Protocol – Microbiological Proficiency Testing

Drinking water and Food

Livsmedelsverket
Swedish Food Agency

PT micro
Since 1981

This protocol is available at: https://www.livsmedelsverket.se/en/PT-micro

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Contents

1. Introduction 6
2. Organisation 6
2.1. General information 6
2.2. Information on the program webpage 6
2.3. Accreditation 6
2.4. Areas of responsibility 7
2.5. Advisory group 7
2.5.1. Subcontracting services 7
2.6. The two schemes 7
2.6.1. The drinking water scheme 7
2.6.2. The food scheme 7
2.7. PT round schedule and activities 7
2.7.1. Participant activities 8
2.7.1.1. Registration/cancellation 8
2.7.1.2. Instructions and analyses 8
2.7.1.3. Reporting method information 8
2.7.1.4. Reporting analytical results 8
2.7.1.5. Collusion and falsification of results 8
2.7.2. PT provider activities 9
2.7.2.1. Reporting reminder 9
2.7.2.2. Corrections 9
2.8. Confidentiality and participant identity 9
2.8.1. Participant code and password 9
2.8.2. The principle of public access to information 9
2.9. Conditions and obligations 10
2.9.1. General 10
2.9.2. Who can participate? 10
2.9.3. Which methods may be used? 10
2.9.4. Participant obligations 10
2.9.5. Obligations of the Swedish Food Agency 10
2.9.6. Limited responsibility 10
2.9.7. Complaints and deviations 10
2.10. Filing 11
3. Test material 11

3.1. Type of material 11
3.2. Microorganisms 11
3.3. Hazards 11
3.4. Manufacture 11
3.4.1. Strain purity 11
3.4.2. Freeze-drying 12
3.4.3. Storage 12
3.5. Quality control 12
3.5.1. Timing of quality control 12
3.5.2. Selection of vials 12
3.5.3. Analytical methods 13
3.5.4. Homogeneity 13
3.5.4.1. Order of analysis 13
3.5.4.2. First criterion: fulfilment of ANOVA 13
3.5.4.3. Second criterion: comparison with s_R 13
3.5.4.4. Evaluation of homogeneity 13
3.5.4.5. Underestimation of within-vial variation 14
3.5.4.6. Exclusion of vials with deviating results and high variation 14
3.5.5. Target microorganism concentrations 14
3.5.6. Quantitative analyses and special vials 15
3.5.7. Stability 15
3.5.8. Vacuum test 15
3.6. Distribution to participants 15
3.6.1. Labelling of samples 15
3.6.2. Packaging of samples 15
3.6.3. Packaging of samples 16
3.6.4. Transport 16
3.6.4.1. Damage during transport 16
3.6.5. Instructions 16
3.6.6. Safety data sheet 16
4. Data analysis and performance assessment 17
4.1. False results 17
4.2. Results transformation 17
4.3. Assigned value and standard deviation 17
4.4. Accepted results and outliers 18
4.5. Measurement uncertainty for the assigned value 18
4.6. Coefficient of variation 18
4.7. Non-robust statistical measures 19

4.8. Exceptions for small datasets 19
4.9. Z scores 19
5. PT reports 20
5.1. Preliminary report 20
5.2. Final report 20
5.2.1. Histograms and tables 20
5.2.2. Box plots 20
5.2.3. Quality control results 20
5.2.4. Appendices 20
5.2.5. Photos 21
5.2.6. Inaccuracies in the report 21
5.2.7. Use of report by participants 21
5.3. Performance assessment 21
5.3.1. Final assessment 21
5.3.2. Follow-up samples 21
6. References 22

1. Introduction

Laboratories that perform analyses need to ensure that they obtain realistic results. They also need to prove this to their clients to be trustworthy. One means of this is to participate in interlaboratory comparative tests. Participation in such tests is also a requirement according to the standard EN ISO/IEC 17025 [1], where the name Proficiency Testing (PT) is used for these interlaboratory comparisons.

The Swedish Food Agency provides PTs in the areas of food microbiology and drinking water microbiology and is accredited for this according to ISO/IEC 17043 [2]. The PT's are mainly intended for accredited laboratories, but are suitable also for non-accredited laboratories that want to compare their analytical results with other laboratories.

The purpose of this protocol is to give participants, and other interested laboratories and parties, a description of the organisation of the microbiological PTs, and how some basic tasks are performed.

2. Organisation

2.1. General information

The Swedish Food Agency is the central Swedish authority for food issues, including drinking water. The Swedish Food Agency organises microbiological proficiency testing, divided into one scheme for food and one scheme for drinking water.

