sciensano

healthy all life long

IMPLEMENTATION OF ISO/CEI 17025:2017 FOR CAPRIPOX DIAGNOSTICS

(SPECIAL FOCUS ON ELISA & RT-PCR ASSAYS)

October 3, 2019

.be

EU REGULATION 625/2017

(50) Laboratories designated by the competent authorities to carry out analyses, tests and diagnoses on samples taken in the context of official controls and other official activities should possess the expertise, equipment, infrastructure and staff to carry out such tasks to the highest standards. To ensure sound and reliable results, those laboratories should be accredited for the use of these methods according to standard EN ISO/IEC 17025 on ‘General requirements for the competence of testing and calibration laboratories’.

Benefits of ISO 17025 and accreditation

sciensano

.be

REQUIREMENTS CONCERNING

ISO/CEI 17025:2017 7.2 : SELECTION, VERIFICATION AND VALIDATION OF METHODS

EU REGULATION 625/2017ANNEX III CHARACTERISATION OF METHODS OF ANALYSIS

sciensano

.be

ISO/CEI 17025:2017 Chapter 7.2

7.2.1.4 When the customer does not specify the method to be used, the laboratory shall **select an appropriate method** and inform the customer of the method chosen.

7.2.1.5 : The laboratory shall verify that it can properly perform methods before introducing them by ensuring that it **can achieve the required performance**. Records of the verification shall be retained. If the method is revised by the issuing body, verification shall be repeated to the extent necessary

7.2.2.1 : The laboratory shall validate non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified. The validation **shall be as extensive as is necessary to meet the needs of the given application or field of application**

ISO/CEI 17025:2017 Chapter 7.2

What must be done :

- Selection of appropriate methods
- Verification of standard methods witch are:
  - Methods published either in international, regional or national standards, or by reputable technical organizations or in relevant scientific texts or journals and generally accepted within a discipline / sector;
  - Methods published, validated and made mandatory within the framework of specific regulations by the competent authority;
  - Methods published by kit manufacturers as long as they have been validated and officially recognized by a competent body.
- Validation of non-standard methods

Verification

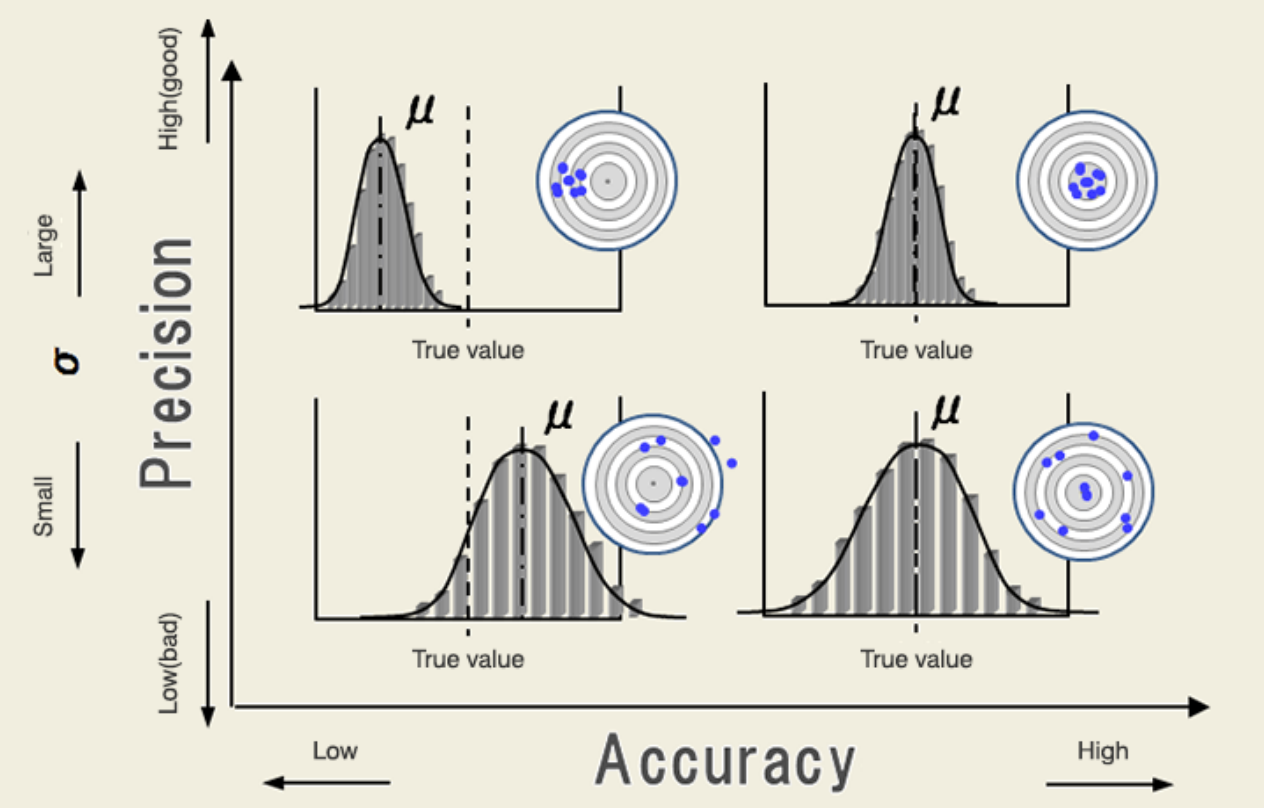
Verification is to provide evidence that the laboratory is capable of achieving the **required performance** characteristics of the method;

