



# Method validation and verification

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# Method Validation

- What is it?
- When is it required?
- Why is it necessary?
- What are required?
- How much is adequate?
- How should it be done?

## Method Validation – what is it

- **Definition** – validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (*ISO/IEC 17025:2005 cl. 5.4.5.1*)
- A process of evaluating method performance and demonstrating that it meets a particular requirement

## Method Validation – what is it

- Specific intended use – why are you analysing the samples and what are the resulting data used for
- Objective evidence – set of results from validation studies
- Confirmation – compare information obtained with customer's requirements

## Method Validation – when is it required

- When is validation not required
- standard methods on condition that
  - used within their scope of applicability
  - without modifications (including QA plan and reporting)
- Otherwise, required

## Method Validation – when is it required

- Non-standard methods
- Laboratory-developed methods

## Method Validation – when is it required

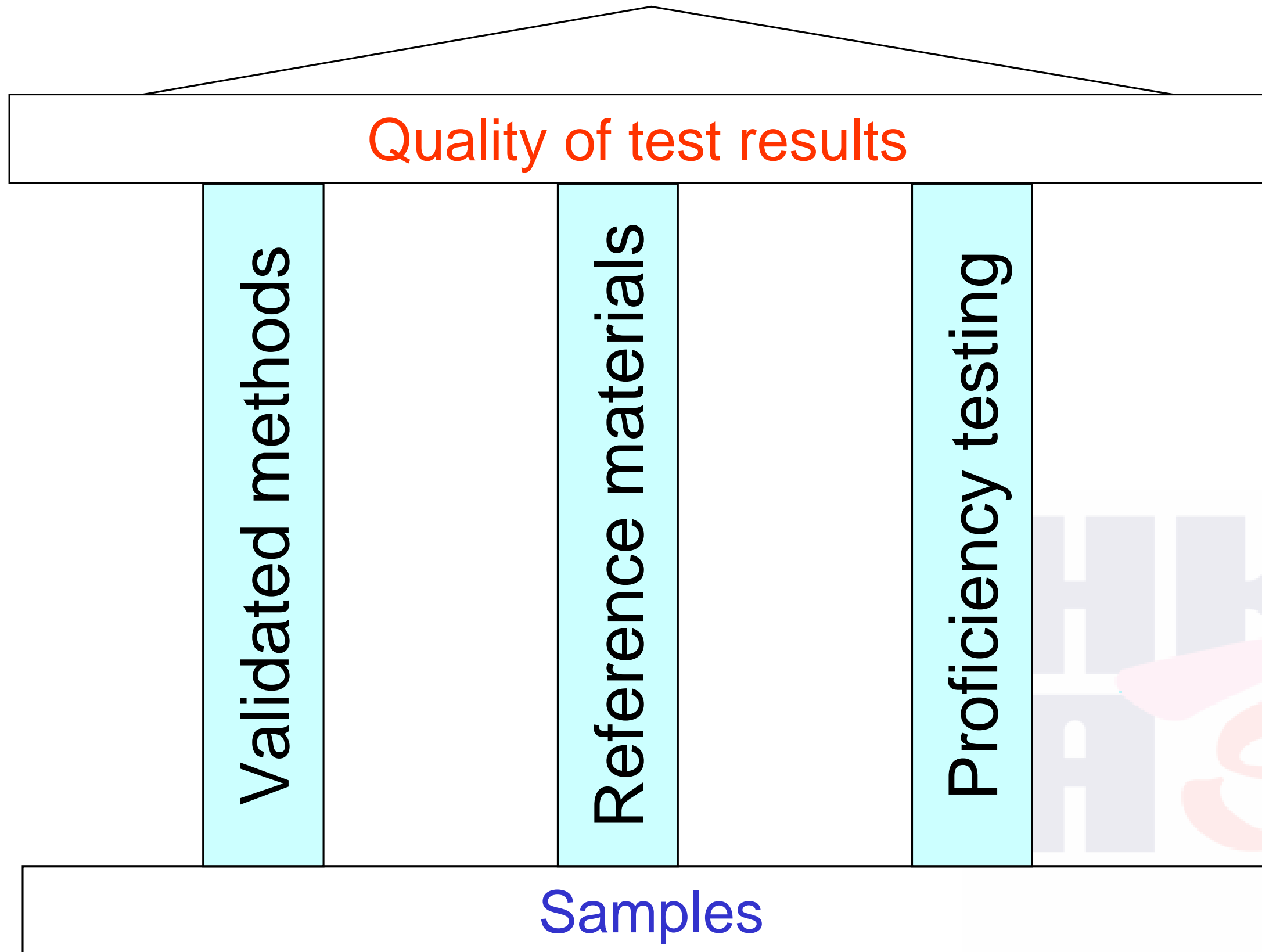
- Standard methods and **validated methods** which are:
  - used outside their intended scope (e.g. matrices, ranges, etc)
  - amplified or modified (e.g. analytical techniques, etc.)