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2.2. Information on the program webpage

General information about the schemes is available on the program webpage:

https://www.livsmedelsverket.se/en/PT-micro

The webpage is combined with a web portal, where – after login – participants can manage their participation, and report results and method data:

https://laboratory.livsmedelsverket.se

2.3. Accreditation

The Swedish Food Agency is accredited for arranging microbiological proficiency testing, according to the standard EN ISO/IEC 17043 [2]. The accreditation is approved by Swedac, the accreditation body in Sweden.

2.4. Areas of responsibility

For each scheme, a dedicated **scheme coordinator** has the overall responsibility but also specific responsibilities for planning, correspondence, processing of results and reports. For both schemes, dedicated staff are responsible for the manufacture, quality control and storage of the test material, as well as dispatch of test items.

The overall responsibility for the quality management system of the program is assigned to the head of the Unit for Microbiology.

2.5. Advisory group

The PT program has an advisory group composed of representatives with expertise in drinking water and food microbiology. Their role is mainly advisory, and to give opinions on for example analytical parameters, frequency, costs, accepted methods and the content of the reports.

2.5.1. Subcontracting services

Various aspects of the proficiency testing scheme can from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and the proficiency testing provider is responsible for this work.

2.6. The two schemes

2.6.1. The drinking water scheme

The Drinking water scheme currently consists of two PT rounds per year, each with 2–4 test items.

The scheme includes about 13 quantitative analytical parameters of bacteria, moulds and yeasts with a focus on indicator organisms, including some that may cause illness. Some parameters are part of both PT rounds.

2.6.2. The food scheme

The Food scheme currently consists of three PT rounds per year, each with 2–4 test items.

The scheme contains more than 25 different quantitative and qualitative analytical parameters – bacteria, moulds and yeasts – including the analyses of pathogenic bacteria. Some parameters are part of two or all testing rounds.

2.7. PT round schedule and activities

The time schedule and analytical parameters for current testing rounds are listed on the webpage.

As a PT provider, the Swedish Food Agency aims to keep the time frames stated on the webpage and in this protocol. In case of unforeseen events, PT rounds may however be rescheduled, or even cancelled. Participants will be informed about such actions prior to the originally scheduled dispatch date of a round.

2.7.1. Participant activities

2.7.1.1. Registration/cancellation

Participants are expected to register for individual PT rounds on the web portal. The registration deadline for each PT round is shown on the webpage. A reminder to administer the participation is regularly sent by e-mail by the Swedish Food Agency to (potential) participants.

2.7.1.2. Instructions and analyses

Participants are expected to read and follow the instructions. Analyses should as far as possible be performed in the same manner as the participant's routine analyses but taking into account any potential restrictions or addenda stated in the instructions.

2.7.1.3. Reporting method information

Participants are required to report method information on the web portal, for all analytical parameters for which they report results.

The method information provided by the participants is used to distinguish and present method differences in the reports. It will normally be used in the way it was stored in the database at the reporting deadline. Although significant statistical differences may be difficult to prove, trends and possible disparities will be discussed in the report, to assist the participants in the interpretation of varying results.

2.7.1.4. Reporting analytical results

All results are saved in the database exactly as entered by the participant. There is no processing in between that can cause incorrect registration. Unreasonably large results will however be excluded from the results evaluation (blunder removal).

Results must be reported on the web portal before the stipulated deadline. Results can be entered, checked and changed up until the reporting deadline. After the deadline, changes to the reported data can no longer be made by the participant.

Participants can, e.g. due to technical or other problems, report results via e-mail or ordinary mail, and the Swedish Food Agency will manually enter them into the database. These results should be checked by the participant, and corrections must be sent to the Swedish Food Agency before the reporting deadline.

Results reported by participants after the deadline, are only included in exceptional circumstances (e.g. when there are problems with the web portal). With a few exceptions, incorrectly reported results are not corrected, but are considered part of the proficiency test.

2.7.1.5. Collusion and falsification of results

Collusion or falsification of results defeats is unscientific, unethical, unprofessional, and defeats the entire purpose of the PT. It is strongly discouraged by the Swedish Food Agency. Participants that are suspected of collusion or falsification will be contacted and may be refused to participate in future PT rounds.

Measures used to by the Swedish Food Agency to prevent collusion in the PT include:

- Keeping the identify of participants confidential
- Not disclosing any information about the PT samples prior to the reporting deadline

- Not accepting results – or changes to submitted results – after publication of the preliminary report.
- The use of a consensus value as the assigned value makes it difficult to collaborate to find the "true" value.

Ultimately, it is however the responsibility of the individual participant to act in an ethical and professional manner.

2.7.2. PT provider activities

2.7.2.1. Reporting reminder

The Swedish Food Agency will normally as a courtesy remind participants by e-mail a few days before the reporting deadline. However, the final responsibility to report results lies on the individual participant.

