

Method Validation Procedure

Risk Assessment [2 x 2 = 4]
 This procedure has been examined under COSHH Guidelines, Manual Handling and VDU Regulations and has been assessed as **LOW RISK** if carried out as written.



This procedure could involve the handling of fresh biological material and as such should be regarded as a **POTENTIAL BIOHAZARD**. However the risk is minimal if the procedure is carried out as written

Area of CPA Standard

F1 Selection and validation of examination procedures

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Purpose of the Examination

Method validation is an important requirement in the practice of an analytical process. One can interpret method validation as the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires.

If the result of a test cannot be trusted then it has little value and the test might as well have not been carried out. Therefore a laboratory and its staff have a clear responsibility to justify that the analytical part of the problem is correct, in other words results have demonstrable “fitness for purpose”.

Principle

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem .eg

- New method developed for particular problem
- Established method revised to incorporate improvements or extended to a new problem
- When quality control indicates an established method is changing with time
- Established method used in a different laboratory, or with different analysts or different instrumentation
- To demonstrate the equivalence between two methods, e.g. a new method and a standard

The laboratory has to decide which method performance parameters need to be characterised in order to validate the method. Characterisation of method performance is an expensive process and inevitably it may be constrained by time and cost considerations.

Method development can take a number of forms. The majority of methods used within the Pathology department have been developed/published in the past. Therefore the department is required to consider two issues.

1. Is the existing validation data adequate for the required purpose or is further validation necessary.
2. If the existing validation data is adequate, is the laboratory able to achieve the level of performance claimed possible in the method.

CE Marked Products/Methodologies

The introduction of the In-Vitro Diagnostic Medical Devices Directive 98/79/EC applies to devices and accessories used in diagnostics and includes the related software. This includes Diagnostic and monitoring equipment for alleviation of disease. Since all methodologies that are bought by the laboratory now have to be CE marked we can assume that No1 above i.e. existing validation data is

adequate for the required purpose. Therefore with all bought kits that are CE marked the laboratory need only prove that the level of performance, claimed by the manufacturer is achievable within the department.

Non-CE Marked Methodologies reproduced from published methods

A large number of laboratory tests are not CE marked, as they are “in-house” methodologies reproduced from previous publications. Depending on the analyte and the methodology the laboratory has to decide which method performance parameters need to be characterised in order to validate the method. This may involve, as above, the laboratory proving the level of performance, claimed by the published method is achievable within the department. However, if any modification of the methodology is made, or a totally new method enhancement is being introduced into the laboratory then a full extensive method development/validation will be required. If this is the case then refer to “The Fitness for Purpose of Analytical Methods” (available in Haematology BMS 4 Office). This should include some or all of the below

- Confirmation of Identity and selectivity/specificity
- Limit of detection
- Limit of Quantitation
- Working and Linear Ranges
- Accuracy
- Trueness
- Interpreting Bias Measurements
- Repeatability
- Reproducibility
- Measurement Uncertainty
- Sensitivity
- Ruggedness
- Recovery

Personnel Requirements

This procedure can be carried out by BMS grade 1 and above, following the appropriate training.

Specimen Requirements

Dependant on analyte being measured

Equipment

Dependant on analyte being measured

Reagents

Dependant on analyte being measured

Procedure

Validation of Quantitative CE Marked Products/Methodologies (Examples TSH, Ferritin, Vitamin B12 etc....)

1. Before the laboratory decides on the method and degree of validation required, the company from which the product is purchased should be contacted and all method validation information requested.
2. The laboratory can then use this information to decide on the extent of method validation required.
3. However, the following is considered to be the minimum requirements for method validation.
4. It is essential that the analyst make himself or herself completely familiar with a new method before using it for the first time. Ideally someone already expert in its use will first demonstrate the method to the analyst. Questions regarding preparation of reagents need to be understood. Do they need to be made in advance?
5. Secondly, an assessment needs to be made how many samples can be conveniently handled at a time. It is better to analyse a few samples well than to try to analyse a large number and have to repeat most of them.
6. Thirdly, make sure that every thing needed for the method is available before work is started. This involves gathering together the right sort of equipment, reagents and standards.
7. The procedure should initially be run using the methodology, reagents, calibrants and controls supplied to assess the *Trueness* of the method and allow the analyst to become familiar with the procedure.
8. Once the analyst is satisfied that the kit is working correctly (i.e. the quality control is within limits) then the following assessments should be made.

- **Accuracy**

Accuracy expresses the closeness of a result to a true value.

Method validation seeks to quantify the likely accuracy of results

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by assessing both systematic and random effects on results. Accuracy is, therefore normally studied as two components: *Trueness* and *Precision*.

Trueness

Two techniques are available and if possible both should be performed.

a) Method Comparison

If you are introducing a new method into the department, which has already been validated, compare the results from the 2 methods for the same samples. It is recommended that a minimum of 10 samples are compared, however, the more results the better the comparison. If an analyser is being used then this number should be at least 30 samples. Use a statistical package to compare the two methods using linear regression, thereby calculating any bias.

b) Reference Comparison

Obtain reference material from the relevant External Quality Assessment scheme along with the statistical results showing all method mean and method mean, or if available a certified reference material. Run the EQA samples as many times as possible (depending on available sample) up to a maximum of ten and determine the mean and standard deviation of these replicate tests. Compare the results with the method mean obtained nationally.

Precision

Precision (in this case meaning Repeatability) is usually stated in terms of Standard Deviation. Precision is generally dependent on analyte concentration, and therefore should be determined at a number of concentrations and if relevant, the relationship between precision and analyte concentration should be established.

