Verification/Validation of Test method

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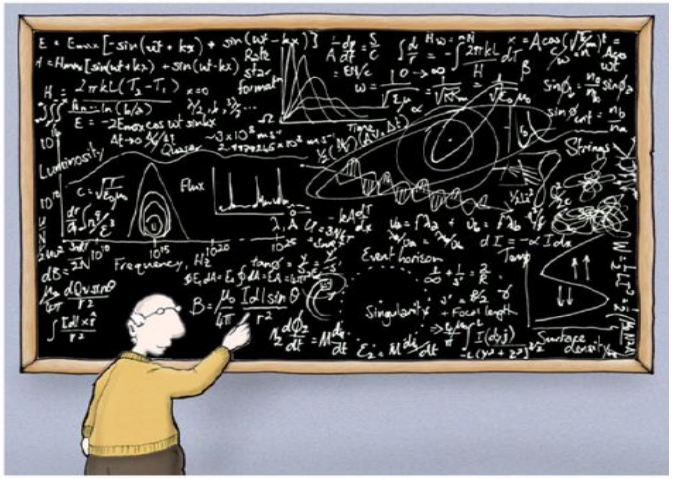


Introduction

- The aim of any analytical measurement is to obtain **accurate, reliable, and consistent data** to find the nature of a sample.

- These properties can be **judged** by the results obtained through method verification / validation, which since long is an integral part of any **good analytical practice**.

- In this aspect **method verification/validation** is an important requirement in the practice of testing

Introduction

Clause 5 of ISO/IEC 17025:2005

5.1 General
5.2 Personnel
5.3 Accommodation and environmental conditions
5.4 Test and calibration methods and method validation
5.5 Equipment
5.6 Measurement traceability
5.7 Sampling
5.8 Handling of test and calibration items
5.9 Assuring the quality of test and calibration results
5.10 Reporting the results

Introduction

Standardized- methods

↓

Verification

Modified standardized and inhouse method

↓

Validation

Introduction

Equipment validation

IQ

Soft and hardware

OQ

PQ

Method validation

… different items

Terminologies in measurement science

Term	What is it	Comments
Error	Result minus the reference value	The true error is unknown because the true value is unknown. Since the true value cannot be known, a conventional value, such as the reference value for a certified reference material, can be used giving a practical value for the error. It has two components, systematic and random
Random error	Component of error that varies in an unpredictable way.	There may be more than one component (source) of random error. It is not possible to correct for random error. The size of the random error can be reduced by reporting the mean of replicate measurements. The standard deviation for the mean is its standard error of the mean
Bias	Total Systematic error Component of error which varies in a predictable way	There may be more than one component of systematic error. Bias can be estimated by the difference of the mean value of several measurements from the reference value. It can be estimated by measuring the value of one or more reference materials several times under repeatability or intermediate precision conditions and calculating the mean. The difference between the mean and the reference value is the bias. In many cases, a correction can be used to remove the effect of known systematic errors (bias). Bias is determined in the method validation experiments.
Trueness	Closeness of agreement between the average of an infinite number of results and a reference value	Trueness is a hypothetical indication of the ability of the method to yield results close to the expected reference value. It is hypothetical because an infinite number of results cannot be obtained and the true value cannot be known. Thus, trueness cannot be expressed numerically. Accuracy should not be used for trueness.

Terminologies in Method Validation

Term	What is it	Comments
Accuracy	Closeness of agreement between a result and a true value	Accuracy describes how close a single result is to the true value. Therefore, accuracy includes the systematic error and the random error that impacts that result. Stated another way, accuracy includes trueness and precision. Since the true value is not known, accuracy cannot be given a numerical value, but is a descriptive and comparative term for a method. A method that has less random error, or a smaller bias, or both is called "more accurate."
Precision	Closeness of agreement between results obtained by replicate measurements on the same object under specified conditions	Precision is related to random errors only; see random error above. Precision is usually expressed numerically as a standard deviation or variance. The specified conditions can be, for example, repeatability, intermediate precision, or reproducibility conditions.
Uncertainty		Measurement uncertainty (MU) comprises many components, including random components and the uncertainty associated with the systematic effects. MU is expressed as standard uncertainty which is a standard deviation. MU is the parameter that includes uncertainties from the most possible effects; it is the most suitable way to describe the accuracy of results.

Verification

Verification is the confirmation, through the provision of objective evidence that specified requirements have been fulfilled (ISO 9000: 2005).

Method verification gives a confirmation to the laboratory that it can properly operate standard methods before introducing the tests. If the standard method changes, the confirmation shall be repeated (ISO 17025 cl. 5.4.2).

Verification

Standard Methods- Why import

- All the method development work has been done.
- Method performance understood.
- "Official" methods used to settle disputes.
- Inter-laboratory proficiency testing may already be in place.

Standard method- Once imported

Must show you can do the method correctly at your site! using

- the equipment
- the required reference materials/cultures, standard, reagents, media
- the environmental conditions
- testing staff member competence to perform the test
- capability to achieve the method performance

Verification

Slightly modified method

- It's always wise to have supporting data.

You Never Know!

- Prove or state that modifications have **no or understood effect**
- Must have supporting documentation
- May use **validation data**

Modified method

Supporting data is a must!

Examples

- different column,
- different detector,
- change of sample weights, dilutions or other sample preparation steps,
- solvent switch.