2.7.2.2. Corrections

As a general rule, after the reporting deadline, the only allowed adjustments are those that are due to technical reasons (e.g. computer errors) or due to ambiguities/errors made by the Swedish Food Agency, e.g. due to unclear or incorrect instructions. Corrections are normally accepted only after careful individual considerations.

Reporting mistakes made by the participants are thus generally not accepted and therefore not corrected. Such errors include mistakes made when entering the results, results reported for the wrong sample/analysis/dilution, calculation mistakes and results reported in any other way than described in the instruction, such as use of the wrong numeric scale. The Swedish Food Agency does however reserve the right to – exceptionally – allow corrections even after the reporting deadline, e.g. for special circumstances not covered here.

2.8. Confidentiality and participant identity

2.8.1. Participant code and password

Participant codes and passwords for login to the web portal are treated as confidential by the Swedish Food Agency. They are never given to a third parties, except after permission by the participant.

Passwords can be changed by participants directly on the web portal. Participant codes will be changed upon written request by a participant, or when they have been used by either part in such a way that the identity of the participant has been revealed.

Correspondence with a participant where its PT results, participant code or user password is revealed is treated as confidential by the Swedish Food Agency.

2.8.2. The principle of public access to information

The Swedish Food Agency is a government agency. This means that according to the principle of publicity, all communication to us is in principle considered to be public documents. In accordance with ISO 17043 [2], participant identities and PT results are treated as confidential, and are not shared with third parties, unless permission to do so is given by the participant or when Swedish law requires handing out documents or information about a participant. In the latter case, the principle of publicity is tried against the participant's need for confidentiality on an individual basis.

2.9. Conditions and obligations

2.9.1. General

The general conditions for participation and the obligations of the participants and the Swedish Food Agency are stated on the webpage: https://www.livsmedelsverket.se/en/PT-micro

Contracts with special conditions and obligations can be established between the Swedish Food Agency and an individual participant when necessary.

2.9.2. Who can participate?

- Laboratories that perform analyses within the frames of the schemes and that are using relevant methods.
- Laboratories to which consignments will be available in time by use of ordinary mailing facilities or a carrier service, and that are able to report results and pay invoices in due time.

2.9.3. Which methods may be used?

- All methods adapted for the analytical parameters that are evaluated in the PT schemes. The methods should, preferentially, be used as routine methods.

2.9.4. Participant obligations

- To visit the program webpage and actively register/unregister for participation in the PT rounds.
- To report results according to the written instructions.
- To keep their contact information updated on the web portal.

2.9.5. Obligations of the Swedish Food Agency

- To keep the information on the webpage up-to-date, e.g. information regarding PT rounds, analyses, dates and prices.
- To publish the original and preliminary processed results on the webpage within the stated period of time.
- To publish a final report as a pdf document on the webpage within the stated period of time.

2.9.6. Limited responsibility

The Swedish Food Agency has no liability regarding third party claims depending on a participant's participation and performance in any of the PT schemes run by the Swedish Food Agency.

2.9.7. Complaints and deviations

Complaints and deviations on the work performed within the PT schemes are documented and investigated. If required, corrective actions and measures to avoid re-occurrence will be taken.

Definitions and procedures for managing complaints and deviations are described in internal instructions at the Swedish Food Agency. All documentation, including any corrective and preventive measures taken, are documented in a database at the Swedish Food Agency.

2.10. Filing

In general, all documents derived from PT participation, e.g. correspondence related to or generated by PT participation, are filed for a period of at least 1 year.

All results that are reported in the test rounds are filed in the Swedish Food Agency's participant database, for at least 4 years after publication of the final report.

3. Test material

3.1. Type of material

For PT purposes, natural samples or specifically manufactured test items may be used. Another option is to add cultured test microorganisms to a natural or artificial product ("spiking" of a food or drinking water).

The Swedish Food Agency uses manufactured (freeze-dried) PT test items, which after re-constitution constitute artificial food and drinking water samples, respectively.

3.2. Microorganisms

Cultures of the microorganisms used in the test items are stored at −70 °C in a collection at the Swedish Food Agency (SLV). All strains are identified by specific SLV numbers. The strains have either been isolated from food or water samples or have been bought from established culture collections. Bacterial strains are characterized either internally by the API system or by other means at external culture collections like ATCC (American Type Culture Collection), CCUG (Culture Collection University of Gothenburg) CBS-KNAW (Centralbureau vor Schimmelcultures), SVA (Swedish Veterinary Agency) and FoHM (the Public Health Agency of Sweden).

