

## **CALCULATION PERFORMANCE EVALUATION CRITERIA**

### **Laboratory code and confidentiality**

Confidentiality is guaranteed. Participants are identified in the Final Report by a randomly assigned code.

The laboratory codes were communicated to each participant through his web restricted area.

### **Confirmatory and screening result: concentration value**

In confirmatory analysis, the compounds are separated by chromatographic techniques (GC, HPLC, UPLC, LC...); afterwards they are detected by MS, FLD, DAD, etc...

In screening analysis, participants use techniques as ELISA, lateral flow, etc...

Each participation includes the possibility of being evaluated for 2 different results (obtained with different methods or different technicians).

Participants were asked to report quantitative or semiquantitative ("less than..." or "greater than...") results corrected for recovery.

In case of confirmatory quantitative results, if correction is not applied, the value is not included in the calculation of the assigned value.

Results can be given as:

- "=" means that the analyte was detected and quantified
- "< of..." means that the analyte was not detected
- "> of..." means that the analyte was detected but not quantified
- "Not Searched-NS" means that the laboratory did not perform the analysis

In reference to the method used, screening results can be expressed for a single analyte or for an analyte group.

### **Limits of Detection (LOD/DL), Limits of Quantification (LOQ/QL) and uncertainty/MU(%)**

For mycotoxins: the limits of detection, limits of quantification and uncertainty for the relative compounds were claimed by the laboratories.

For acrylamide: the limits of quantification were claimed by the laboratories.

If the DL and/or QL and/or MU(%) are not reported by participants, in the tables the box will be empty.

### **False positive results**

If it is possible, false positive results obtained by the laboratories are shown.

- "None" means that no false positive results were reported. This corresponds to a good performance for both screening as well as confirmatory methods.
- "-" means that evaluation was not applicable (e.g. the laboratory search only the compound that was present)

It is not possible to classify as false positives concentrations lower than the assigned value of an intended blank test material. In this case concentrations are reported just for information.

### **False negative results**

In case of false negative confirmatory results, no evaluation is given.

If the laboratory does not detect the analyte that is effectively present, as its method does not allow it, this is an information concerning the capability of the method. The result is "congruent" but the participant should take into consideration if his method has the appropriate capability in respect of his requirement.

In case of false negative screening results, evaluation is given as described (see screening assessment).

### **Elaboration of laboratory data**

In the statistical data processing, all the data submitted by the participants are elaborated considering two decimal places. In case there are not declared decimal places, they are considered as corresponding to "zero" (E.g. 25=25.00 – 25.3=25.30 – 25.32=25.32).

## **CALCULATION OF THE ASSIGNED VALUE**

### **The Assigned Value $x_{pt}$**

The Assigned Value  $x_{pt}$ , is the value attributed to a particular property of proficiency test items (definition from ISO13528:2022).

In the routine, the results from the confirmatory analysis (chromatographic techniques) are considered a reference; they are used with legal purpose (as regulatory requirement). Instead, screening methods have the purpose to analyze in a short time a wide quantity of samples; in case of "positive" results, the data will be verified through the use of chromatographic techniques.

Because of above described, the Assigned Value  $x_{pt}$  derives just from participants' quantitative results obtained with confirmatory analysis. The screening results are compared to the Assigned Value  $x_{pt}$  obtained from the confirmatory data.

The procedure for determining the Assigned Value  $x_{pt}$  is described below.

After excluding results that are identified as invalid the data population was checked for normality and for the presence of outliers by applying appropriate statistics.  $x_{pt}$  represents the value of concentration obtained from Algorithm A or from the median.

The chosen value will be reported in the specific test material tables, where Algorithm A will be indicated as "ISO5725-5 (Alg.AS)" and median as "MADe/Median".

Sometimes very low concentrations are quantified. When it occurs, the concentration value is assigned only if statistics described in this paragraph are applicable.

The value is not assigned when  $p < 8$ , where "p" is the number of data after invalid results rejection, when there is a bimodal distribution data or when the standard uncertainty is not negligible (based on PTP experience).