## Method Validation – why is it necessary

- To meet accreditation requirement
- Ensure that the test method give “correct” results
- Objective evidence for defence against challenges
- Customers want to be assured of the correctness of results



# Method Validation – why is it necessary



## Method Validation – what are required

- **ISO/IEC 17025:2005** Section 5.4 *Test and calibration methods and method validation*
- Selection of methods (cl. 5.4.2)

Laboratory-developed methods or methods adopted by the laboratory may also be used if they are appropriate for the intended use and if they are **validated**.

## Method Validation – what are required

### Laboratory-developed methods (cl. 5.4.3)

- introduction of test methods for its own use
- planned activity
- assigned to qualified personnel
- equipped with adequate resources
- plans updated
- effective communication

(Process requirements)

## Method Validation – what are required

### Non-standard method (cl. 5.4.4)

- methods not covered by standard methods
  - agree with customers
  - include clear specifications of customer's requirements and purpose of test
  - validated appropriately before use
- (a laboratory-developed method is a non-standard method but the reverse may not be true)

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.2)

- as extensive as is necessary
- record
  - results obtained
  - procedure used
  - statement that method is fit for intended use

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.2 Note 1)

- May include procedure for sampling, handling and transportation

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.2 Note 2)

- techniques for method performance determination include
  - Calibration using reference standards and Reference Materials
  - Comparison of results achieved with other methods
  - Interlaboratory comparisons
  - Systematic assessment of the factors influencing the result
  - Assessment of uncertainty of results based on scientific understanding of the theoretical principles of the method and practical experience

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.2 Note 3)

- When changes are made in the validated non-standard methods, the influence of such changes should be documented and, if appropriate, a new validation should be carried out.



## Method Validation – what are required

### Validation of methods (cl. 5.4.5.3 )

- Range and accuracy of values obtained from validated methods, as assessed for the intended use, shall be relevant to the customers' need

# Method Validation – what are required

## Validation of methods (cl. 5.4.5.3 )

Examples:

- Uncertainty of results
- Detection limit
- Selectivity
- Linearity
- Repeatability
- Reproducibility
- Robustness/cross-sensitivity

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.3 Note 1 )

- Validation includes
  - Specification of the requirements
  - Determination of characteristics of method
  - Check requirement fulfilled by method
  - Statement on validity

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.3 Note 2)

- Regular review as method development proceeds to verify customer needs fulfilled
- Modifications to development plan due to change in requirements should be approved and authorised

## Method Validation – what are required

### Validation of methods (cl. 5.4.5.3 Note 3)

- Validation is a balance between costs, risks and technical possibilities

## Method Validation – what are required

### Validation of methods

(5.4 H HOKLAS Policy (c))

- Requirements differ significantly from one technical discipline to another
- Should commensurate with intended use
- Confirm fit for intended use

## Method Validation – what are required

### Validation of methods

(5.4 H HOKLAS Policy (d))

- HOKLAS classifies test methods into 3
  - standard method
  - standard method with modifications
  - in-house method

## Method Validation – what are required

### Validation of methods

(5.4 H HOKLAS Policy (d))

- standard method
  - Conforms exactly to the standard



## Method Validation – what are required

### Validation of methods

#### (5.4 H HOKLAS Policy (d))

- standard method with modifications
  - Differs from standard but deviations unlikely to affect test results
  - Need supporting evidence, i.e. validation required
  - Modifications stated in scope of accreditation and test reports

