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 and https://www2.slv.se/absint

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Recommended citation

Protocol – Microbiological Proficiency Testing, Swedish Food Agency, Uppsala, Sweden.

Edition

Version 7.2 (2023-05-08)

Editor in chief

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Webpage

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SWEDAC ACKREDITERING
Accred. no. 1457
Proficiency testing
ISO/IEC 17043

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Contents

1. Introduction

Laboratories that perform analyses need to know that they obtain realistic results. They also need to prove this to their clients to be trustworthy. They can obtain this knowledge by performing different kinds of controls. Internal controls may be used to see that no unexpected changes have occurred within the laboratory. Since microbiological analytical results are dependent on the analytical method used, it is also important for the laboratory to be able to compare their analytical results with those of other laboratories. One means of this is to participate in interlaboratory comparative tests. These tests, when they are available, are compulsory for laboratories that aspire to become – or already are – accredited for their analyses. This is e.g. a requirement according to the standard EN ISO/IEC 17025 [1], where the name Proficiency Testing (PT) is used for these interlaboratory comparisons.

PTs are normally organised by a third party, independent of the laboratories and their clients. The evaluations are made by this party and the process is defined as an external assessment of the analytical competence of the laboratory.

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 PTs are mainly directed to accredited laboratories within these analytical areas. They are also suitable for non-accredited laboratories, e.g. laboratories that perform controls in production lines of food or drinking water, but want to be able 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. In particular this protocol includes general parts that are not described elsewhere, including the general aspects of production and handling of test material and the statistical processing of results. General information as well as specific information about the testing rounds is available on the program webpage: www2.slv.se/absint.

2. Organisation

2.1. General information

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

E-mail for questions and opinions concerning the schemes: PT-micro@slv.se

2.2. Information on the program webpage

2.2.1. Structure of the webpage

A general description of the schemes can be found on the webpage: www2.slv.se/absint.

The webpage consists of two parts: one public and one for participants only, the latter where a user identity is required to log in.

2.2.2. The public section

This section contains pages with:

- General information about the proficiency testing, including the most recent edition of this document as a pdf file.
- Basic information regarding the drinking water and food schemes, e.g. which analytical parameters they comprise.
- The content of current and coming testing rounds along with the time schedule.
- The conditions for participation.
- The current prices.
- Addresses to contact the Swedish Food Agency.
- A web form to apply for registering as a new participant in a scheme.
- The login to participants' pages.

2.2.3. The participant section accessible by login

This section contains:

- A page to report analytical results in ongoing PT rounds.
- A page to report method data for each analytical parameter.
- A page with reported results and statistics from previous rounds that are presented as:
    1. **preliminary results** shortly after the reporting deadline
    2. if necessary: as **preliminary results** during processing of the results, when only the reported results and the preliminary median values are presented
    3. **final results** when a PT round has been fully evaluated

    (Note: as the webpage is not as manually flexible as the written report, in cases of discrepancies, the written report overrules the webpage.)
- A page for managing participation in coming PT rounds.
- A page with previous PT reports and general information to the participants available as pdf-files.

2.3. 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 laboratory staff are responsible for the manufacture, quality checks and storage of the test

material. There is also administrative staff responsible for the participant database, contacts regarding invoices and participation, as well as dispatch of test items.

The overall responsibility for the quality management system of the program is assigned to the team manager together with the head of the Department of Biology.

The head of the Department of Biology is also the editor-in-chief of the final reports and this scheme protocol.

2.4. Advisory group

2.4.1. Composition and meetings

The PT program has an advisory group composed of representatives for both drinking water and food microbiology. The members are from the countries with the largest numbers of participants, so far the Nordic countries. The advisory group meets semi-regularly, discussing both issues common to the two schemes and issues specific to the individual schemes.

2.4.2. Duties

The advisory group members can come from different organisations and countries, and represent both these and themselves. Their role is mainly advisory, with opinions on e.g. analytical parameters, frequency, costs, accepted methods and the content of the reports. Major changes within a scheme should be discussed with the advisory group. This can be accomplished by e-mail conversation.

2.5. Accreditation

The Swedish Food Agency is since December 2004 accredited for arranging microbiological proficiency testing. The accreditation has been approved by Swedac, and is currently performed according to the standard EN ISO/IEC 17043:2010 [2].

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.

3. The two schemes

3.1. The Drinking water scheme

The Drinking water scheme has been ongoing with participants from several countries since 1992. Currently, two PT rounds with 2–4 test items each are organised each year.

The scheme includes about 15 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. The parameters are presented on the webpage.

3.2. The Food scheme

The Food scheme has been ongoing with participants from several countries since 1988. Currently, three PT rounds with 2–4 test items each are organised each year.

The scheme contains more than 25 different quantitative and qualitative analytical parameters – bacteria, moulds and yeasts – including the analyses of pathogenic bacteria normally searched for in food. Some parameters are part of two or all testing rounds. The parameters are presented on the webpage.