It may happen that only few participants confirm the presence of some analytes in the test materials. In such cases, the presence of an analyte is considered:

- "unconfirmed", when less than 25 % of participants detect the compound;
- "confirmed", when 25% of participants, or more, detect the compound (the minimum number of positive results is anyway three).

In case of "blank" test materials, it is provided a threshold above which the analyte should not be present, which is based on the capability of participants to determine the analyte. Among the participants' data that indicate that the analyte was not detected, it is established the mode, which is the limit of quantification (QL) most used by participants: the mode will be chosen as the estimator. In case more than one mode is present, the PTP will use as estimator a data deriving from its experience on the most frequently limit of quantification used by participants for the analyte. The laboratory whose QL is greater than the threshold value, should take into consideration if his method has the appropriate capability in respect of the requirement.

### **The standard uncertainty of the assigned value $u(x_{pt})$**

The standard uncertainty  $u(x_{pt})$  is calculated as:

$$u(x_{pt}) = 1.25 [s^* / \sqrt{p}]$$

where:

- $s^*$  is the robust estimate of the participant standard deviation;
- $p$  is the number of participants;
- 1.25 is the expanded factor

In case of median as estimator, the standard deviation is calculated as  $s^* = \text{MADe}$  (where MADe is the Median Absolute Deviation).

In case of  $u(x_{pt}) \geq 0.3 \sigma_{pt}$  the standard uncertainty of the assigned value is not negligible, so the effects of uncertainty are introduced in the calculation of the z-score, that will be calculated as z'-score.

### **z-score, z'-score and $\sigma_{pt}$ (standard deviation for proficiency assessment):**

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When the number of confirmatory quantitative data is  $p \geq 8$ , the participant's result (confirmatory and screening) are converted into a

z-score according to the equation:

$$Z\text{-score} = (x_i - x_{pt}) / \sigma_{pt}$$

where:

$x_i$  is the analyte concentration value reported by the laboratory;

$x_{pt}$  is the assigned value (obtained with confirmatory methods);

- $\sigma_{pt}$  is the standard deviation for proficiency assessment calculated from  $b x_{pt}$
- $b = \%RSD / 100$ , (RSD = Relative Standard Deviation)

unless otherwise specified the %RSD value comes from the Horwitz equation (Horwitz, W., 1988, *Pure Appl. Chem.* 60, 855-864)

$$\%RSD = 2^{(1-0.5 \log x_{pt})}$$

where  $x_{pt}$  is expressed as a dimensionless concentration.

$\sigma_{pt}$  is related to the concentration of the analyte: it comes from Horwitz equation (unless otherwise specified); in case of contamination less than 10 ppb the Thompson equation modified Horwitz equation (Thompson, M., 2000, *Analyst* 125, 385-386). In particular circumstance  $\sigma_{pt}$  is chosen from Proficiency Test provider's (PTp) experience, derived from previous rounds.

The adopted criteria is reported in the specific test material table. In the result table, Thompson will be indicated as "Truncated Horwitz".

In case of an inhomogeneous measurand the effects of inhomogeneity are introduced in the calculation of  $\sigma_{pt}$ , that will be calculated according to the equation:

$$\sigma'_{pt} = \sqrt{(\sigma_{pt}^2 + S_s^2)}$$

where:

- $\sigma_{pt}$  is the standard deviation for proficiency assessment calculated from  $b x_{pt}$
- $S_s$  is the between-sample variance obtained in the homogeneity test

In this case the standard deviation for proficiency assessment will be indicated as  $\sigma'_{pt}$

If  $u(x_{pt}) < 0.3 \sigma_{pt}$  is not met, participants' results are converted into a z'-score according to the equation:

$$z'\text{-score} = (x_i - x_{pt}) / \sqrt{(\sigma_{pt}^2 + u^2(x_{pt}))}$$

where:

$x_i$  is the analyte concentration value reported by the laboratory;

$x_{pt}$  is the assigned value (obtained with confirmatory methods);

- $\sigma_{pt}$  is the standard deviation for proficiency assessment calculated from  $b x_{pt}$
- $u^2(x_{pt})$  is the standard uncertainty calculated as previously described.