## Method Validation – what are required

### Validation of methods

(5.4 H HOKLAS Policy (d))

- In-house method
  - Methods other than the above two
  - Validation required
  - Cannot make reference to other standard method, i.e. cannot claim “based on” a standard method

## Method Validation – what are required

### Validation of methods

(5.4 H HOKLAS Policy (f))

- Non-standard methods shall be fully documented and validated

## Method Validation – what are required

### Validation of methods

#### (5.4 H HOKLAS Policy (g))

- Shall have policy and procedure for design, development and subsequent validation of
  - in-house methods,
  - laboratory designed/developed methods,
  - standard methods used outside their intended scope
  - amplifications and modifications of standard methods

## Method Validation – what are required

**HOKLAS Supplementary Criteria No. 20** *“Chemical Testing”, “Chinese Medicine”, “Construction Materials”, “Food”, Toys and Children’s Products” – Chemical Testing*

**Section 5.3 Validation of methods**

## Method Validation – what are required

### **HOKLAS SC No. 20** *Section 5.3*

- Concentration range
- Sample matrices
- “more advanced” techniques may be a deviation
- Confirmation of identity

## Method Validation – what are required

### **HOKLAS SC No. 20 *Section 5.3***

- LoD
- Precision and trueness
- Applicable concentration range
- Applicable sample matrices

## Method Validation – what are required

### HOKLAS SC No. 20 *Section 5.3*

- Method bias assessed by appropriate matrix CRMs
  - Levels of analytes
  - Matrix matches intended sample matrix
  - Uncertainty of assigned values suitable



## Method Validation – what are required

### **HOKLAS SC No. 20 *Section 5.3***

- Method performance characteristics review, and revised, regularly

## Method Validation – what are required

### **HOKLAS SC No. 20** *Section 5.3*

- For food analysis, the method validation required depends very much on the analytes of interest and the matrices. Common food matrices include those rich in protein, carbohydrate, oil, dietary fibre, liquid, etc.

## Method Validation – what are required

### **HOKLAS SC No. 20 *Section 5.3***

- If a method is to be accredited for general food, satisfactory validation data shall be obtained for at least five different food matrices with at least three kinds of food for each food matrix.

## Method Validation – what are required

### **HOKLAS SC No. 20 *Section 5.3***

- The range of matrices shall also in line with those listed in relevant regulations. Due consideration shall also be taken for the food matrices with potential interferences, e.g. high chloride effect on the ICP-MS determination.

