Technical Session: New Technologies in Testing and Calibration contributing to Global Supply Chain

Relevance of Method Validation and Laboratory Quality Assurance in Test Programmes

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The Challenge…

- With the fast ingress of newer technologies coming into the analytical field, newer methods are required to utilize these technologies
- Developing, standardizing and publishing test methods is a time consuming task and standard publishing bodies are hard pressed in publishing methods in keeping pace with these fast developments
- With fast emerging contaminants (food sector) and newer detection technologies available to detect them, and lack of availability of standardized methods, **laboratories are now developing their own methods** using these newer instrumentation/techniques. This is specifically true globally for handling challenges in analysis of toxic residues & contaminants in food.

The challenge

- Some of the critical questions to be asked in using these laboratory developed methods are:
  - Since different laboratories are developing their own methods, ***will the results be comparable***?
  - Are these methods **fit for the purpose** they are being utilized for ?
  - How will the laboratory personnel develop **competence and confidence** in using these newly developed methods?
  - How will you ensure that these methods perform well during their routine use?
- Also, even if the laboratory adapts a standardized published method, how does it ensure that:
  - This standardized method performs are per requirements under **its own laboratory conditions**
  - The **personnel are competent** to carry out this new method with equal efficacy as is desired to get accurate results
  - The **equipment** available with the laboratory is fit for achieving the desired results?

To overcome these challenges, the relevance of method validation becomes critical

- Structure of the Presentation
  - What is method validation?
  - Why is method validation necessary?
  - What is the difference between method validation & method verification
  - How should methods be validated?
  - What are the method performance characteristics
  - How to use your validation data to design an effective in-house quality control program

Establishing a new Method

What is method validation?

- Method validation is the process of defining an analytical requirement, and confirming that the method under consideration has capabilities consistent with what the application requires.
- Requirement is covered in Clause 5.4.2 of ISO/IEC 17025:

  "*The laboratory shall use test and/or calibration methods, including methods for sampling, which meet the needs of the customer and which are appropriate for the tests and/or calibrations it undertakes...*"
- Validation basically involves evaluation of method performance characteristics especially after development of a new method.
- Method validation is applicable to both quantitative and qualitative (e.g. screening etc.) methods

Why is method validation necessary?

- For an analytical result to be fit for its intended use it must be sufficiently reliable so that any decision based on it can be taken with confidence
- Method validation enables chemists to demonstrate that a method is 'fit for purpose' and gives a fairly good idea on the limitations of the method
- Additionally, method validation provides a solid knowledge and experience of the practical details of performing the method, including awareness of any critical steps in the process. Validation gives the laboratory and its employees a greater confidence in their own results
- Valuable data generated during method validation can be utilized to design an effective quality control program

What is the difference between validation and verification?

Q: *Does a laboratory need to validate a method if it has adapted a validated procedure which has been published as a standard?*

- In such a case, basic validation work has already been carried out but the laboratory will **still need to confirm** its ability to apply the method. This is called **method verification**
- **Method verification** involves some experimental work to be done to demonstrate that the method works in the end-user's laboratory.
- the **workload** for carrying out method verification is **considerably less** compared to validation of a method that has been developed in-house.
- Method verification is even more critical where quantities measured are in very low levels, for example residue analysis, that typically have high uncertainties

Validation or Verification?

Method	Requirement
1. Standard published method 2. Method published in scientific journals with validation data 3. Instrument manufacturer's published technical paper with validation data 4. Commercial test kit third party validated or approved by regulatory agencies	**No validation required**. **Verify to confirm the performance characteristics** are achievable. Validation may be required if the method changes (revised) and if the revision/s are significant.
1. Standard published method subject to in-house modification 2. Standard method applied outside the scope of the standard method (e.g., different matrices, analytes or conditions)	**Validation is required.** The rigor of validation will depend on the extent of the modification/s.
1. In-house developed method 2. Method published in scientific journals without validation data 3. Instrument manufacturer's published technical paper without appropriate validation data 4. Commercial test kit with no performance data or incomplete	**Full validation required.**

Differentiated in ISO/IEC 17025

- As per **Clause 5.4.5.2 of ISO/IEC 17025**, a method should be validated when it is:
  - non-standard methods
  - laboratory-designed/developed methods
  - standard methods used outside their intended scope
  - amplifications and modifications of standard methods
- For standard(ised) methods, such as those published by, e.g. ISO or ASTM, validation by the laboratory using the method is not necessary. However, the laboratory needs to verify the performance of the method as detailed in ISO/IEC 17025 Clause 5.4.2