This include:

- **Accuracy of measurement**
- **Evaluation of the measurement uncertainty**

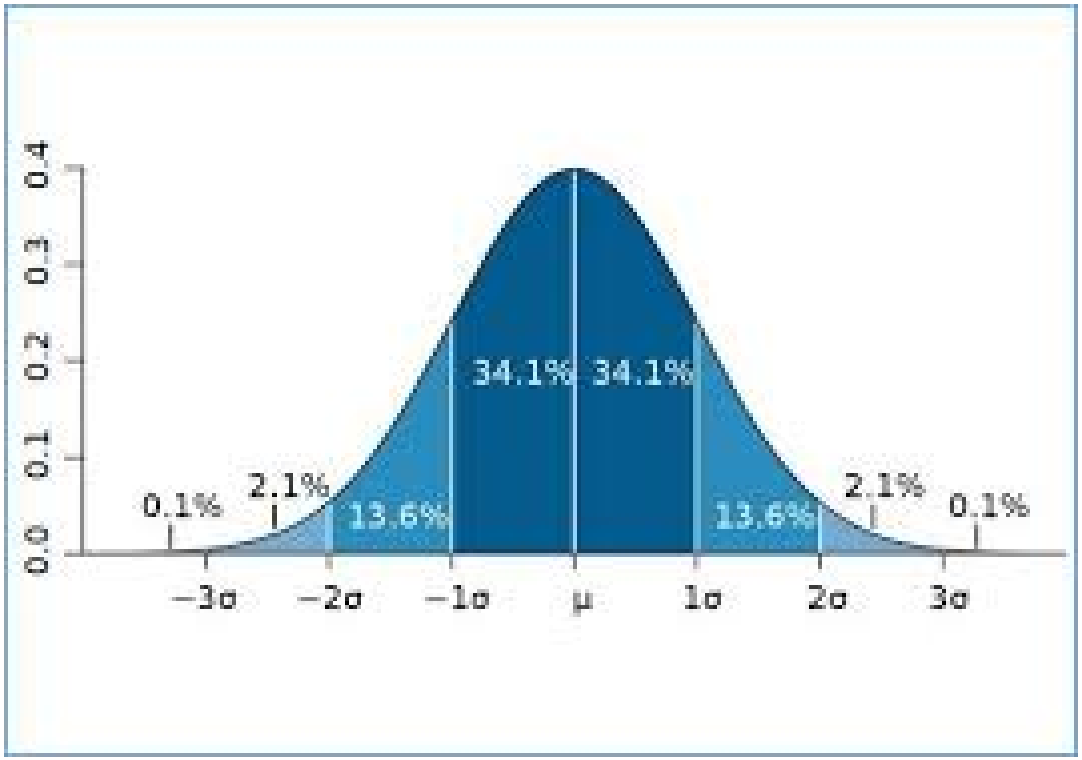
Verification

Accuracy is the closeness of agreement between a test result and an agreed upon reference for comparison. It is expressed by two components: bias and precision