3.3. Hazards

All microorganisms used in the PT belong to hazard groups 1 and 2, as classified by the Swedish Work Environment Authority [3]. The Swedish Food Agency's stipulation regarding handling and transport of the PT [4] is based on a risk assessment by The Public Health Agency of Sweden on the microorganisms used in the PT [5].

3.4. Manufacture

3.4.1. Strain purity

The purity of all strains is controlled during all stages of production by culture onto non-selective medium. If there is any doubt in the purity of a strain, it is either not included in the sample, or the affected parameter is not evaluated in the PT.

3.4.2. Freeze-drying

The PT test items consist of 0.5 ml aliquots of microorganism suspensions, freeze-dried in 2 ml glass vials. Manufacture is done according to the description by Peterz and Steneryd [6]. Dispension of the microorganism suspension into vials is monitored by weighing. A maximum variation of 0.015 g between the dispensed amounts is allowed, corresponding to 3.0 % of the 0.5 ml target volume. The final PT sample for testing (simulated water and food homogenate, respectively) is obtained after reconstituting the material in a specific volume of suitable diluent.

3.4.3. Storage

The PT test items are transferred to a freezer (−20 °C) and tested for the microorganism content. Accepted batches are checked for vacuum and are sealed with aluminium caps. The test items are thereafter stored at −55 °C. This temperature is chosen to minimize the risk of "glassing", i.e. stiffening, of the rubber stoppers causing air inlet into the vials, that might occur at a temperatures somewhere below −60 °C.

3.5. Quality control

The PT test items are manufactured and undergo quality control in the same way as the reference materials (RM) produced at the Swedish Food Agency: https://www.livsmedelsverket.se/en/RM-micro

Characterization and assessment of homogeneity and stability is therefore done in accordance with the recommendations in ISO 17034 [7] and ISO Guide 35 [8].

Three different types of quality controls are performed:

- Preliminary quality control is performed in connection with manufacture, with the aim to establish approximate property values as a *guidance for further testing*.
- Primary quality control is performed in connection with manufacture and is aimed at *assessing homogeneity* and determining the concentrations of target microorganisms.
- Stability tests are performed when PT test items are used in more than one PT. They are normally performed in the same ways as the primary quality control, but with fewer vials, and is mainly aimed at monitoring the *stability* of the PT test item.

3.5.1. Timing of quality control

Quality control of the PT test items is normally performed within 6 months prior to dispatch of the samples to participants. Occasionally, this may not be possible, and samples may be shipped to participants before the quality control is completed. If the completed quality control is not approved by the reporting deadline, the affected parameters and/or samples will be excluded from evaluation in the PT.

3.5.2. Selection of vials

For the primary quality control, at least 10 vials from the whole filling process are used (stratified sampling). For the continuous quality control, 5 randomly selected vials are used.

3.5.3. Analytical methods

Analyses are performed with appropriate analytical methods for the property values to be tested and are done by subsequent duplicate analysis of the vials by the same person. The two duplicates from a single vial are analysed within a relatively short period of time; first all media from one series and thereafter all media from the second series.

In general, accredited analytical methods are used for characterization and assessment of homogeneity. Non-accredited methods may be used when a new parameter is tested or in certain special cases.

3.5.4. Homogeneity

The aim of the homogeneity test is to establish the relative variation, both *between* and *within* vials, and to determine whether individual vials can reliably be considered to be identical subsamples of the same material.

A large variation *between* vials is often correlated with a large variation between the results of the participants as well. However, this is compensated for by the fact that the standard deviation for calculation of z scores is not fixed, but is a robust measure based on the results obtained by the participants.

For food analyses, results are $\log_{10}$-transformed prior to calculations of concentrations and for assessment of homogeneity. For drinking water analyses, results are square-root-transformed prior to calculations of concentrations and for assessment of homogeneity.

3.5.4.1. Order of analysis

A linear regression analysis is performed in order to determine if the order in which the vials are analysed has an impact on the results. If a statistically significant trend ($\alpha = 0.05$) is identified, it is corrected for and the corrected values are used in subsequent calculations (homogeneity, uncertainties, acceptance limits etc.)

3.5.4.2. First criterion: fulfilment of ANOVA

A one-way analysis of variance (ANOVA) is performed to determine the between-vial and within-vial variations (s_{bb} and s_{wb}, respectively). If the p value of the ANOVA fulfils the criterion $p \geq 0.05$, the results from different vials are considered comparable, i.e. no significant variation between the vials.

3.5.4.3. Second criterion: comparison with s_R

The between-vial standard deviaion (s_{bb}) from the ANOVA is compared with the expected between-laboratory variation (s_R). Estimates of s_R are based on historical distribution and analysis of PT samples, and are therefore considered to include the uncertainty contributions from both transport and the use of different analytical methods.

