Method validation and verification

W. W. Wong
Senior Accreditation Officer
HKAS
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Method Validation and verification

- *What is it?*
- When is it required?
- Why is it necessary?
- What are required?
- How much is adequate?
- How should it be done?
- Any questions on the questions?

Method Validation – what is it

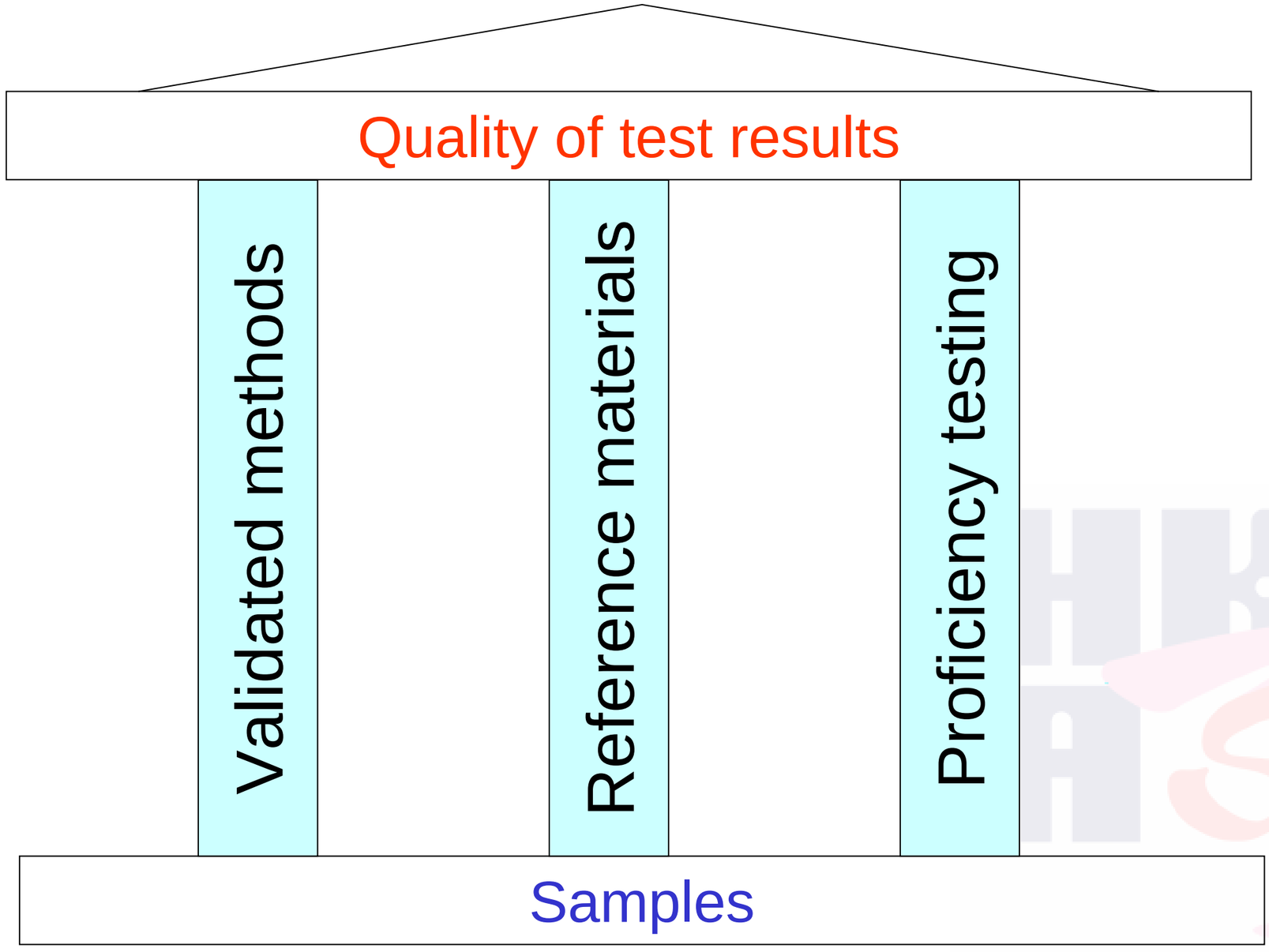
Definition –

- 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)*
- verification, where the specified requirements are adequate for an intended use (ISO/IEC Guide 99:2007)

Method Verification – what it is

- Verification – provision of objective evidence that a given item fulfils specified requirements (ISO/IEC Guide 99:2007)

Method Validation – why is it necessary



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.

