



Guidelines PT ARP AE 2025

Battery of ecotoxicological tests

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1 INTRODUCTION

The purpose of the Proficiency Tests (PT) is to verify the participants' performance in accordance with the official methods used.

ARP AE RA has initiated the accreditation process as a Proficiency Test Provider (PTP), which will finish in October 2025 with the scheduled inspection visit. The proposed schemes will be subject to accreditation and will be carried out in compliance with the standard UNI CEI EN ISO/IEC 17043:2023.

This guideline and all the documentation sent to the participants, are an integral part of the Quality Management System (QMS) documents, drawn up in compliance with the UNI CEI EN ISO/IEC 17043:2023 standard.

The proficiency tests are organized by ARP AE for the determination of the ecotoxicity of chemical substances in aqueous solution.

The organization of the proficiency tests aims to improve the quality and accuracy of ecotoxicological tests data, as well as to evaluate the ability of each laboratory to obtain comparable results at national and European level.

All analytical determinations concerning the test samples are carried out by an Arpae laboratory accredited according to the UNI CEI EN ISO/IEC 17025:2018 standard.

2. CONFIDENTIALITY

The laboratories are identified only and exclusively through a numerical code (Lab.id), that is assigned at the time of registration and it is maintained for subsequent PTs.

The communication of a participant's results to third parties occurs only with the prior knowledge and written authorization of the participant.

This authorization is not foreseen if the request is required by law, however the Provider must give written notice to the participant.

3. SAMPLES FOR PT BATTERY OF ECOTOXICOLOGICAL TESTS

The preparation for the proficiency test (PT) includes:

- **the selection of the matrix:**

- Milli-Q water, grade 1 water with conductivity <10 $\mu\text{S}/\text{cm}^2$, for the EC50 determination with *Daphnia magna*, *Pseudokirchneriella subcapitata*, and *Aliivibrio fischeri*.

- ISO test water (ISO 6341 - 6.3 Dilution and culturing water; LG OECD 202 – Annex 3 ISO test water) for the % of immobilisation with *Daphnia magna* and for the OECD 236 - Fish Embryo Test (FET)

● **the selection of the active substance:** the substance to be used as a toxicant in the test is selected based on the following characteristics:

- availability of literature data
- low toxicity to the operator
- easy availability and accessibility
- low cost
- easy disposal at the end of the test

A defined quantity of active substance is homogenized with an appropriate volume of matrix; subsequently, it is divided into containers. If requested, ten of these containers, selected at random, are analyzed in duplicate to assess homogeneity. Often a particular test material does not required homogeneity assessment prior to distribution: such sample types includes standard solutions in water whose homogeneity was already assessed in previous PT round.

The samples are kept at a refrigerated temperature before shipping and they are shipped at room temperature.

To evaluate sample stability, three containers, selected at random, are analyzed in duplicate upon sample shipping, 48 hours post-shipping, and at the deadline of the result submission .

4. COMMUNICATIONS

Communications between ARPAE and PT participants take place using the following Annexes:

- *Annex 1: PT Program for 2025* - sent by email with the presentation letter.
- *Annex 2: Costs of the Proficiency Test 2025* - sent by email with the presentation letter.
- *Annex 3: PT registration form for 2025* - sent by email with the presentation letter; each participant must give it back by the specified deadline.
- *Annex 4: list of active substances.*
- *Data return sheet:* for each test, an Excel spreadsheet will be sent for the insertion of results as well as other useful information

If, during the year, changes should arise concerning company names, shipping addresses, billing data, email addresses and reference personnel, it is the responsibility of the participating laboratory to notify the coordinator by completing a new *Annex 3 - PT registration form* for the year 2025.

5. SUBCONTRACTS

Not applicable

6. ASSIGNED VALUE, ROBUST RELATIVE STANDARD DEVIATION AND PERFORMANCE ASSESSMENT

Before proceeding with the calculation of the assigned value and PT standard deviation, the distribution of the results is assessed using a Kernel density plot to highlight any bimodal trend and to evaluate the asymmetry of the distribution. This preliminary analysis allows for the identification of gross errors, also known as blunders. Gross errors are defined as results that deviate by one unit of measurement or more from the median of the data. Such results will be excluded from subsequent calculations of the assigned value and PT standard deviation. In the final report/data statistics tables, the excluded data will be highlighted.

Additionally, a box plot is employed to evaluate the presence of outliers.

Outliers are not excluded from the calculation, as the influence of anomalous results on statistical evaluation is minimized by applying robust statistics analysis through Algorithm A, as described in Annex C of ISO 13528:2022. As stated in section 6.5.2 of ISO 13528:2022, Algorithm A can compensate for the presence of anomalous data up to a maximum of 20% of the entire dataset. In the case of a percentage greater than 20%, the outliers are removed and the mean and standard deviation were calculated.

At the organization's discretion, the assigned value is calculated either as robust mean using the Algorithm A as described in Annex C of ISO 13528:2022 or as mean when Algorithm A is not applicable. The uncertainty of the assigned value is always calculated and included in the report.

The PT standard deviation (σ_{pt}) is in general set as 30% of the assigned value. The PTP provider could also calculate the σ_{pt} as standard deviation or robust standard deviation.