For the **Food scheme**, it is assumed that $s_R = 0.25$, though exceptionally $s_R = 0.35$ may be used for property values where historical data imply that a higher variation may be expected. The assumption that $s_R = 0.25$ is based on historical results for participants in the Swedish Food Agency's PT, recommendations in ISO 22217 [9], and similar observations and principles used by other PT organisers.

For the **Drinking water scheme**, where *square-root transformed* results are used, it is instead assumed that $s_R = 1.25$, though exceptionally $s_R = 1.50$ may be used for property values where historical data imply that a higher variation may be expected. Similar values for s_R are used by other PT organisers.

3.5.4.4. Evaluation of homogeneity

If the ANOVA yields $p \geq 0.05$, the PT test item batch is considered homogenous.

If the If the ANOVA yields $p < 0.05$, the PT test item batch is still considered homogenous, if $s_{bb} < s_R/3$. The variation is in this case considered fit-for-purpose.

If the results from the initial homogeneity study do not fulfil the homogeneity criteria, the analyses for the affected parameters are repeated. If the homogeneity criteria are still not met after a second homogeneity test – and no cause other than inhomogeneity is considered a likely explanation – the production batch not used as an PT test item.

3.5.4.5. Underestimation of within-vial variation

In some instances, the ANOVA may not give an accurate representation of the (in)homogeneity. In particular, there may sometimes be an underestimation of the variation within vials. This may for example occur when – by chance – the duplicate results *within* one or a few vials are identical or very similar. If – in such cases – artificially increasing the within-vial variation leads to an accepted ANOVA, the homogeneity criterion may still be considered to be fulfilled. This exception is used sparingly.

3.5.4.6. Exclusion of vials with deviating results and high variation

In a homogeneity test, one or both values from a duplicate analysis of a single vial may sometimes *deviate* substantially from the remaining values. In such a case, the Swedish Food Agency reserves the right to re-evaluate the homogeneity with these values excluded. This will be done if it can reasonably be assumed that the divergent results are not due to non-homogeneity of the test mixture, and instead due to e.g. a pipetting error or the analysis of the wrong dilution or volume. If the results from re-calculations with such values excluded fulfil the criteria for homogeneity, the PT test item batch will be approved. This exception is used sparingly.

Cochran's *C*-test- (*C*) is used as a *guidance* to identify if, among the set of tested vials (p), the variation within a single vial (s) is significantly larger than the variation in the set of vials. When this is the case, the homogeneity is re-evaluated with both values from this vial excluded.

$$C = \frac{s_{max}^2}{\sum_{i=1}^{p} s_i^2}$$

3.5.5. Target microorganism concentrations

Target microorganism concentrations are determined by a single measurement procedure. The error contributions from heterogeneity and stability are – based on experience – assumed to be negligible. The concentration values thus consist of the characterised values, i.e. the mean value of the 10 individual mean values (each from a duplicate analysis) for each analytical parameter. Likewise, the standard deviation of the property value is the standard deviation of the 10 individual mean values.

The standard uncertainties of the concentration values include uncertainty contributions from characterisation, homogeneity, and method differences. The first two are determined experimentally for each PT test item and analytical parameter, whereas the latter is assumed to be identical to the expected between-laboratory variation (s_R). Estimates of s_R are based on historical distribution and analysis of PT samples and are therefore considered to include the uncertainty contributions from both transport and the use of different analytical methods.

The uncertainty contribution from long term stability is by experience considered to be negligible.

3.5.6. Quantitative analyses and special vials

Quality control of quantitative analyses, and analyses with a low concentration of the target organism compared to the competing background, present special challenges. For example, stress and competition may in these cases lead to a lower colony recovery than the actual concentration in the vials, and an unreasonably large dispersion of the results.

In these cases, quality control is carried out on separate vials containing a pure culture ("special vials"). These vials are freeze-dried *in parallel* with the vials containing the main PT test item (the main microorganism suspension). The volume ratios used in the manufacture of the “special vials” are identical to those in the test item; the same final concentrations of microorganisms are therefore assumed in both.

3.5.7. Stability

Some test items are used in more than one PT round. When vials from such test items are used again in a subsequent PT round, and more than 6 months has passed since the last determination of homogeneity, the concentration and homogeneity is re-assessed.

Such a stability control is performed in the same way as the initial determination of concentrations and homogeneity, except 5 vials are analysed instead of 10. If the results from these 5 vials do not fulfil the homogeneity criteria, the analyses for the affected parameters are repeated on 5 new vials. If the homogeneity criteria are still not met after those additional 5 vials have been analysed, the test item is either not used, or the affected individual parameter(s) is excluded from evaluation in the PT.