Verification

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

To demonstrate you can repeat the **method performance**, including:

- Detection limits
- Precision
- Bias/Accuracy

Verification- Detection limit

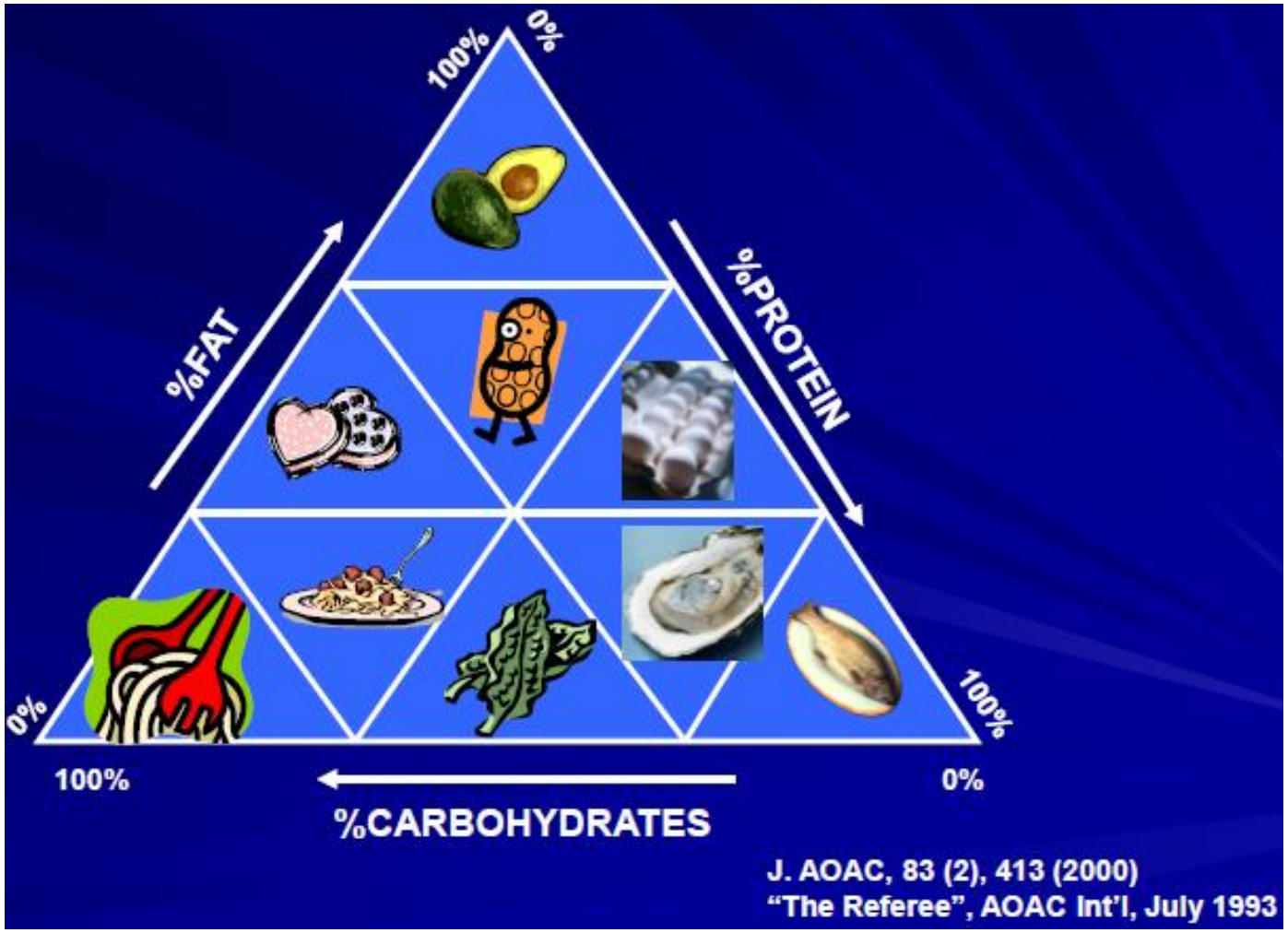
- **Spike** matrix blanks at the level close to the method detection limit given in the standard method
- Perform the analysis on the spike matrix blank at least **7 times over a period of at least 3 days**
- Calculate the **recoveries and the RSD**
- **Compare** the values with those given in the standard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

Verification- Detection limit

Factors to consider:

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

Verification- Detection limit



Verification- 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

Verification- Detection limit

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

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

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 standard method
- ✓ Should use repeatability std. dev.
- ✓ 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.

Verification- Precision

Comparison of precision of the two methods

Method	Mean (ppm)	Standard Deviation (ppm)
1	6.7	0.8
2	8.2	1.2

As an example, assume we want to see if a method (Method 1) for measuring the arsenic concentration in a sample is significantly more precise than a second method (Method 2).
Each method was tested ten times, with, yielding the following values:

A method is more precise if its standard deviation is lower than that of the other method.

So we want to test the null hypothesis H_0: $\sigma_2^2 = \sigma_1^2$, against the alternate hypothesis H_A: $\sigma_2^2 > \sigma_1^2$.
Since $s_2 > s_1$,

$F_{calc} = s_2^2/s_1^2 = 1.2^2/0.8^2 =$ **2.25**.