Laboratory internal validation and verification

Existing information	Laboratory requirement
Fully validated standard methods (have been studied in a collaborative trail)	Verification (Secondary validation)
Standard methods – amplifications and modifications e.g. new instrument	Validation
Standard methods – outside their intended scope	Validation
Laboratory – developed and non-standard methods	Validation (Primary validation)

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

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

Method Verification

To demonstrate you can repeat the method performance, including:

- Detection limits
- Precision
- Bias

Method Verification – Method detection limit

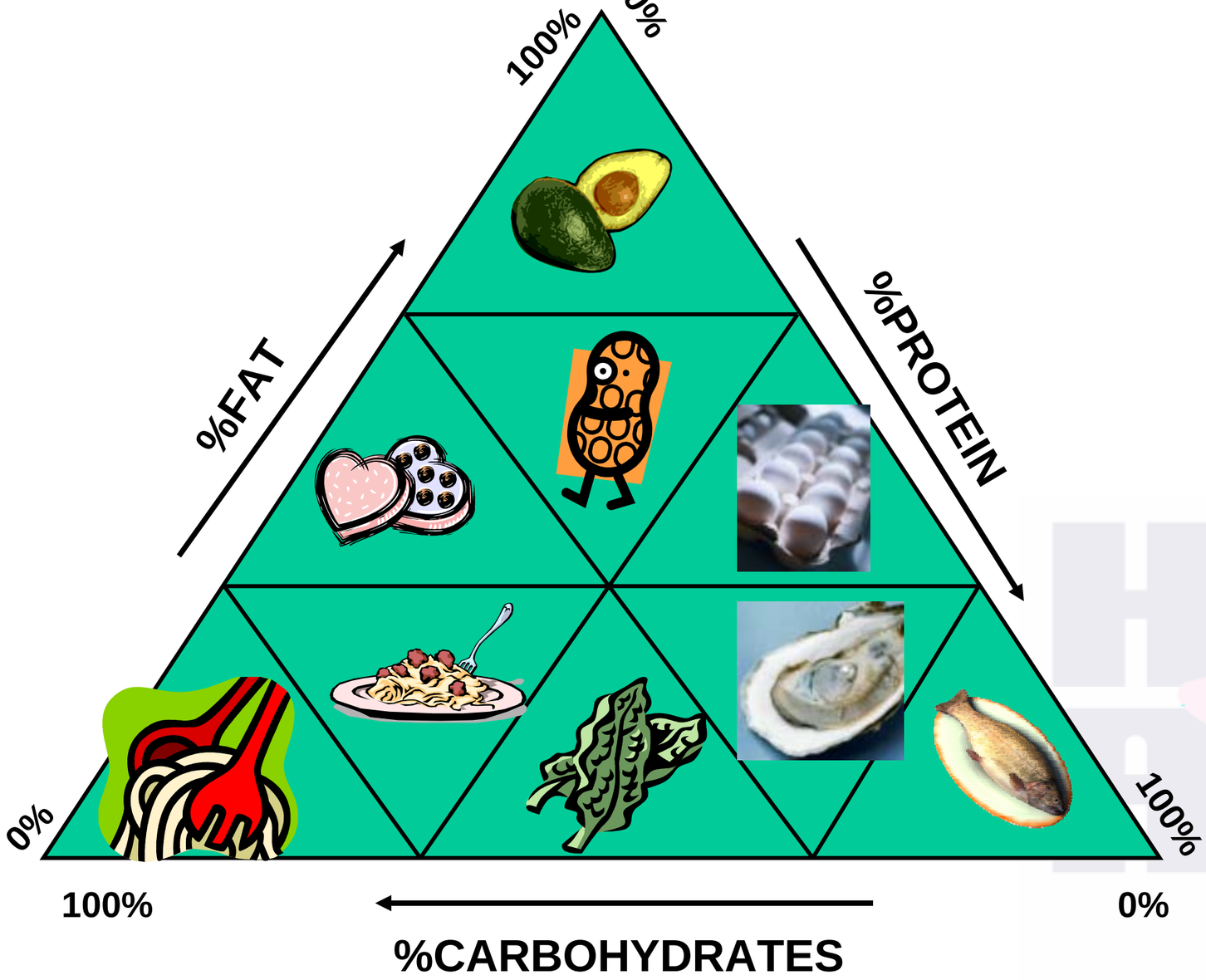
- Spike matrix blanks at the level close to the method detection limit given in the standard method
- Perform the analysis on the spike matrix blank at least 7 times over a period of at least 3 days
- Calculate the recoveries and the RSD
- Compare the values with those given in the standard method