3.5.8. Vacuum test

An inert environment is necessary in order to maintain the viability and concentrations of the micro-organisms in the PT samples. The freeze-dried material therefore needs to stay under vacuum after the vials are sealed and capped. Each individual vial is tested for vacuum before storage, performance tests or delivery. Vials without vacuum are discarded. Normally, very few of the newly produced vials need to be discarded.

If more than 4 months have passed since the original vacuum test and the sample dispatch date, a new vacuum test is made on at least 10 % of the remaining vials (however, never less than 50 vials).

3.6. Distribution to participants

3.6.1. Labelling of samples

Before dispatch to the PT participants, the samples are labelled. This activity is carried out on a separate work bench for each PT test item in order to avoid labelling errors.

3.6.2. Packaging of samples

The samples are packaged according to international regulations in a secondary packaging (a transportation tube or a safety jar containing a shock- and liquid-absorbing material) and an outer packaging for shipping (either a protective envelope or a cardboard box, respectively). A safety data sheet, as well as a delivery note, are also added to the package.

3.6.3. Packaging of samples

Sending samples to the wrong participant address is possible but is avoided by careful comparison of label addresses with the list of participants. If a mistake occurs, it is usually detected upon arrival at the destination. Since participant codes are not sent in the packages, confidentiality will be maintained even in these instances.

Delivery addresses can be updated by participants directly on the web portal.

3.6.4. Transport

Dispatch of the PT samples is usually done 1–3 weeks ahead of the starting date for analyses in a PT round.

Based on the risk assessment (above), the test items are sent via ordinary postal means. In addition, a tracking number or courier service is used for certain destinations.

The PT test items are kept at ambient temperature during packaging and transport. Participants are however recommended to keep the received material in darkness and in a freezer (−20 °C) until use.

When the test material is to be used shortly after delivery, as in PT rounds, the needs of a long shelf life is relatively small. Storage at room temperature (≤ 25 °C) for up to 3 weeks is therefore in general not critical. The test material should however always be kept in the dark.

3.6.4.1. Damage during transport

In theory, the testing material could be damaged during transport if it is subjected to very high temperatures or strong x-rays. So far however, this does not appear to have been a problem. Test items have e.g. been transported for long distances to warmer countries, without problem. According to PostNord (the main Swedish postal service) only very low doses of x-rays (<1/100 of the dose for dental x-rays) are used for domestic and international goods at Arlanda (the international airport of Stockholm). Since no general negative effect from transportation has been noticed, it seems likely that neither temperature, nor the doses of x-rays utilized at airports are a problem for the freeze-dried test items.

3.6.5. Instructions

Instructions for sample preparation, analysis and reporting are sent to participants by e-mail latest the day after dispatch. The instructions also often contain recommendations for which dilutions and to volumes to analyse.

3.6.6. Safety data sheet

A safety data sheet with information regarding storage, disposal, health hazards etc. is included with the samples. It is also available on the PT program webpage.

4. Data analysis and performance assessment

4.1. False results

A **false positive result** is an analytical result where a microorganism/analyte is reported as detected even though it was not present in the sample.

A **false negative result** is an analytical result where a microorganism/analyte is reported as not detected even though it is present in the sample.

The number of reported false results varies depending on which parameter is analysed, the composition of the sample and the degree of difficulty, e.g. concentration and/or background flora.

When the average concentration of a target organism is very low (e.g. <10 cfu/ml for the drinking water scheme), zero results are expected to be obtained randomly. In these cases, they are usually not identified as outliers or false negative results.

4.2. Results transformation

Unless otherwise stated, evaluation of the participants' results and statistical calculations are carried out on $\log_{10}$ transformed or square root transformed results for the food and drinking water analyses, respectively.

For the drinking water scheme, as a practical assistance to the participants, *re-transformed* statistical measures (i.e. in the normal cfu scale) are often shown in the report.

4.3. Assigned value and standard deviation

Algorithm A with iterated scale as described in ISO 13528 [10] is used to determine the robust mean (m_{PT}) and robust standard deviation (s_{PT}) of the participants' results.

Results that are obviously erroneous are excluded prior to determining m_{PT} and s_{PT} (blunder removal).

For evaluated parameters, the assigned value consists of m_{PT}. It is regarded as the true, normative value.

An alternative to the robust m_{PT} as the assigned value, is to use a value determined by expert laboratories. This is not done for two reasons:

a) Microbiological quantitative results are strongly dependent on the method, and therefore a "true value" strictly does not exist.

b) Different brands or batches of dehydrated culturing media may give different colony appearances and recovery. A systematic recovery bias of a particular culturing medium should not be allowed to have an impact on the outcome for participants using another medium.