The tabulated value for d.o.f. $\nu = 9$ in each case, and a 1-tailed, 95% confidence level is $F_{9,9} =$ **3.179.**

In this case, $F_{calc} < F_{9,9}$, so we accept the null hypothesis that the two standard deviations are equal, and we are 95% confident that any difference in the sample standard deviations is due to random error.

We use a 1-tailed test in this case because the only information we are interested in is whether Method 1 is more precise than Method 2.

$\nu_1 = n_1 - 1$	2	3	4	5	6	7	8	9	10
$\nu_2 = n_2 - 1$									
2	19.000	19.164	19.247	19.296	19.329	19.353	19.371	19.385	19.396
3	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.785
4	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964
5	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735
6	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060
7	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637
8	4.459	4.066	3.838	3.688	3.581	3.500	3.438	3.388	3.347
9	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137
10	4.103	3.708	3.478	3.326	3.217	3.135	3.072	[illegible]	2.978

Verification- Bias/Accuracy

- To demonstrate the **absence** of lab bias
- **Proficiency tests/inter laboratory** comparisons
- Analysis of **CRMs/Spike samples**

Validation is the **confirmation** by examination and the provision of **objective evidence** that the particular requirements for a specific intended **use** are fulfilled (ISO 17025 cl. 5.4.5.1).

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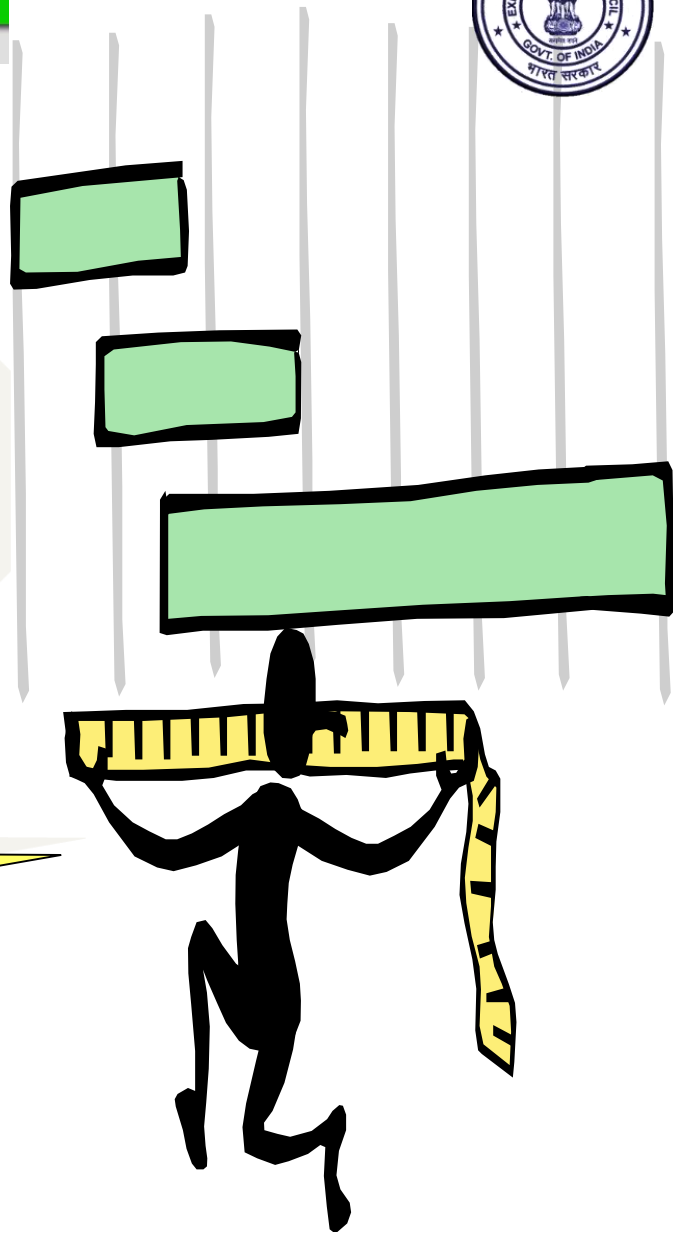


Methods must meet the needs of the customer

Validation is the process of establishing the

- Performance characteristics/perfomance criteria
- Limitations of a method
- Identification of the influences which may change these characteristics and to what extent.