To evaluate the performance of each participant, the z-score parameter is calculated using the following mathematical calculation:

$$z = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

where

x_i : represents the value detected by the laboratory

x_{pt} : represents the value detected by the robust statistical analysis

σ_{pt} : represents the PT standard deviation

For tests where the uncertainty of the assigned value, $u(x_{pt})$, is not negligible compared to the PT standard deviation, σ_{pt} , i.e., $u(x_{pt}) > 0.3\sigma_{pt}$, the z'-score is calculated as described in ISO 13528, paragraph 9.5.1:

$$z' = \frac{(x - x_{pt})}{\sqrt{u^2(x_{pt}) + \sigma_{pt}^2}}$$

The meaning of 'z-score' and 'z'-score' is presented in the following table:

z-score value	assessment
$ z \leq 2$	satisfying
$2 < z < 3$	questionable
$ z \geq 3$	unsatisfying

Only one value is used for each participating laboratory; in the case of submitting more than one result, the participating laboratory must designate one result from those submitted, which will be used for performance evaluation. Any other results submitted will be evaluated based on the statistical data obtained in the PT and communicated via email to the participating laboratory. The results provided by the participants are never rounded and are used in the calculations as submitted by the participants; the descriptive and performance statistics are calculated and reported in the report with 4 significant figures. The z-score is reported with three significant figures.

Since the assigned value of the PT is calculated as the consensus value among participants, in the case of a bimodal distribution, the z-score will not be calculated, highlighting this in the final report and still providing participants with as much information as possible, such as descriptive statistics and graphical analysis.

If the number of participants is small, the PTP may decide not to calculate the quantitative performance of the participants or may still provide it as a z-score (or z'-score), making sure to clarify in the final report the meaning to be attributed to any performance values provided. The uncertainty of the assigned value must always be calculated and reported in the final report, where the approach chosen for managing these data should also be stated.

In some cases, the analysis of the % of immobilization can be complex due to the strong asymmetry of the distribution of the results. In these cases, the PTP may decide not to provide a quantitative

performance through the calculation of the z-score, but instead issue a qualitative judgment based on the predicted result.

7. STEPS TO FOLLOW

For the year 2025, participation in the proficiency test involves 4 essential steps:

- 1 Each laboratory must complete and return to ARP AE the Annex 3, the registration form, by the specified deadline; the tests of interest must be clearly indicated. The email address provided through the participation form is the only one that will be used for communications. Any changes must be notified.
- 2 Participating laboratories must respect the deadlines indicated for sending the results, using exclusively the Data Return Sheets.
- 3 Once all the results have been submitted, ARP AE evaluates the results and sends to the participants the Final Report in pdf format. This report will include information on the test preparation, statistical evaluation and graphical representation of the results, and any other relevant information.
- 4 Participating laboratories will receive by 31/12/2025 the invoice for the PT participation that includes the costs for samples and shipping. Participating laboratories must pay the amount reported in the invoice. The costs are those reported in *Annex 2: Costs of the Proficiency Test 2025*.

8. SAMPLE QUANTITY

Participating laboratories, depending on the chosen tests, may receive:

1. For round 1S-E25 PT FET:

- A PET bottle containing 1000 ml of sample

2. For round 2S-E25 PT ECOTOX:

- A PET bottle containing 500 ml of sample for the determination of the % of immobilization.
- A PET bottle containing 1000 ml of sample for the EC50 determination tests with *Daphnia magna*, *Pseudokirchneriella subcapitata* and *Aliivibrio fischeri*.

9. SAMPLE SHIPMENT

All the test samples are stored at refrigerated temperature until shipping. The expected shipping dates are those indicated in *Annex 1: PT Program for 2025*. In case of damage or loss of the items, the participating laboratory may request a second shipment at no cost.

10. SAMPLE HANDLING

Once received, the test samples must be kept under refrigeration until analysis to prevent any deterioration. All the participants may use their own standard analytical procedures and reference materials for testing. The sample should be handled as a routine sample.

11. RESULTS

The results are returned by an Excel spreadsheet (Data Return Sheet) for each of the selected determinations. Until the deadline, an unlimited number of changes and/or additions can be made by submitting a new Excel spreadsheet (Data Return Sheet) to replace the one previously sent. After the deadline, it is no longer possible to modify the results sent.

The samples received are considered to be at a concentration of 100%, and any dilution must be referred to this concentration.

The results must be expressed as a percentage of the initial sample, using a period as decimal separator; no separator is needed to identify thousands.

12. APPEALS AND COMPLAINTS

In accordance with ARP AE's Quality Management System (QMS), participants to the PT have the opportunity to submit appeals or complaints to the PT coordinator by sending a written communication via email to the following address: *interconfronto.arpae@gmail.it*.

The nature of the complaint is related to the service provided: delays in the scheduled timeline, damaged samples.

An appeal can be submitted after the final report has been issued. The participant may contest the evaluation of their performance by presenting objective documentation of their reasons. Once an appeal or complaint has been received, ARP AE has 30 calendar days to respond via email and, if necessary, amend the final report.

13. REMAINING SAMPLES

As reported by ARP AE QMS, at the conclusion of each PT scheme, remaining samples are stored at a controlled freezing temperature, for a period of 30 calendar days from the sending date of the Final Report revision 0.

The samples will remain available to the participants who may request an additional aliquot to use the sample for their own purposes.

Please note that the stability of the material is guaranteed until the results transmission deadline, that is communicated by email and specified in *Annex 1: PT Program for 2025*. The participating laboratory is responsible for the shipping logistics and costs of the samples.

14. ARCHIVING

All the records related to PT schemes (e.g., results sent by participants, statistical analysis, reports, documents and any relevant communication) are stored on a computer medium by the PTP, for at least 5 years. The electronic storage of these files is managed in accordance with I71101/LM.