  …The laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations

Approaches to method validation

- **Interlaboratory approach**
  - Dedicated Interlaboratory comparisons often referred to as 'collaborative studies' or 'cooperative studies'.
- **Single-laboratory approach**
  - The entire validation work is carried out within the same laboratory. There is no requirement of inter-laboratory comparisons.
  - Several protocols exist for single laboratory validation studies and some specify the performance criteria. Some of them are:
    - **AOAC Guidelines** for Single Laboratory Validation of Chemical Methods
    - The **IUPAC** method validation protocol
    - **Commission Decision 2002/657/EC**: Performance of analytical methods and the interpretation of results for residues in products of animal origin
    - **SANTE/11945/2015:** Analytical quality control and method validation procedures for pesticides residues analysis in food and feed

Performance Characteristics

Performance Characteristics	Reason
Selectivity or Specificity	Ensure that the substance quantified is the intended analyte
Limit of Detection (LOD)	Lowest level of detection of an analytical method
Limit of Quantitation (LOQ)	Lowest quantification level
Linearity or working range or calibration	Range of operation, lowest end is the LOQ
Accuracy	Measure of Systematic & Random errors
Trueness or Bias	Closeness between measured value & reference value (systematic)
Precision (Repeatability)	Random errors under repeatable conditions
Precision (Within-lab reproducibility	Random errors under reproducible conditions within the same lab
Robustness	Measure of a method's capacity to remain unaffected by small variations in method parameters
Measurement Uncertainty	Strictly, not a performance characteristic but a property of the results obtained using that measurement procedure.

All parameters above are required for method validation but for verification, evaluation of parameters is **BOLD** are sufficient

Validation report

After successful validaition of a method, a report should be prepared, covering at least the following:

- Validation plan
- Raw data
- Interpretation of raw data
- Conclusions a statement of ‘fitness of purpose’ of method is also added as a concluding remark

Scenario 1

Q. *Can a method developed and validated at one laboratory be used in another laboratory without re-validation?*

As per current protocols & understanding, No, since it is **not a standardized or published method**. A standardized method ideally is one that has been accepted by a standard publishing authority based on certain method evaluation criteria that generally include single lab validation, collaborative studies to test for **method ruggedness and peer evaluation**.

Scenario 2

Q. *What if the method has been validated at one lab & is not a standard published method, but:*

- the **method ruggedness** been established **through collaborative studies** as per established interlaboratory comparison protocols to provide reliable information on the method's performance (defined in ISO 5725).
- The facilities including the required equipment are available at the other lab

Q. *Can this method be then transferred to another lab, where the other lab does not carry out a full method validation but can adapt the method if it is able to verify the performance characteristics only, i.e. carry out a simple method verification ?*

This is something that the standard making bodies and the accreditation bodies need to think about. This will reduce a lot of repetitive work and save cost and time

What happens after validation is successfully completed?

- After the validation report is ready, there are 2 options:
  - **Option 1:** File the report in a nice folder and forget about it until the assessor comes for the audit ? OR
  - **Option 2:** Put this validation data that has been generated using a lot of efforts and money, to good use ?
- Generally the first option is what we usually follow and waste the valuable data that has been generated.
- Therefore, the correct approach is to go for the second option and put the validation data to good use !!

Using validation data to design internal quality control

- Internal QC refers to procedures undertaken by laboratory staff for the **continuous monitoring of operations and measurement results** in order to decide whether results are reliable enough to be released. It is covered in **clause 5.9 of ISO/IEC 17025:2005**.

- During the validation stage the method was largely applied to samples of known content. Once the method is in routine use it is **used for samples of unknown content.**