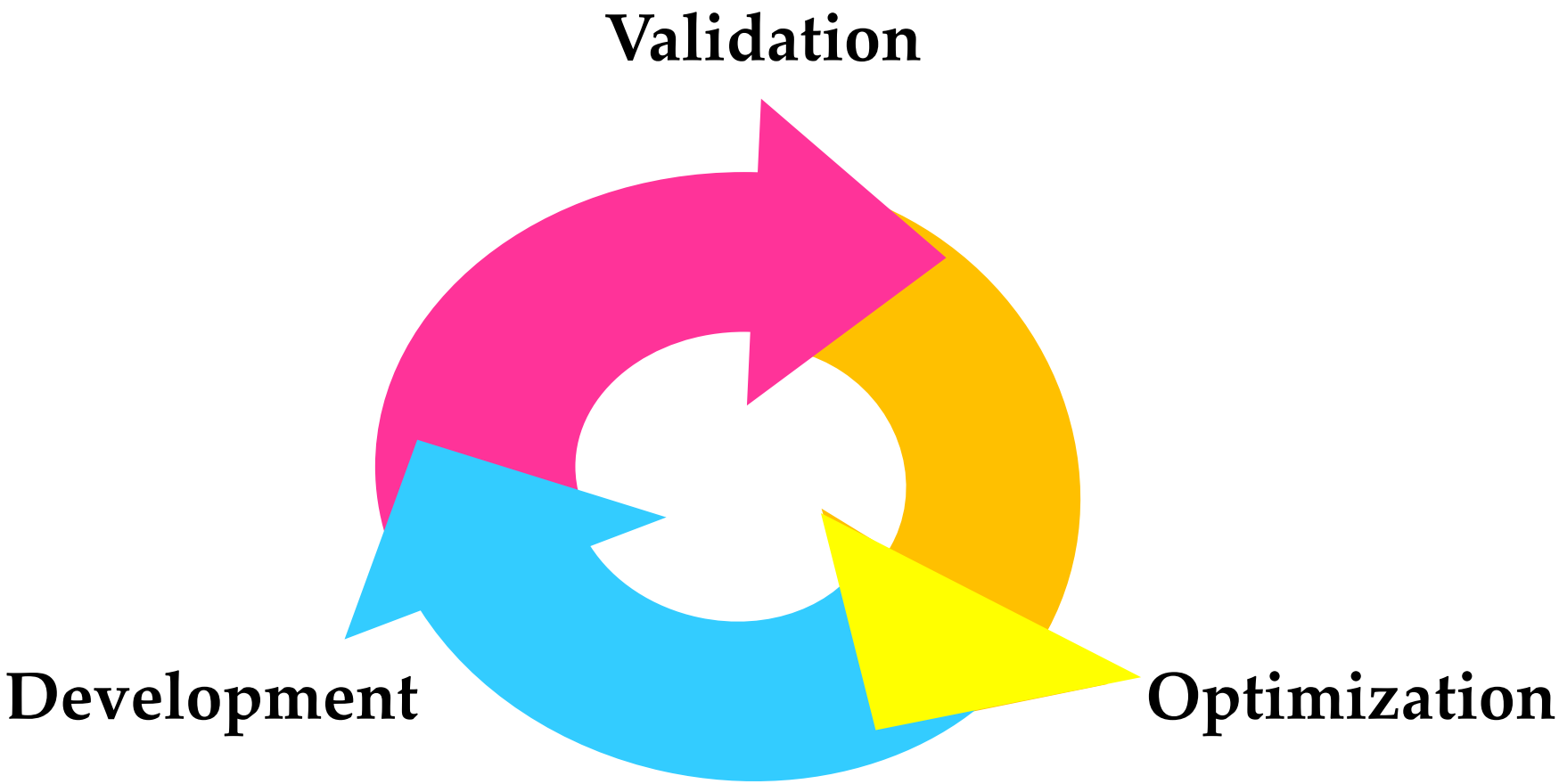
Which analytes can be determined in which matrix in the presence of which interferences?

Within these conditions what levels of precision and accuracy can be achieved?

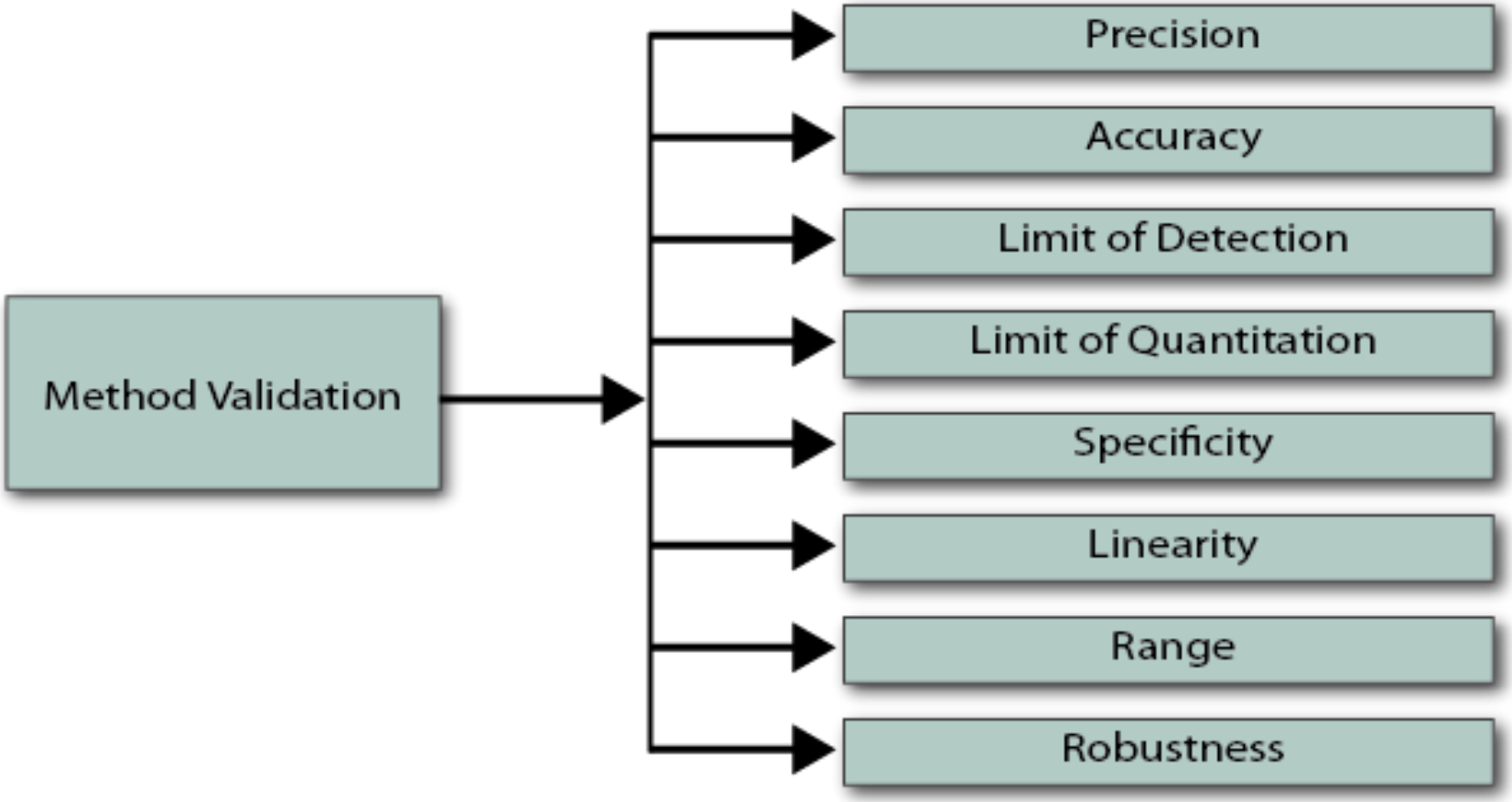
To measure is to know!