3.3. Time schedule and analytical parameters for a testing round

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.

4. The test material

4.1. 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 has chosen to use manufactured test items. These simulate food or drinking water samples containing mixtures of microorganisms, with each test material having a certain purpose.

The content of the test items varies depending on the parameters of the PT round. An individual test item may include both bacteria and fungi (moulds and/or yeasts). Some test items include pathogenic bacteria, while others contain microorganisms possessing specific indicative properties only. Currently, no test material containing protozoa or virus is manufactured.

The test items consist of 0.5 ml freeze-dried serum broth with different microbial mixtures in 2 ml glass vials. The material is manufactured according to the description by Peterz and Steneryd [3]. The sample for testing (simulated water respective food homogenate) is obtained after reconstituting the material in a specific volume of suitable diluent.

Freeze-dried test material without matrix in vials has a number of advantages compared to natural samples or “spiked” samples, but also some disadvantages.

4.1.1. Advantages

- The vials do not require much space, which facilitates storage, package and transport.
- The vials are fairly robust and are therefore easy to transport.
- Several organism groups (analytical parameters) may be tested in parallel by the same test item.

- The test material has a good stability and may be used in more than one PT round without a substantial change in the microbial concentrations.
- It is possible to manufacture a large number of identical sub-samples where the organisms are Poisson distributed at lower concentrations or log-normal distributed after dilutions at higher concentrations.
- Expenses are kept low by having a standardised and relatively simple procedure of manufacture.
- Dissolved test material may be used for "spiking" of natural matrices.

4.1.2. Disadvantages

- The freeze-drying procedure and machinery need to be sufficiently stable to ensure that the process can be repeated.
- The microorganisms require a protective substance, cryoprotectant, which helps them survive the freeze-drying.
- The material has to be dissolved in liquid, which requires a certain amount of work and can induce mistakes.
- The ingredients of the material may cause some foaming when it is dissolved. Therefore, the sample is likely to be distinguished from real samples, which means that the analyst may know it is a control sample.
- For food analyses, natural matrices are absent during preparation of the test material, since the dissolved test material mimics a ready-to-use homogenate.

4.2. Production and product control

4.2.1. Composition

Freeze-dried 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 internally by API system or by other means at external culture collections like ATCC (American Type Culture Collection) and CCUG (Culture Collection University of Gothenburg). The characterization of yeasts and moulds, *Salmonella* strain and *E. coli* O157 strains is performed externally by, the Centralbureau vor Schimmelcultures (CBS-KNAW Collection, The Netherlands), the National Veterinary Institute (SVA, Sweden) and the Public Health Agency of Sweden, respectively.

ISB (Inositol Serum Broth) is used as cryoprotectant to protect the organisms during the freeze-drying process. ISB is composed of sterile-filtered horse serum mixed with inositol and a small amount of nutrient broth. After washing or diluting the microorganism cultures from nutrient rich medium, the component SPG (Saccharose Phosphate Glutamate), which also possesses organism-protective characteristics in the freeze-drying process, is added. Peptone water or a potassium phosphate buffer containing magnesium salt (see e.g. ISO 8199:2018 [4]) is used when diluting and washing. The same solutions can be used when dissolving ready-to-use material before analysis.

4.2.2. Manufacture and storage

Each microorganism is cultured individually in suitable nutrient medium, usually TSB (Tryptone Soy Broth) or BHI (Brain Heart Infusion) Broth/Agar for bacteria, and MEA (Malt Extract Agar) for fungi. The cultures are used after a predetermined time period, after which the nutritious medium is washed or diluted off. The test mixture of microorganisms is obtained by transferring predetermined volumes of each diluted/washed culture (or spore suspension for moulds and other spore formers) to a specific volume of ice-cold ISB.

The test mixture is kept on ice and continuously stirred while aliquots (0.5 ml) are transferred into sterile glass vials. The vials are then freeze-dried and thereafter closed by rubber stoppers while under vacuum.

The produced test items are transferred to a freezer (−18 to −24 °C) and tested for the microorganism content shortly after the manufacture. Accepted batches are checked for vacuum and are sealed with aluminium caps. The test items are thereafter stored at −55 °C until used. 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. Labelling of the vials is made in connection with the dispatch.

4.2.3. Quality checks

Quality control of samples is normally performed 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. In these cases, if the completed quality control is not approved, the affected parameters and/or samples will be excluded from evaluation in the PT.

4.2.3.1. Purity of the strains

The purity of all strains included in the test items is controlled by direct culture from the strain collections onto non-selective medium. In addition, the purity of each obtained culture is checked by streaking an aliquot onto non-selective medium. If there is any doubt in the purity of a strain, it is excluded from use and the production of the test mixture is usually postponed.

4.2.3.2. Amount of test material

While dispensing the test mixtures into vials the dispensed volume is monitored by weighing. The total range and measure of dispersion (coefficient of variation) are calculated from the weights. The range may be at most 0.015 g between the dispensed amounts, which corresponds to 3.0 % of the average dispensed amount of material (the target volume 0.5 ml). The weight monitoring is done in parallel to the dispensing, in order to be able to adjust or interrupt the dispensing process, if the weights vary too much.

