can be designed which can be used to evaluate the performance of candidate methods.

The plan will include details on what is going to be analysed at each stage, what degree of replication is required. It is possible that several parameters may be examined in one set of experiments in which case, the order in which things are done can be important.

Once the plan is finalised the method parameters are evaluated and the data used to decide whether the method is fit-for-purpose. The statement of validation is the positive assertion of fitness-for-purpose.

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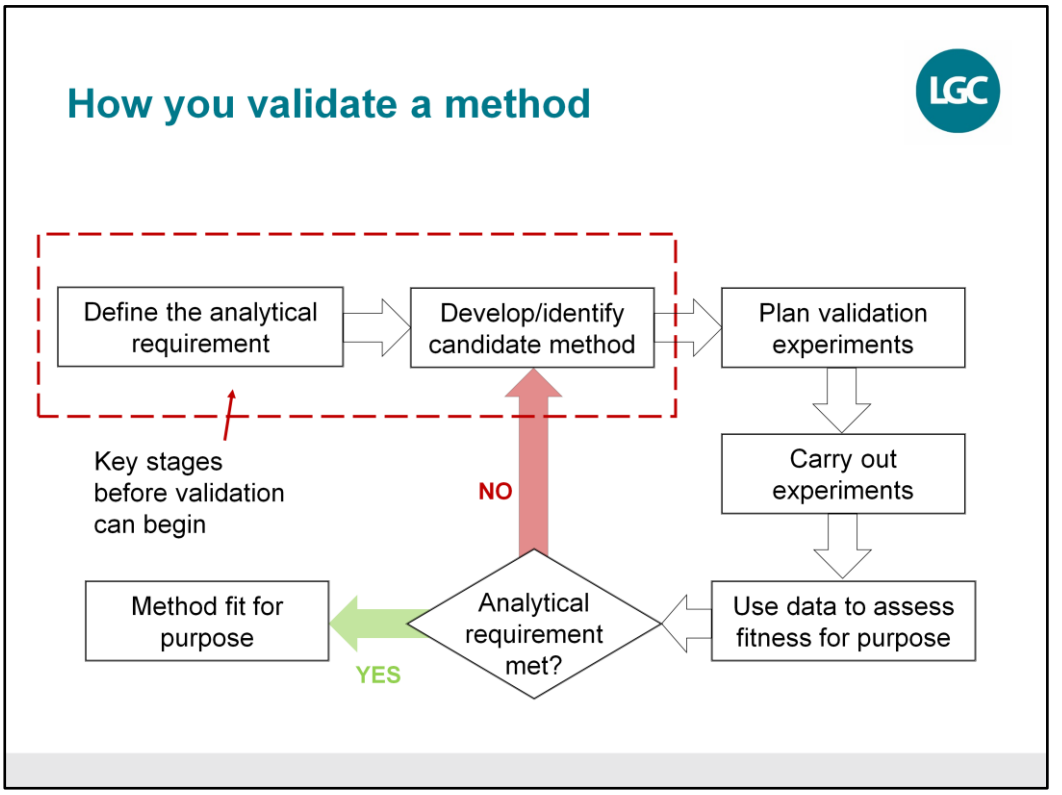

Method validation is not an accidental activity, it should be both deliberate and planned.

The first stage is to examine the problem presented by the customer. Look at the reasons behind carrying out the analysis and find out what it is that the customer hopes to establish from having the work carried out. From this it should be possible to decide which method to select, which method performance parameters are relevant to the work and what target values are required. From this, a suite of experiments can be designed which can be used to evaluate the performance of candidate methods. If a published method is being used it is still necessary to do some testing because you need to verify that the published performance can be achieved in your laboratory.

The validation plan will include details on what is going to be analysed at each stage and the degree of replication required. It is possible that several parameters may be examined in one set of experiments in which case, the order in which things are done can be important.

Once the plan is finalised the method parameters are evaluated and the data used to decide whether the method is fit for purpose. The statement of validation is the positive assertion of fitness for purpose.