The term "Validation" raises questions/doubt on an analyst

Method Life Cycle



Performance characteristics



Specificity

Specificity is the ability of a method to distinguish between the analyte/pathogen being measured/identified from other substances/interferences. Specificity is mainly a function of the measuring technique described, but can vary accordingly to class of compounds/pathogen or matrix. It will be referred to as selectivity in few documents.

Power to discriminate:

Isomers

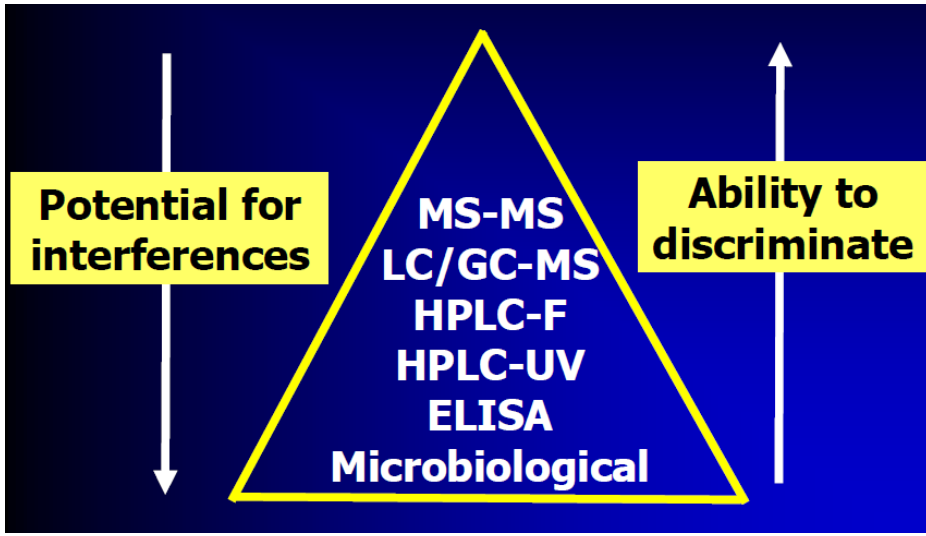
Metabolites

Degradation products

Matrix components

False positive/False negative

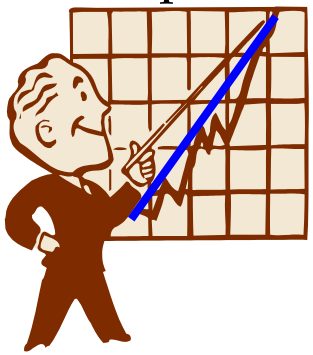
Discrimination depends on method use:



Linearity

Linearity

"Is the relation between the concentration level and response factor linear"



How to go about doing Linearity?

- **At least 3 to 5 levels** should be used for construction of a calibration curve.
- The **working range** of the curve to be described.
- Mathematical formula of the curve and **goodness-of-fit** of the data to the curve to be described.
- **Acceptability range for the parameters of the curve should be described.**

Detection Limit (LOD)/ Quantitation Limit (LOQ)

Limit of Detection (LOD)

- ✓ Lowest amount of analyte in a sample that can be detected but not necessarily quantitated.
- ✓ Estimated by Signal to Noise Ratio of 3:1.