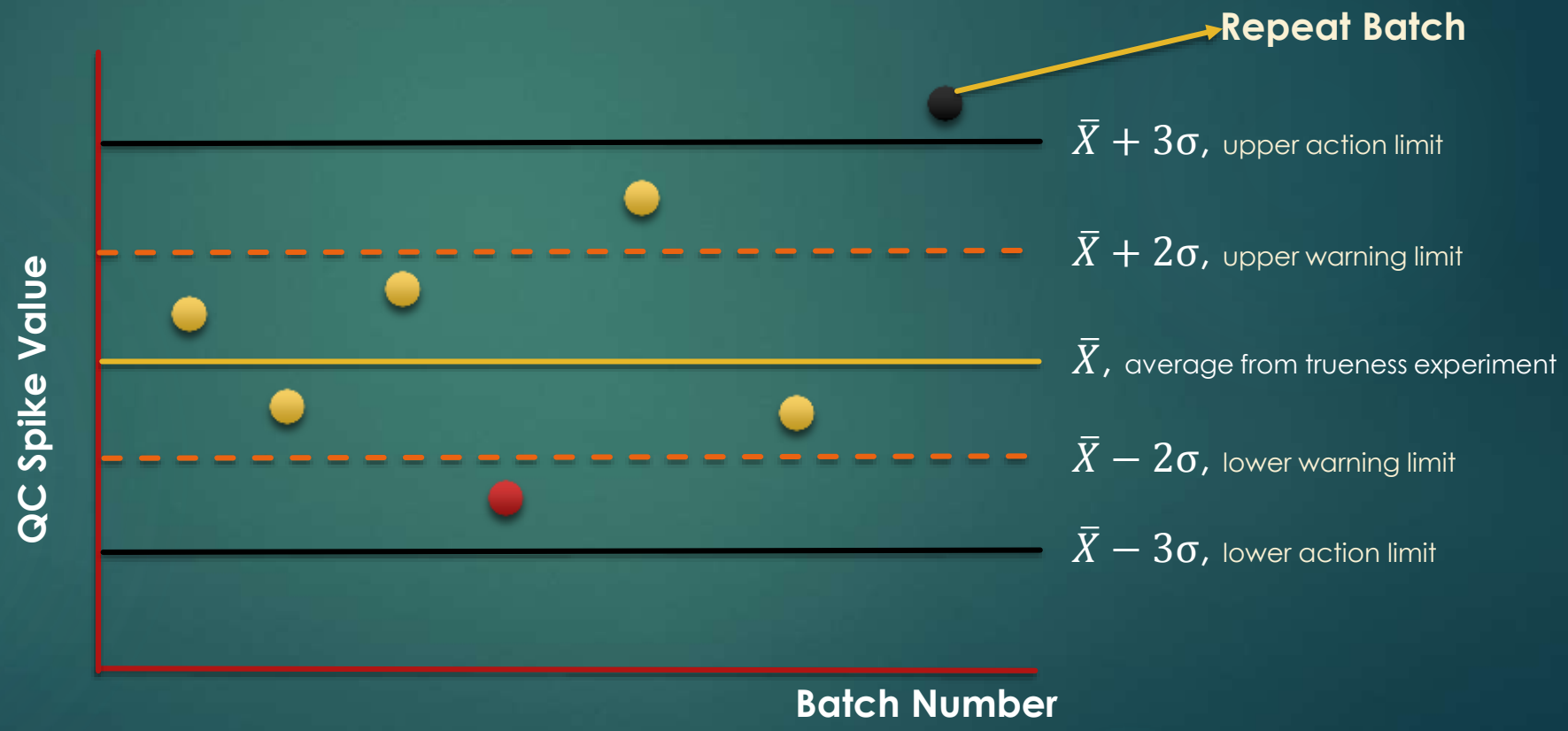
- Therefore, **continuously monitoring the performance** of the method becomes all the more critical

Control Charts – A critical tool for evaluating method performance

- One such critical tool to monitor the ongoing method performance is a **control chart**.
- Control charts use your validation data as defined criteria to monitor your method performance.
- Control charts allow the analyst to decide whether **unexpected and unwanted changes** are occurring in the method performance on an ongoing basis.
- Control charts are created using **QC batch spikes or pre-spiked samples of known analyte content**
- In practice these **QC batch spikes** should be measured with every batch of samples as part of the on going quality control process
- One such control chart that is predominantly used as a QC tool in laboratories is the **Shewart Chart**

Shewart Control Chart

- A control chart is a graphical method for **monitoring the day to day performance** of an analytical process



Control criteria example for Shewart Control Chart

- Point falling outside the 3s limit
- 2 subsequent points between 2s and 3s limit (on the same side)
- 11 subsequent points on the same side of the mean

Some other takeaways of the Shewart Chart

- A Shewart control chart is actually a graphical presentation of the lab's **QC efficiency**
- Keeps a control on the day-to-day **accuracy** of the analysis
- Data points over a period of time (7-10 days) can give you a value of **precision** in the form of **within-lab reproducibility** which can be continuosly compared to the within lab reproducibility values derived from your validation data
- Provides the tool for distinguishing the pattern of **random variation** from the **systematic variation**. trends from assignable or systematic causes can be corrected
- Hence, if the procedure is *in-control*, the results will almost always be within established control limits

Use of validation data for QC

- **% RSD** can also be calculated using replicate results and compared to % RSD determined from repeatability analysis during method validation. This will not be very accurate though, as only 2 values would be used for %RSD calculation, but will still give you an idea on precision.
- Similarly, **% RPD** can be calculated between pair of values obtained during method validation and this value can be used as a criteria for evaluating % RPD achieved during on-going QC checks.
- The above exercise can also be done in the reproducibility mode for retesting of retained items, using the within-lab reproducibility results estimated during method validation
- This way the method validation data provides an acceptance criteria to the various internal qc checks carried out as per clause 5.9 of ISO/IEC 17025 especially for replicate testing and retesting of retained items.

To summarize……

- With the rapid growth in analytical technologies, not supplemented with published methods, laboratory developed methods are being widely used globally
- Method validation is a critical tool that can give credibility and acceptance to laboratory developed methods.
- For published or standard methods, only initial demonstration of lab's capability to run the method is sufficient.
- Method validation and method verification data can be used by the laboratory to design its internal quality control program to continuously monitor the performance of its methods.

Thank you for your kind attention