Verification

Measurement uncertainty parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand



sciensano

.be

Verification

In the context of ELISA and RT-PCR tests

Repeatability = Intra-run Repeatability = CV Intra-run

$$CV = \frac{\sigma}{\mu}$$

Intermediate precision = Inter-run Repeatability = CV Inter-run

Reproducibility : obtained from a collaborative trial

Uncertainty = 2 times the standard deviation σ (e.g. the 95 % confidence intervals) of the inter-run repeatability

Verification

Case of RT-PCR tests

In addition

Limit of detection (LOD) of the method

The LOD is the lowest amount of analyte (copies of genomic material) in a sample that can be detected with (stated) probability (e.g. in 95% of cases), although perhaps not quantified as an exact value.

Validation

Validation is the process used to confirm that an analytical procedure employed for a specific test is **suitable for its intended use.**

Validation shall be as extensive as is necessary to meet the needs of the given application or field of application (e.g. screening or confirmation).

Planning a validation by having a systematic approach.

Judge which factors are of most importance and deserve most attention.

Validation

In the context of ELISA and RT-PCR tests

The same parameters as verification and in addition:

Diagnostic sensitivity (Dse) and specificity (DSp)

DSe : proportion of samples from known infected reference animals that test positive in an assay

DSp : proportion of samples from known uninfected reference animals that test negative in an assay

Validation

Analytical specificity (inclusivity and/or exclusivity)

Inclusivity is the capacity of an assay to detect several strains or serovars of a species, several species of a genus, or a similar grouping of closely related organisms or antibodies thereto.

Exclusivity is the capacity of the assay to detect an analyte or genomic sequence that is unique to a targeted organism, and excludes all other known organisms that are potentially cross-reactive.

Aspecific or cross reactions will be examined.

In the case of RT-PCR the analytical specificity (inclusivity and exclusivity tests) must be experimentally evaluated by PCR amplification.

Validation

Negative deviation, positive deviation and relative trueness

	Positive (Reference method)	Negative (Reference method)	
Positive (Alternative method)	A	B	A+B
Negative (Alternative method)	C	D	C+D
	A+C	B+D	A+B+C+D

Positive deviation: [b / (b+d)] x 100

Negative deviation: [c / (a+c)] x 100

Relative trueness: [(a+d) / (a+b+c+d)] x 100

Validation

Analytical sensitivity = Limit of detection (LOD) of the method or minimum required performance limit

The LOD is the estimated amount of analyte in a specified matrix that would produce a positive result at least a specified percent of the time.

Estimated LOD will be based on spiking of the analyte into the target matrix.