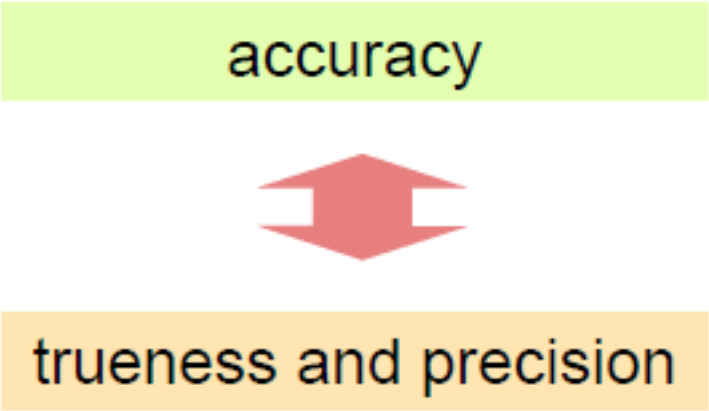
Limit of Quantitation (LOQ)

- ✓ Lowest amount of analyte in a sample that can be quantified with suitable accuracy and precision.
- ✓ Estimated by Signal to Noise Ratio of 10:1.

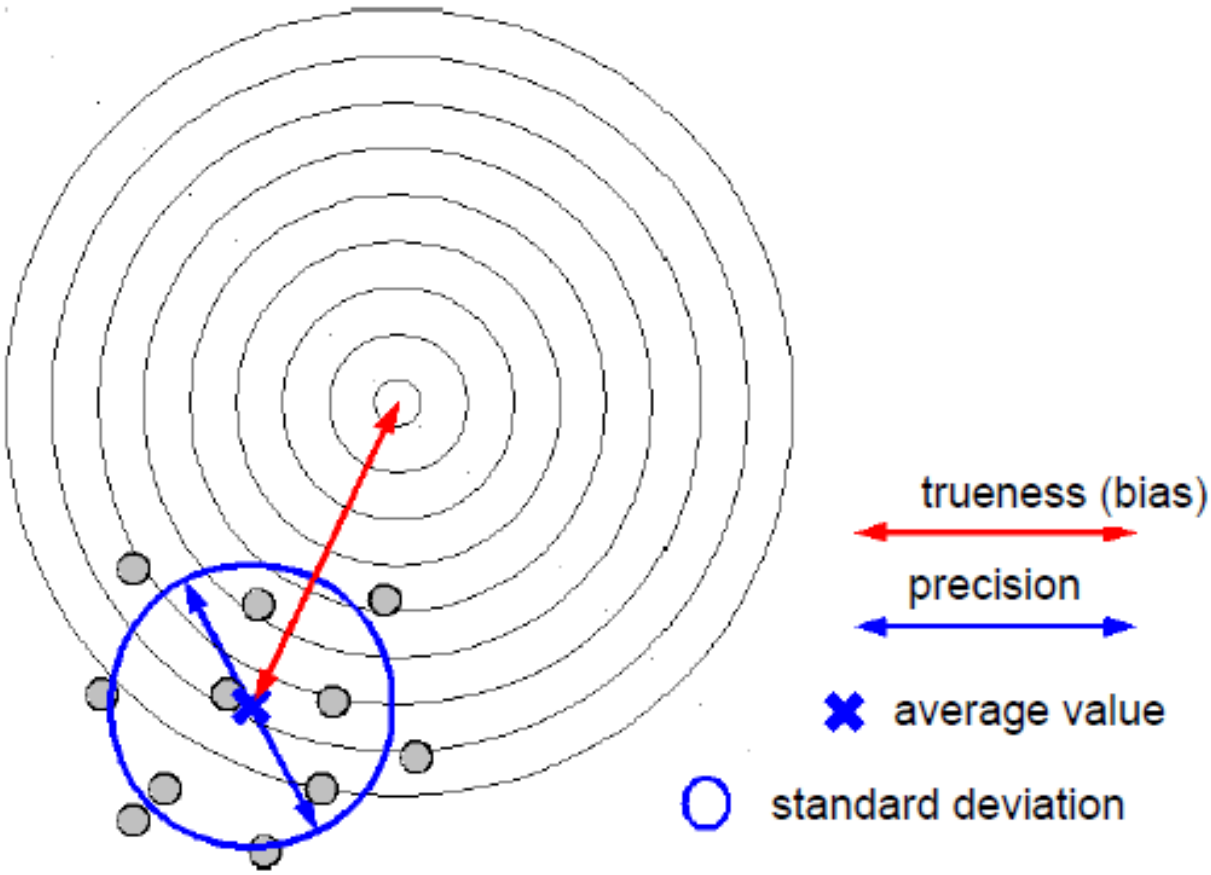
Trueness/Accuracy

- **Trueness:** Closeness of agreement between the average value obtained from a series from test results and an accepted value

- **Accuracy:** Closeness of agreement between a test result and the accepted reference value



Trueness



[Source: wikipedia.com]

Recovery

Recovery is the amount measured as a percentage of the amount of analyte(s) (active substance and relevant metabolites) originally added to a sample of the appropriate matrix, which contains either no detectable level of the analyte or a known detectable level. Recovery experiments provide information on both **precision and trueness (bias)**, and thereby the **accuracy** of the method.

How to go about doing Recovery?

Recovery has to be determined by spiking on a blank matrix.