The chosen score is reported in the specific test material table.

The laboratory performance evaluation was established taking into account the following criteria for z-score:

acceptable (satisfactory)	when	$ z  \leq 2.0$
warning signal (questionable)	when	$2.0 <  z  < 3.0$
action signal (unsatisfactory)	when	$ z  \geq 3.0$

### Screening assessment

Participants who use screening methods, have to provide quantitative answer (see z-score and  $\sigma_{pt}$  chapter); if they provide a

semi-quantitative data (“lower than...” or “greater than...”) they receive a qualitative evaluation:

The results are classified as “satisfactory” in the following cases:

- The laboratory detects the analyte or the group of analytes that are effectively present in the test material.
- The laboratory does not detect the analyte or the group of analytes that are not effectively present in the samples.

The results are classified as “unsatisfactory” in the following case:

- The laboratory does not detect the analyte or the group of analytes that are effectively present in the sample, but according to the method specifications the analyte/analytes is/are detectable. It means that a false negative has been reported.

The results are classified as “questionable” in the following case:

- The laboratory detects an analyte or a group of analytes that were not effectively present in the sample. It means that false positive has been detected. The false positive results are not considered unsuitable, because routine screening positive results should be confirmed by chromatographic methods.

The results are classified as “congruent” in the following case:

- The laboratory does not detect the analyte or a group of analytes that is effectively present, as its method does not allow it. This is an information concerning the capability of the method. The participant should take in consideration if his method has the appropriate capability in respect of his requirement.

The results are not classified, therefore “not applicable” in the following case:

- The laboratory detects the analyte or a group of analytes that were not effectively present in the sample, but the level detected is lower than declared value. In this case it is not possible to evaluate its results.
- The laboratory does not detect the analyte that is effectively present, but his capability corresponds exactly to the assigned value.

Because of the different country legislations, the regulatory limits are not considered in the results evaluation.

### Evaluation screening table

Example of the performance criteria for the screening are reported in the table below.

**Table d:** example of evaluation.

TEST MATERIAL CONTAMINATION	RESULT (PROVIDED BY PARTICIPANT)	EVALUATION	Z-SCORE
Contaminated material (the analyte is present above or below the regulatory limit)	=5 ppb or =7ppb (quantitative result)	not provided	provided
	>5 ppb or >7 ppb (semi-quantitative result)	satisfactory	not provided
	<5 ppb (semi-quantitative result)	unsatisfactory	not provided
	<7 ppb (semi-quantitative result)	congruent	not provided
Assigned Value $X_{pt}$ = 6 ppb (from confirmatory methods)	<6 ppb (semi-quantitative result)	not applicable	not provided
Blank material	<5 ppb or <7 ppb (semi-quantitative result)	satisfactory	not provided
	=6 ppb or =7 ppb (quantitative result)	questionable	not provided
Value < 6 ppb (from confirmatory methods)	>5 ppb or >7 ppb (semi-quantitative result)	questionable	not provided
	= 5 ppb (quantitative result)	not applicable	not provided

### Graphical presentation

Confirmatory and screening results are shown in different tables. This approach takes into account the different purpose of the methods.

When confirmatory participants receive a score it will be shown in the “bar-chart of z-score”.

Where there is an invalid data, it will be mentioned in the table of assigned value and target standard deviations.

## REFERENCES

“Progetto Trieste” is managed in agreement to:

- UNI CEI EN ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing
- EURACHEM Selection, Use and Interpretation of Proficiency Testing (PT) Schemes, 3rd edition, 2021
- ISO 13528:2022 Statistical method for use in proficiency testing by interlaboratory comparisons
- UNI CEI EN ISO 17034:2017 General requirements for the competence of reference materials producers
- IUPAC Technical Report The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, 2006