## Method Validation – what are required

### **HOKLAS SC No. 20** *Section 5.5*

- Estimation of uncertainty of measurement

## Method Validation – how much is adequate

Depends on

- the criticality of the measurement
- The scope of the method

## Method Validation – how much is adequate

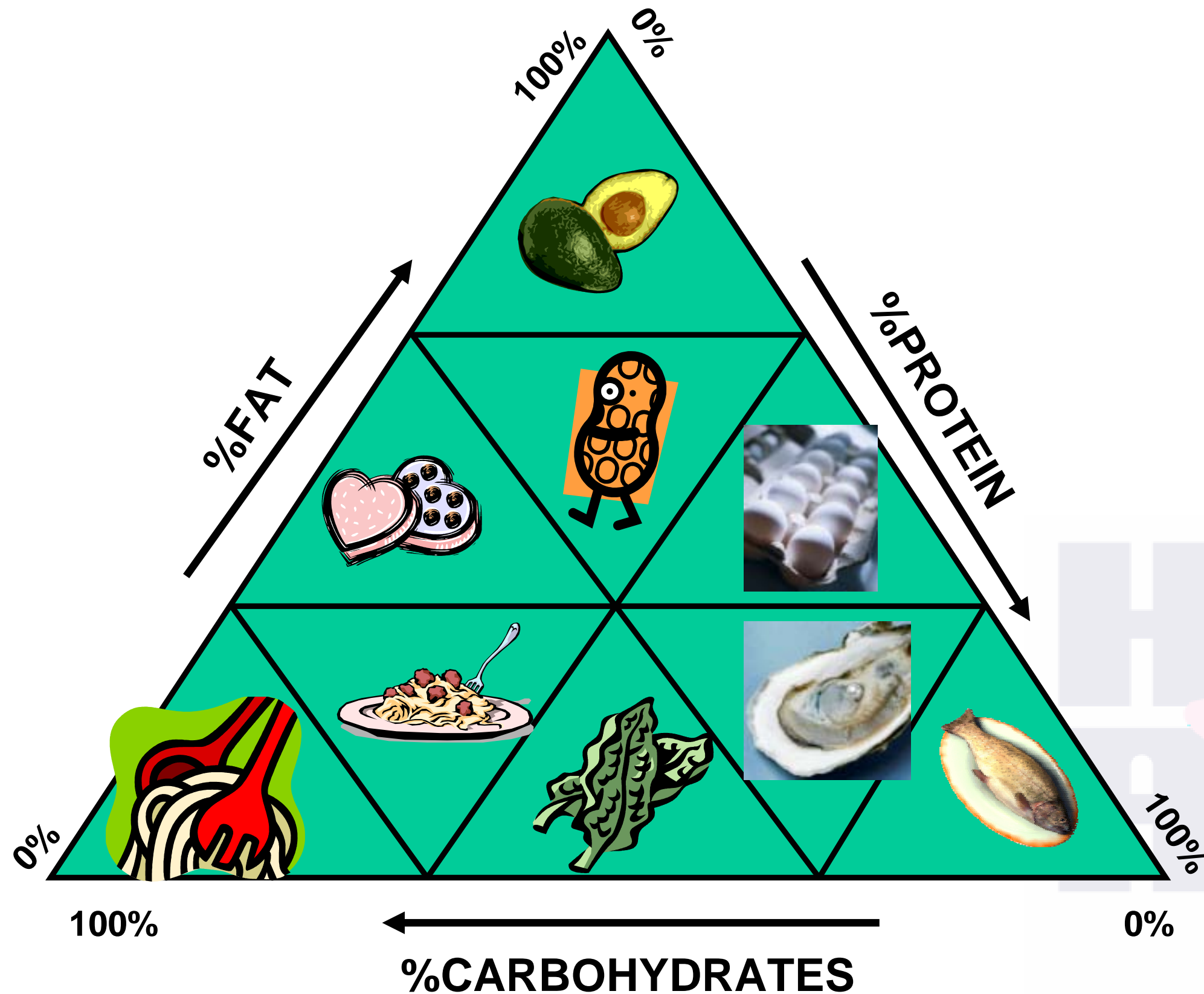
- How far can the light shine?
- How far can the validation data on some matrices be extended to other matrices?
- Need professional judgment based on technical knowledge of the limitations of the methodology used.

## Method Validation – how much is adequate

- Example: General foodstuff
- Can never validate using all possible foods
- Classify food according to matrix from an analytical chemistry point of view
- Main components of food – fat, protein, carbohydrate, (fibre, water)
- To obtain reliable data, 3 food types for each matrix
- Performance at specification limits should be available



# Method Validation – how much is adequate



# Laboratory internal validation and verification

## Existing information

Fully validated standard methods (have been studied in a collaborative trail)

Standard methods – amplifications and modifications e.g. new instrument

Standard methods – outside their intended scope

Laboratory – developed and non-standard methods

## Laboratory requirement

Verification (Secondary validation)

Validation

Validation

Validation (Primary validation)

# Definition

- ***Validation*** is the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO/IEC 17025 clause 5.4.5.1)
- ***Verification*** – confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (ISO 9000: 2005)

Note: intended use vs specified requirements

# Method Verification

## **ISO/IEC 17025 *cl. 5.4.2***

- The laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations. If the standard method changes, the confirmation shall be repeated.

## Method Verification – what are required

### **HOKLAS SC No. 20** *Section 5.4*

#### Verification of methods

- A laboratory using standard methods has to confirm that it has the ability to carry out those methods... Verification is usually carried out by comparing the performance data obtained by the laboratory when performing a standard method with those claimed by the same method.