- Spike at various level considering the range of testing and limits
- Analyse the samples and calculate the concentration in each sample.
- Calculate the **recovery and CV** from six results at each level
- % Recovery= 100 x Measured content/fortification level
- The sample should be corrected for recovery (wherever applicable)

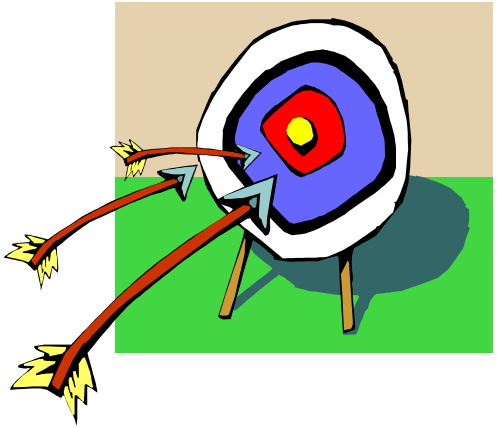
Precision- Repeatability

Repeatability

Closeness of agreement between independent test results obtained under the **same conditions.** **Repeatability conditions** means, conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment

How to go about doing Repeatability?

- Levels to check the repeatability has to be decided based on range of testing and limits
- At each level, analysis to be performed with **minimum 6 replicates (ideally 7)**
- Analyse the sample and calculate the concentration in each.
- Find the mean, SD and CV%.
- Calculate the overall mean concentration and CVs for the fortified sample.



Typical repeatability criteria

Content (µg/kg)	Repeatibility C.V.
1	30 *
10	23 *
100	15
1000	11

*** As low as possible**

Precision- Within lab reproducibility

Within-lab reproducibility

Closeness of agreement between independent test results obtained under **different conditions**. **Reproducibility conditions** means conditions where independent test results are obtained with the same method on identical test items in the same laboratory by a different operator (analyst) using the same equipment on different days.

How to go about doing within lab Reproducibility?

- Level fixed during repeability should be considered to check within lab reproducibility
- At each level, analysis to be performed with **minimum 6 replicates** (ideally 7).
- **Repeat these steps on two other occasions with different operators, different environmental conditions like different batch of reagents, room temperature, different instruments, etc., if possible.**
- Analyse the sample and calculate the concentration in each.
- Find the mean, SD and CV%.
- Calculate the overall mean concentration and CVs for the fortified samples.

Range

The range of an analytical procedure is the **interval between the upper and lower concentration (amounts) of analyte** in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

*The **'working range'** is the interval over which the method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ. The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed.*

Robustness

- The 'ruggedness' ('robustness') of an analytical procedure is "a measure of its capacity to remain unaffected by **small, but deliberate variations** in method parameters.

- A 'ruggedness test' involves making deliberate changes to the method, and investigating the subsequent effect on performance

- Ruggedness provides an indication of the method's reliability during normal usage"

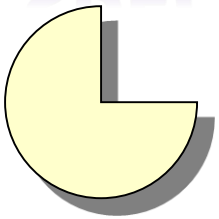
Methods requiring Validation

- ✓ **New or original** methods
- ✓ **Modifications** to validated methods
- ✓ **Extension of scope** to include additional analytes, matrices, or changes in intended use Changes involving new technology or automation
- ✓ Significant **parameter changes**: reagents/apparatus, time /temperature incubation periods, enrichment media, etc.
- ✓ **Specific requirements** of regulatory bodies or customers

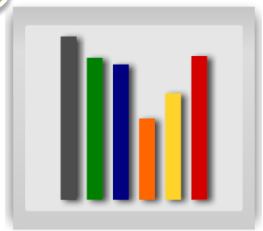
Method Validation- Practical Work

Loads of work, but…

SPECIFICITY



Limit of Quantitation



Accuracy

Range

RUGGEDNESS

Precision

False Positive Rate

Method Validation- Personnel (MAN)

- Personnel designing and approving verifications and validations should be **knowledgeable** about the technology as well as the needs of the lab
- Personnel carrying out verification and validations should be **competent** in all techniques
- Work on verification and validation studies can be used to demonstrate competency but should not be used as training

Pit-falls of Method Validation/Verification

- **Repeating** tests until you get the result you want
- **Drifting** from method validation into method development
- **Adjusting criteria** for acceptance AFTER the data is obtained

Overcoming Pit-falls

- Have a well thought out, documented and **approved plan** and stick to it
- **Go back and start** over, incorporating any changes into new validation
- All data should still be included in evaluation

"Great plan. Could we get some more details?"