Note the subtle difference between the following terms.

Validation establishes method performance values which are fit for an intended use

Verification demonstrates that published performance can be achieved

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Method performance parameters

Provide the evidence that the method is fit for purpose

- Confirmation of identity (specificity/selectivity)
  - are you measuring what you think you are measuring?
- Trueness (bias)
  - how far from the estimated truth are your results?
- Precision (repeatability, reproducibility)
  - how close to one another are your results?
- Limit of Detection, Limit of Quantitation (LOD, LOQ)
  - what is the minimum amount your method can detect and measure?
- Working range (Linearity)
  - what is the range over which your method is going to be used?
- Ruggedness/robustness
  - what are the key experimental parameters affecting your method?
- Sensitivity
  - rate of change of response with concentration/property value

Different method performance parameters will be important in different situations.

Trueness will be important for calculating absolute values of properties or analytes. Note that the term 'accuracy' is often used in documents/standards referring to method validation. Under current ISO definitions, accuracy is defined as the closeness of agreement between a measured value and a true value, and therefore includes the effect of both precision and trueness.

Precision is important for all measurements but particularly in comparative level studies.

Working range will be of interest in most cases. For trace level work, limits of detection and quantitation may be relevant. For planning calibration strategies it may be useful to know over what range the response is linear.

The meaning of "sensitivity" depends on the sector in which it is used. In an instrument sense it refers to the rate of change of response with analyte concentration/property value. Medical and clinical chemists often use it as an alternative to limit of detection.

Ruggedness studies, carried out mainly during method development will indicate which parameters need to be controlled in order to preserve performance. This in turn enables suitable quality control strategies to be devised.

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Confirmation of performance

- Once performance parameters have been evaluated, a judgement on whether the performance is acceptable is required
- Target values can be:
  - defined in standards/regulations
  - stated in a standard published method (can you match the stated performance?)
  - based on performance of similar procedures that are known to be fit-for-purpose
  - defined as the current sate-of-the-art (what is the method capable of)?
- Performance should be consistent with analytical requirement

Once the method performance parameters have been evaluated they can be compared with the analytical requirement. In some cases the required performance level will be specified in legislation.

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Declaration of fitness-for-purpose

- Analyse data from method performance parameters
- Are target values achieved?
  - YES - method is fit-for-purpose
  - NO - more development required
- Method is validated by the declaration of fitness-for-purpose

If the method performance parameters requirements are achieved, then the method can be declared as fit-for-purpose and considered validated.

If the target values are not achieved further development of the method will be necessary, followed by reassessment against the target value.

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Summary

- Method validation is necessary to produce meaningful data
- Extent of validation varies with application and sector
- Plan and perform studies in line with analytical requirements
- Make judgement of validation using objective evidence
- Apply common sense
- Assumptions
  - staff competent and equipment suitable and calibrated
  - environmental conditions and reference materials stable

Validation of a method is necessary for that method to have any value for analysis of samples. Validation adds a considerable burden to the costs of analysis but equally it can be argued that without validation, analytical data produced by a method has little or no value.

How much validation you do and what form it takes will depend very much on the circumstances and the requirements of the particular analytical problem. Cost considerations suggest you do as little as is adequate. Even if you cannot afford to do as much validation as is statistically ideal, some validation is better than none at all.

There is no single fixed way of doing validation. Particular performance parameters will be very important in some situations but irrelevant in others.

The accepted way of carrying out validation studies often varies according to the sector to which the work relates. Special guidance is often available in particular sectors. It is recommended that a laboratory follows whatever guidance is available in their own sector of work so that any validation is compatible with other methods validated in the same sector by other laboratories. Any particular interpretations made, or conventions followed, should be stated.

Validation should always be a planned activity. This makes it easier to ensure that all parts of the analytical requirements are adequately covered in the most efficient way. The extent of data recorded should be sufficient to enable objective and common sense decisions of fitness for purpose.

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