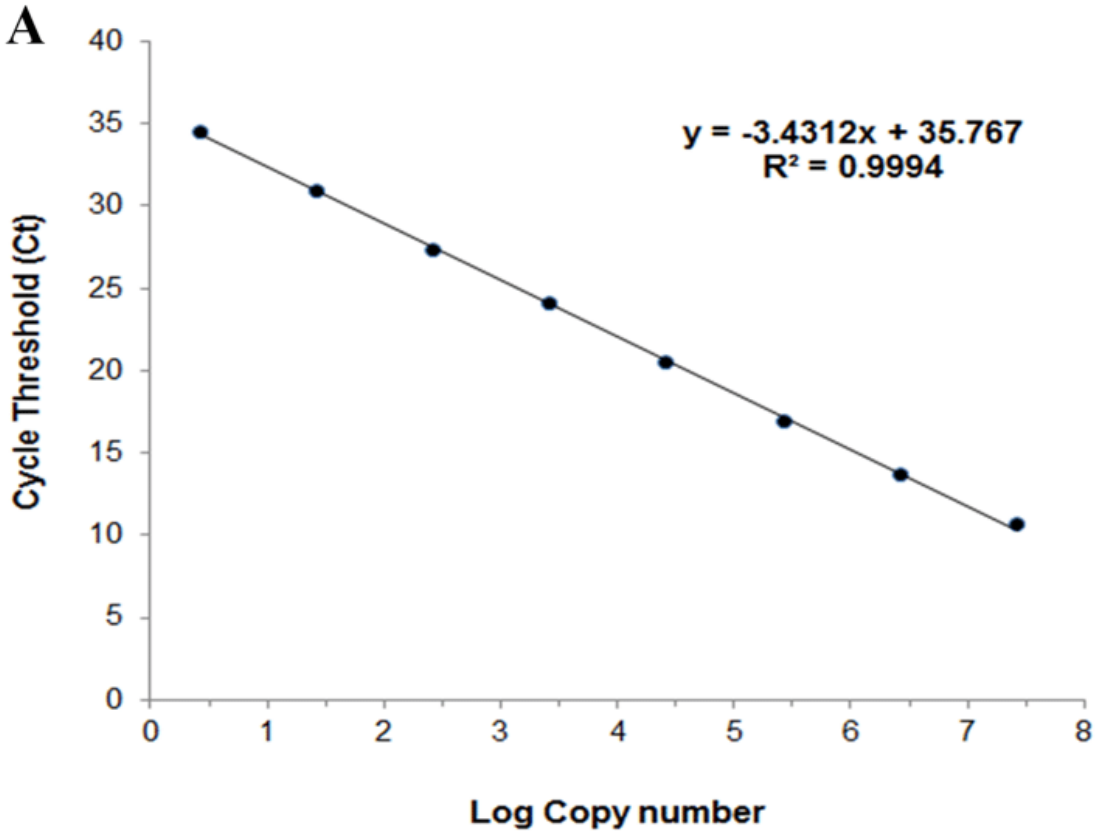
Alternative : determination of the minimum required performance limit using reference material specified in the legislation which must be absolutely positive in the assay.

Validation

Case of RT-PCR tests In addition

LODPCR

Linearity (efficiency)



sciensano

.be

Verification - Validation

Matrix effect (applicability)

Must systematically be analyzed

Robustness

Over time evaluation

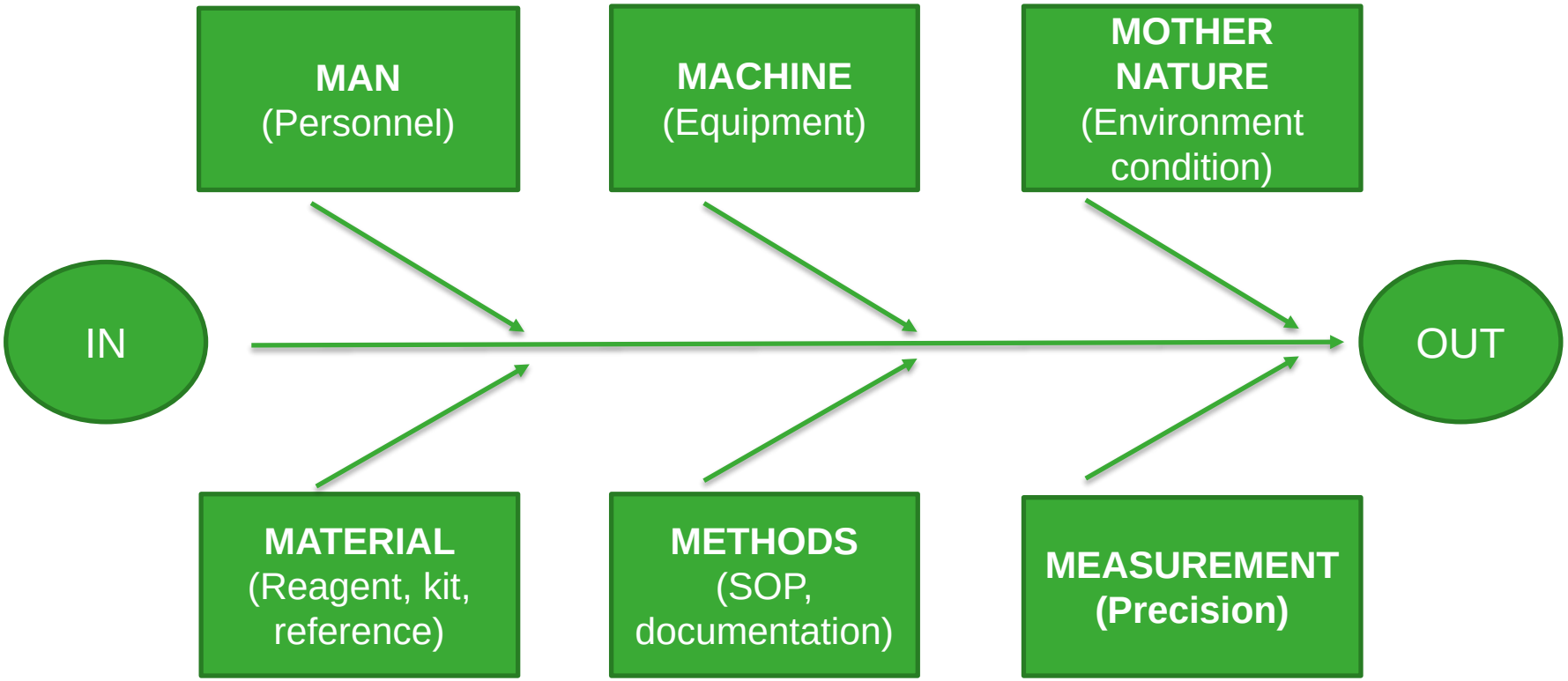
VALIDITY AND RELIABILITY OF TEST RESULTS