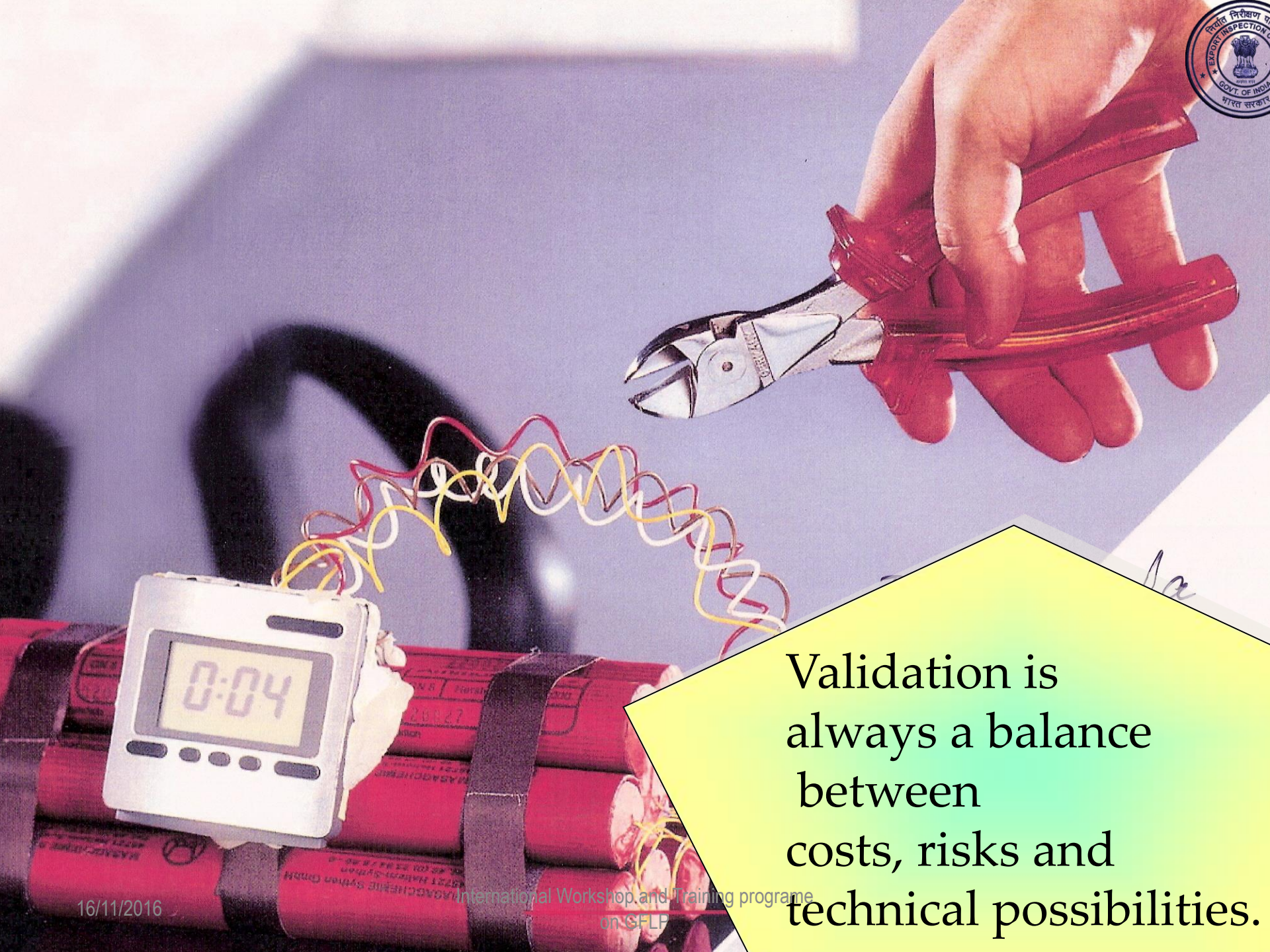
Use of validation data

'Quality assurance' (QA) and 'quality control' (QC) are terms whose meanings are often varied according to the context.

- ✓ Method validation gives an idea of a method's capabilities and limitations which may be experienced in routine use while the method is in control.
- ✓ During the validation stage the method was largely applied to samples of **known content (generally artificially added-spiked)**. Once the method is in routine use it is used for **samples of unknown content (naturally present matrix bound)**
- ✓ **Control charts-** Control charts using Validation data generated against the routine inhouse control check to ensure that the method is **reliable.**
- ✓ Participation on **proficiency testing** to ensure **reliability** of the method and an indication of the reproducibility of method validation done.

Documentation

- Approval of plan prior to running
- Raw Data
- Interpretation of raw data
- Final approval from customer
- Reference of validation in SOP
- If SOP is a modified Standard Method, list deviations from the Standard Method in a section in the SOP



Validation is always a balance between costs, risks and technical possibilities.

Conclusion

Validation — Demonstrate the **METHOD** is "equivalent" to the reference method [for the matrices validated] based on defined method criteria

Verification — Demonstrate in the hands of the **USER** that the method can be performed to meet the defined method criteria

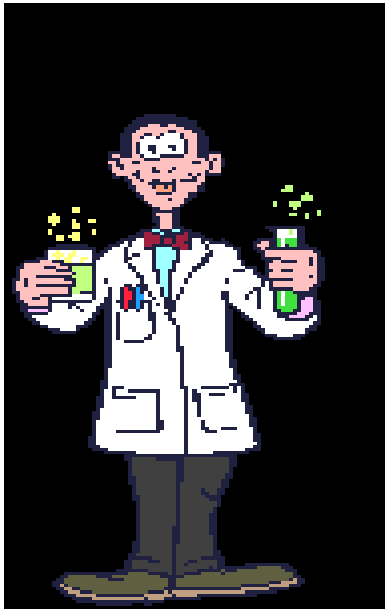
Method validation and verification are **tools** for a **Good Food Laboratory Practice**

MAN –MACHINE- METHOD

Reference for validation/verification

- International Union of Pure and Applied Chemistry (IUPAC)
- AOAC_Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals
- ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD) GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT
- CODEX - CAC/GL 74-2010 Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific dna sequences and specific proteins in foods http://www.codexalimentarius.org/standards/list-of-standards/
- ISO/DIS 16140-1 Microbiology of food and animal feed — Method validation — Part 1: Vocabulary Currently under revision
- US FDA: Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf
- EU regulations (CD/657/2002/EC, SANTE 11945/2015

(**eur-lex**.europa.eu/homepage.html)



Thank you for your kind attention- Question!!!!!!

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