*(note: specified requirements include the method performance of the std methods)*

# Method verification

- Released early 2008
- [http://www.aoac.org/al/acc\\_guide\\_2008.pdf](http://www.aoac.org/al/acc_guide_2008.pdf)



# Method Verification

- importing a validated method
- show that laboratory can do it at its site
- demonstrate that laboratory can repeat the method performance

# Method Verification

Standard methods shall be verified for:

1. the equipment
2. the required reference materials/standard, reagents
3. the environmental conditions
4. testing staff member competence to perform the test
5. capability to achieve the method performance



## How should it be done?

The following are some examples of useful references

- *Harmonised guidelines for single-laboratory validation of method of analysis* (IUPAC Technical Report), 2002
- *How to meet ISO 17025 Requirements for Method Verification*, AOAC, 2007
- **ISO/TS 21748:2004** *Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation*

# How should it be done?

## Validation of methods (cl. 5.4.5.2 Note 2)

- techniques for method performance determination include
  - Calibration using reference stds and RMs
  - Comparison of results achieved with other methods
  - Interlaboratory comparisons
  - Systematic assessment of the factors influencing the result
  - Assessment of uncertainty of results based on scientific understanding of the theoretical principles of the method and practical experience

## How should it be done?

1. calibration using reference standards or reference materials
  - *How many replicate results are required for proper comparison of the certified reference value and the actual analysis result?*
  - *Is it necessary for the mean of test result for a CRM to lie within the uncertainty range of the certified value?*
  - *Is a result outside the uncertainty range acceptable ?*

# How should it be done?

## Useful references

- **ISO Guide 32: 1997**

*Calibration in analytical chemistry and use of certified reference materials*

- **ISO Guide 33: 2000**

*Uses of certified reference materials*

## How should it be done?

2. comparison of results with other methods  
(ISO 5725-6:1994 clause 8)
  - comparison of precision of the two methods

$$F = \frac{S_r^2 B}{S_r^2 A}$$

where  $S_r^2 B$  = repeatability standard deviation of method B

$S_r^2 A$  = repeatability standard deviation of method A

if  $F < F(95\%) (v_r A, v_r B)$ : statistically no significant difference

## How should it be done?

- comparison of the means of the two methods

$$\text{if } \frac{\bar{y}_1 - \bar{y}_2}{S} \leq 2.0 \quad ,$$

then statistically no significant difference

where  $\bar{y}_1$  = mean of method 1  
 $\bar{y}_2$  = mean of method 2  
 $S$  = pooled standard deviation

## How should it be done?

3. comparison of results with another laboratory  
(ISO 5725-6:1994 clause 7.2.4.3)

- the means of the two laboratories are compared

if 
$$\left| \bar{y}_1 - \bar{y}_2 \right| \leq 2\sqrt{2} \sqrt{\delta_R^2 - \frac{\delta_r^2}{2}},$$

then statistically no significant difference

where  $\delta_r^2$  = repeatability standard deviation

$\delta_R^2$  = reproducibility standard deviation

# How should it be done?

## 4. interlaboratory comparisons

- ISO 5725 Parts 2 to 5: 1994
- ISO /IEC Guide 43-1: 1997



# How should it be done?

A lot of statistics

- Useful references
  - **ISO/TR 13425:2006** *Guidelines for the selection of statistical methods in standardisation and specification*

## How should it be done - verification

Worked examples of method verification are available from AOAC

- *Determination of Total, Saturated, and Monosaturated Fats In Foodstuffs by Hydrolytic Extraction and Gas Chromatographic Quantitation: Collaborative Study*
- *Determination of Low-Level Glucose and Fructose in Raw and Refined Crystalline Sugar by High-Performance Anion Exchange Chromatography*

## How should it be done?

Worked examples of method verification are available from AOAC

- *Determination of Cholecalciferol (Vitamin D3) in Selected Foods by Liquid Chromatography*
- *Aflatoxin B1 and Total Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste, and Paprika Powder (AOAC Official Method 999.07)*

## Common deficiencies

- Number of food matrices used not adequate
- Number of food types for each matrix not adequate
- Concentration levels used not adequate
- Reporting limits estimated but not verified
- Food samples chosen not representative of the food matrices

# Common deficiencies

- Confirmation of identity technique not available or adequate
- Validation/verification data analyses not done correctly
- Measurement uncertainty not available/not estimated correctly
- Equipment and/or test procedures not conforming to the test std requirements

# Common deficiencies

- Lack of or inappropriate sampling procedure
- QC plan acceptance limits not appropriate



**Thank you**