sciensano

.be

Factors to managing

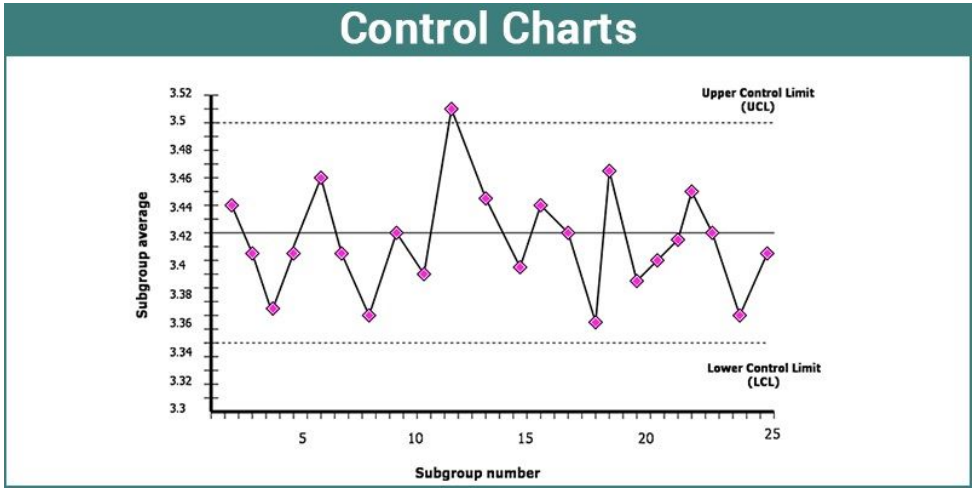
Ishikawa or fishbone diagram



Internal and external Quality Control (QC)

Internal QC

- Control samples



- Retesting retained items

External QC

- Proficiency testing
- Intralaboratory comparisons

sciensano

.be

Literature - Reference documents

- EN ISO/IEC 17025 : 2017 ‘General requirements for the competence of testing and calibration laboratories’
- Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 march 2017
- OIE Manual Chapter 1.1.2. principles and methods of validation of diagnostic assays for infectious diseases
- ISO 5725-1 : 1994 ‘Accuracy (Trueness and Precision) of Measurement Methods and Results - Part 1: General Principles and Definitions’

sciensano

healthy all life long

Contact

Katia Knapen • katia.knapen@sciensano.be • +32 2 379 04 06

Sciensano • Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium
T +32 2 642 51 11 • T Press +32 2 642 54 20 • info@sciensano.be • www.sciensano.be

.be