An alternative to the robust s_{PT} as the assigned value, is to use a *fixed* standard deviation, based on previous suitable PT rounds. This is not done for two reasons:

a) A prerequisite is that the test materials of different PT rounds have the same degree of difficulty. This is usually not the case, and the Swedish Food Agency therefore uses a standard deviation that varies with the difficulty of the test material.

b) The use of a varying s_{PT} means that the z scores are more accurate for the performance of a participant over time, since a compensation for the degree of difficulty of the PT item is made.

4.4. Accepted results and outliers

Outliers are results that deviate from the other results in a way that cannot be explained by normal variation. Participant results within $m_{PT} \pm 3s_{PT}$ are considered acceptable, whereas results outside this interval are considered as outliers.

The interval $m_{PT} \pm 3s_{PT}$ is chosen for a couple of reasons:

a) It empirically gives a similar number of outliers compared to the test used previously (Grubbs test with a 1 % level to detect outliers).

b) As described in ISO 13528 [10], when a z score of ≥ 3 constitutes an action signal, then an error (δ_E) of $\delta_E = 3s_{PT}$ can be considered suitable.

Algorithm A is most reliable when the proportion of outliers is less than 20 %. When this is not the case, it is indicated in the report.

4.5. Measurement uncertainty for the assigned value

The standard uncertainty (u_{PT}) of the assigned value (m_{PT}) is estimated from the standard deviation (s_{PT}) and the number of evaluated results (n) as suggested in ISO 13528 [10]:

$$u_{PT} = 1{,}25 \times \frac{s_{PT}}{\sqrt{n}}$$

The measurement uncertainty is considered negligible compared to the standard deviation (which is used for evaluating the participants' results) when:

$$u_{PT} < 0{,}3 s_{PT}$$

When this criterion is not met, it means that participants might inaccurately receive action and warning signals. In these instances, participants will be informed in the report that the uncertainty of the assigned value is not negligible, and that the assessment of the results should be taken with consideration.

For the drinking water scheme, the relative standard uncertainty of m_{PT} is sometimes also provided:

$$u_{rel,mPT}(\%) = 100 \times \frac{s_{PT}}{\sqrt{n} \cdot m_{PT}}$$

4.6. Coefficient of variation

The coefficient of variation (CV) is a *relative measure* and is calculated as:

$$CV = 100 \times \frac{s_{PT}}{m_{PT}}$$

The *CV* is stated as a measure for dispersion in the drinking water scheme. It is used as an aid in the evaluation of the participants' results. A dispersion of <10 % is regarded as very small, 10−20 % as small, 20−30 % as medium, 30−40 % as large and >40 % as very large.

4.7. Non-robust statistical measures

Non-robust mean values (*m*), standard deviations (*s*) and median values (*Med*) are calculated to assist in the evaluation of the results and may be shown in the report or in connection with the results on the webpage. In these instances, *m*, *s* and *Med* are calculated from the participants' results, with the previously determined outliers and false results excluded.

4.8. Exceptions for small datasets

For small datasets, there is an increased uncertainty associated with determining the robust mean (m_{PT}) and robust standard deviation (s_{PT}) of the participants' results. Therefore, when fewer than 12 participants have reported evaluated results, the statistical measures for performance evaluation will be provided *only as an information* to the participants.

Non-robust median values (*Med*) and standard deviations (*s*) are calculated to assist in the evaluation of the results from different methods. These are shown in tables in the report, in connection with the respective analyses. In these instances, *Med* and *s* are calculated from the respective method groups' results, with outliers and false results excluded. For method groups with fewer than 5 results, only the number of false results and outliers are provided.

4.9. Z scores

All results – including outliers but excluding false results – from the assessed parameters are transformed into standard values (*z* scores) according to the formula:

$$z = \frac{x_{lab} - m_{PT}}{s_{PT}}$$

where x_{lab} is the result of the individual participant.

The *z* scores facilitate comparison of the various analyses with each other, since they are independent of the concentration and expressed on the same scale (the number of standard deviations).

For quantitative analyses, a *z* score is either positive or negative, depending on whether the participants result is higher or lower than m_{PT}. Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism are given a *z* score of 0 (zero). False results do not generate any *z* scores.

Z scores are mainly intended to facilitate comparison of results for different analytical parameters within rounds, and the results of a specific parameter between rounds.