Perform 10 determinations calculating the standard deviation at 3 concentrations (low, medium and high)

The following parameters may be determined depending on the analyte being detected.

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- **Limit of Detection (LoD)**

10 Independent sample blanks measured once each,
Express the LoD as 3SD above the sample blank (this assumes that a signal more than 3SD above the sample blank could only have arisen from the blank much less than 1% of the time, and therefore is likely to have arisen from something else, such as the measurand. Getting a true sample blank can be difficult.

- **Limit of Quantitation (LoQ)**

This is the lowest concentration of analyte that can be determined with an acceptable level of accuracy and precision. This is calculated by analysing 10 independent sample blanks (as above) The LoQ corresponds to the sample blank value plus either 1) 5SD 2) 6 SD or 3) 10 SD. This may involve stripping serum to obtain a sample blank (getting a true sample blank can be difficult)

Another method is to fortify aliquots of a sample blank at various analyte concentrations close to the LoD. Calculate the SD of the analyte value at each concentration. Plot SD against concentration and put a value to the LoQ by inspection.

- **Working and Linear Ranges**

For any quantitative method, it is necessary to determine the range of analyte concentrations over which the method may be applied. The LoD or LoQ will determine the lower end. At the upper end of the concentration range limitations will be imposed by various effects depending on the instrument response system. Within the working range there may exist a linear response range. Note that regression calculations on their own are insufficient to establish linearity. To do this a visual inspection of the line is required.

- a) Plot the measurement response (y-axis) against measurand concentration (x-axis) of 6 concentrations plus blank and visually examine to identify linear range and upper and lower boundaries of the working range. Then
- b) Perform the above in triplicate, plot as above, and calculate the appropriate regression coefficient. Calculate and plot residual values (difference between actual y value and the y-value predicted by the straight line, for each x value). Random distribution about the straight line confirms linearity. Systematic trend indicate non-linearity.

- c) Note: If variance of replicates is proportional to concentration then use a weighted regression calculation rather than none-weighted regression. In certain circumstances it may be better to try to fit a non-linear curve to the data. Functions higher than quadratic are generally not advised.

Validation of Quantitative CE Marked Products/Methodologies (Examples, ENA Screen, tTG etc..)

1. Qualitative analysis can be treated in a slightly different way. Qualitative analysis is effectively a yes/no measurement at a given threshold of analyte concentration
2. Perform steps 1-6 as above
3. Once the method is established Trueness must be established by using the same methodologies however statistical analysis is not required, as trueness cannot be expressed qualitatively by standard deviation. The analyst must therefore assess the results of the method/reference comparisons objectively and make a decision on the strength of the results.
4. Again for qualitative methods, precision cannot be expressed as a standard deviation, but may be expressed as true and false positive (and negative) rates. These rates should be determined at a number of concentrations, below, at and above threshold level

$\% \text{ false positives} = \text{false positives} \times 100 / \text{total known negatives}$

$\% \text{ false negatives} = \text{false negatives} \times 100 / \text{total known positives}$

Validation of non CE Marked Methodologies and “in-house” methods reproduced from published methods.

(Examples, Cytochemical special stains, Histochemical staining, Microbiological testing etc...)

1. As mentioned above a large number of laboratory tests are not CE marked, as they are “in-house” methodologies reproduced from previous publications. Depending on the analyte and the methodology the laboratory has to decide which method performance parameters need to be characterised in order to validate the method.
2. The majority of published methods are well established and only require, the laboratory proving the level of performance, claimed by the published method is achievable within the department.

3. In this scenario one of the above techniques (for CE marked products) is adequate for validating the method (either qualitative or quantitative).
4. However if any modification of the methodology is made, or a totally new method enhancement is being introduced into the laboratory then a full extensive method development/validation will be required. If this is the case then refer to "The Fitness for Purpose of Analytical Methods" (available in Haematology BMS 4 Office).

SPECIAL VALIDATION PROCEDURES

Certain methodologies may have their own specialised validation procedures, which are beyond the scope of this procedure. However, please refer to the guidelines below.

1. Evaluation, validation and implementation of new blood grouping techniques. Transfusion Medicine (1995) 5:145-150.

Documentation

1. Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented.
2. Firstly the validation procedure and results must be documented and a report written and filed within the department.
3. The procedure must then be documented and the SOP Authorised, Signed and (if previous methodology documentation) Archived. (See GENMANPR014 Production Implementation Amendment and Withdrawal of Standard Operating Procedures)
4. When examination procedures are changed so that results or their interpretation may be significantly different, the implications should be explained to the users, prior to the introduction of the change.
5. All BMS staff using the methodology should then be taught the new process, assessed and signed off as competent to perform the procedure

Related documentation

- GENMANPR014 Production Implementation Amendment and Withdrawal of Standard Operating Procedures)
- The Fitness for Purpose of Analytical Methods

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Q-Pulse Reference: Method Validation Procedure.doc

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- Evaluation, validation and implementation of new blood grouping techniques. Transfusion Medicine (1995) 5:145-15
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Cross References

- **ISO/FDIS 15189** 5.5 Examination procedures
 5.6 Assuring the quality of examination procedures
- **ISO/IEC 17025:2000** 5.4 Test and method validation
- **ISO/DIS 9001(E)** 7.2 Customer Related Processes
 7.3 Design and/or development
 7.5.5 Validation of Processes
- **EC4 Essential Criteria** 8.1 Validation
 8.2 Calibration and traceability of methods