Method Verification –
Method detection limit

Factors to consider:

- How many matrices are needed?
- How are the values obtained compared with those of the standard method?

Method Verification



J. AOAC, 83 (2), 413 (2000)
"The Referee", AOAC Int'l, July 1993

Method Verification –
Method detection limit

How many food matrices are needed?

- Less than validation
- Professional judgment needed on the possible matrix effects on the detection limits
- Most difficult food matrices
- How far can the light shine?

Method Verification – Method detection limit

How are the values obtained compared with those of the standard method?

- RSD not significantly different from std method and
- Mean recovery within acceptable limits

Method Verification – Precision

- Perform a precision study by analysing a homogenous sample at least 7 times
- Calculate the std dev
- Use F test to determine if there is statistical significant difference between the std dev found and the std method
- Should use repeatability std dev

Method Verification – Precision

- comparison of precision of the two methods

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

where $S_r^2 B$ = repeatability std dev of lab B

$S_r^2 A$ = repeatability std dev of std method A

if $F<F(95\%)$ (ν_rA,ν_rB): statistically no significant difference

Method Verification – Precision

- comparison of precision obtained against the required value

The precision of the measurement process is assessed by comparing the within-laboratory std dev under repeatable conditions with the required value of the within-lab std dev

Method Verification – Precision

- Compute

$$\chi_c^2 = \{ s_w / \sigma_{wo} \}^2$$

Where σ_{wo} is the required value of the within-lab std dev

$$\chi^2_{table} = \chi^2_{(n-1);0.95} / n\text{-}1$$

= 0.95^{th} quantile of the χ^2 distribution at degrees of freedom (n-1) divided by the degree of freedom (n-1)

Method Verification – Precision

- Decision

$\chi_c^2 \leqq \chi^2_{table}$: No evidence that the process is not as precise as required.

$\chi_c^2 > \chi^2_{table}$: Evidence that process is not as precise as required

Method Verification – Bias

- To demonstrate the absence of lab bias
- Proficiency tests/interlaboratory comparisons
- Analysis of CRMs

Method Verification – Bias

- Estimate of lab bias is, in itself, uncertain
- Choose n such that $\sqrt{s_w}/n < 0.2s_R$
- Certified value, μ, is subtracted from the mean of the results, m, to obtain an estimate of the lab bias, Δ

$$\Delta = m - \mu$$

- Acceptable if

$$| \Delta | < 2s_D$$

Where s_D is the uncertainty of the measurement process and $s_D^2 = s_L^2 + s_w^2/n$

Method Verification – Bias

Given $s_R^2 = s_L^2 + s_r^2$

$\therefore s_D^2 = s_R^2 - s_r^2 + s_w^2/n$

Hence,

$\Delta < 2\sqrt{(s_R^2 - s_r^2 + s_w^2/n)}$

Which is the acceptability test for Δ

Method Verification – Bias

- comparison of results with another laboratory

  - the means of the two laboratories, y_1 and y_2, are compared

if $| y_1 - y_2 | < \sqrt{(2.8\,\sigma_R)^2 - (2.8\,\sigma_r)^2(1 - 1/2n_1 - 1/2n_2)}$,

then statistically no significant difference

where σ_r = repeatability standard deviation

σ_R = reproducibility standard deviation

Method Verification – Bias

- When each lab has obtained only one test result, the absolute difference between the two test results should be tested against the reproducibility limit $R=2.8\,\sigma_{R.}$
- If the absolute difference between the two test results does not exceed R, the two test results are considered to be in agreement.

$$|\ y_1-y_2\ | < 2.8\,\sigma_R$$

Thank you