In evaluations of the analytical results, the following guidelines can be used:

$\|z\| \leq 2$	indicates that the result is acceptable
$2 < \|z\| < 3$	indicates a warning that the result may be deviating, and might motivate an action in the follow-up process

$\|z\| \geq 3$	indicates that the result is regarded as deviating and should lead to an action in the follow-up process

5. PT reports

5.1. Preliminary report

A preliminary report is published within two weeks after the reporting deadline. It is less detailed than the final report and is mainly published to provide participants with a quick initial evaluation of their performance.

The preliminary report normally contains:

- A description of the sample content
- Preliminary statistics and acceptance limits
- Brief comments regarding the outcome.

5.2. Final report

The final report is published within two months after the reporting deadline. The report distributed by e-mail to participants and is also made available on the webpage.

5.2.1. Histograms and tables

The results for parameters with quantitative non-zero results are presented in two histograms. The first histogram provides a visual overview of the result distribution, with outliers and false negative results highlighted. The second histogram visualises the same result distribution, but instead highlights the results from different methods or media. In connection with the histograms, the results result from the different methods are displayed in tables.

5.2.2. Box plots

Box plots are based on the z scores of the individual participants. They illustrate how the participant's z scores are situated as a group in relation to the common, "true", mean value zero. They are included in the report in order to assist in the evaluation of the participants' overall performance.

5.2.3. Quality control results

Results from the final determination of concentration and the homogeneity test – or the most recent stability/homogeneity control – are presented in a table.

5.2.4. Appendices

Two appendices are provided at the end of the report.

Appendix 1 contains a table with the reported results of all participants, with outliers and false results highlighted. The table also contains various statistical measures at the end.

Appendix 2 contains a table with the z scores for each participant. It is intended to assist in the follow-up for the individual participants.

5.2.5. Photos

The report may sometimes contain an annex with photos that show the expected outcome and colony morphology on various media.

5.2.6. Inaccuracies in the report

If a substantial error is found in the final report, the participants will be informed by e-mail. The report is then adjusted and a new version is published on the webpage.

Less substantial errors or inaccuracies, or minor errors that affect only one or a few individual laboratories may – depending on the circumstances – be corrected directly in an e-mail without publishing a new version of the report.

Insignificant errors, e.g. spelling errors that do not affect the report in a meaningful way, do not require neither publication of a new report nor an e-mail with information to the participants.

5.2.7. Use of report by participants

Copyright to all reports remains with the Swedish Food Agency. Participants are however allowed to make copies of the report for their own internal use. The report may be cited; the preferred citation is given in each report.

5.3. Performance assessment

5.3.1. Final assessment

The report provides the basic criteria for participant assessment, e.g. acceptance limits, z scores and other statistical measures. However, the final responsibility to follow up and interpret the laboratory performance lies on the individual participant and – in relevant cases – the accreditation bodies. The Swedish Food Agency's report provides the *tools* for this, but does not take any responsibility for the final interpretation of the individual participants performance.

5.3.2. Follow-up samples

The Swedish Food Agency does not require – or take any responsibility for – that a follow-up of the results is done by the participants. There is also no requirement for participants to report results from such follow-up analyses. Such demands can only be made by the participant itself or by a third party to which the participant is subordinated, e.g. an accreditation body.

The Swedish Food Agency does, however, strive to aid the participants as much as possible in their efforts to understand and correct potential errors. To facilitate this, follow-up samples can be ordered – for a limited time period – and provided that the stocks last. All participants may request one extra vial of each test item free of charge. Additional vials may be requested, but are subject to a charge.

6. References

1. EN ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories.
2. EN ISO/IEC 17043:2023. Conformity assessment – General requirements for proficiency testing.
3. Ordinances of Swedish Work Environment Authority (*Arbetsmiljöverkets författningssamling*), 2018. Risks of infection *(Smittrisker)*. AFS 2018:4. *In Swedish*.
4. Frändberg, E. & Anér, G. Swedish Food Agency (*Livsmedelsverket*), 2002. Judgement on microbiological material for controls. Swedish Food Agency Reg. nr. 2509/02. *In Swedish*.
5. Kallings, I. & Ljungdahl Ståhle, E. Swedish Institute for Infectious Disease Control (*Smittskyddsinstitutet*), 2002. Expert's report regarding proficiency testing and reference samples from the Swedish Food Agency. The Public Health Agency of Sweden (*Fokhälsomyndigheten*) Reg. nr. 527/2002-18. Swedish Food Agency Reg. nr. 2509/02. *English translation.*
6. Peterz, M. & Steneryd, A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. J. Appl. Bacteriol. 74:143-148.
7. ISO 17034:2016. General requirements for the competence of reference material producers.
8. ISO Guide 35:2017 Reference materials – Guidance for characterization and assessment of homogeneity and stability.
9. ISO 22117:2019. Specific requirements and guidance for proficiency testing by interlaboratory testing.
10. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.


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