4.2.3.3. Concentration determination

Concentrations of the included microorganisms are determined after freeze-drying by analysing one or several vials of the test material. For new strains, the concentrations in a test item are often determined before freeze-drying as well. When analyses are performed both before and after freeze-drying, the decrease of the individual strains due to the freeze-drying process can be determined (the reduction factor).

4.2.3.4. Homogeneity

Before the test material is used, the homogeneity must be checked and accepted. In this test of homogeneity, duplicate analyses of 10 vials from the whole filling process (stratified sampling) are made with

appropriate methods for the parameters to be tested. For the test material to be homogenous, certain criteria regarding variation within and between vials must be fulfilled (see below).

4.2.3.5. Vacuum test

An inert environment is necessary in order to maintain the viability and concentrations of the micro-organisms in the test material. To ensure long-time stability of the material, 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.

4.2.3.6. Stability

The stability of many of the organisms included in the test materials has been investigated for several years in different freeze-dried samples. Each PT test material is however not tested in this sense. A renewed concentration and homogeneity check is done only after a longer period of storage. Knowledge of long-term stability of the test material is based on similar material (reference material) that has been manufactured in the same manner, stored for at least 2 years and tested regularly. When stored frozen at −55 °C, most tested bacteria and fungi have shown stability for at least this period of time. Gram-negative bacteria however, tend to decrease somewhat in colony recovery over time, while Gram-positive bacteria and fungal spores are generally unaffected.

With storage at approximately 25 °C, no noticeable negative effects on the stability of the test material has been observed for at least 3 weeks, except for the bacterial genus *Campylobacter*. At 44 °C during the same time, the recovery is somewhat affected, especially for Gram-negative bacteria. This tendency is even more noticeable after storage at 60 °C.

Considering these observations, the following guidelines apply:

- If stored at normal freezer temperature (−18 to −24 °C) the content is stable for at least one year, with the observation for Gram-negative bacteria as described above.
- If stored in a refrigerator (5 ± 3 °C), the content is stable for at least a few months, and usually considerably longer.
- If stored at room temperature (not above 25 °C), the content is stable for at least 4 weeks, and usually longer.

4.3. Hazards

4.3.1. Risk of infection

All microorganisms used in the schemes belong to hazard groups 1 and 2, as classified by the Swedish Work Environment Authority [5]. The Public Health Agency of Sweden (former Swedish Institute for Infectious Disease Control) has made a risk assessment concerning the risks of infection by the microorganisms used in the schemes [6]. In their assessment they concluded that – based on the contents and the way the samples are handled – the risk for illness even after consumption of the contents of one vial is considered extremely small. That assessment is the basis for the stipulation regarding handling and transport made by the Swedish Food Agency [7].

4.3.2. Environmental danger

The test material itself consists of horse serum, inositol and microorganisms, and usually also contains nutrient broth, SPG, peptone, potassium phosphate, and magnesium sulphate. The container consists of glass, rubber, aluminium and has a paper label. Since the container with material does not include any specifically classed or in any other way potentially dangerous chemical compound, it may be discarded in the common waste management after the microorganisms have been rendered harmless by killing.

4.4. Labelling and transport of test items

4.4.1. Randomisation of test vials to laboratories

4.4.1.1. Numbering of vial labels

Before dispatch to the PT participants, the vials are labelled with individual numbers. The numbers are a combination of the 4-digit, confidential laboratory number and a random sample code after a hyphen (e.g. sample 1000-1, 1000-2 etc.). For each laboratory the sample codes are randomly chosen for the different samples. For example, for one laboratory code no. 1 can be from sample A, but, for another laboratory code no. 1 can be from sample C etc.

4.4.1.2. Selection of vials for a laboratory

Before packaging, the vials of each test sample are carefully shuffled. For each laboratory, a vial is randomly picked and labelled with individual numbers, as described above. This activity is carried out on a separate work bench for each test sample in order to avoid errors.

4.4.2. Package of test vials

The samples (glass vials) 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.

4.4.3. Transport of test vials

Based on the risk assessment made by the Public Health Agency of Sweden [6] and the stipulation made by the Swedish Food Agency [7], the test vials are packed as described above, and sent via ordinary postal means. In addition, a tracking number or courier service is used for certain destinations.

4.5. Recommended handling of the test material upon delivery

4.5.1. Storage in connection to dispatch of test material

The material is kept cooled before package and transport, and at ambient temperature during packaging and transport.

Participants are 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 in refrigerator is therefore often satisfactory when the material is received. Storage at room temperature (≤ 25 °C) for up to 4 weeks is also in general not critical. The test material should however always be kept in the dark.

4.5.2. Preparation of samples

Instructions for sample preparation and analysis are distributed to the participants latest the day after dispatch. The standard procedure is to reconstitute the vial content in a given volume (e.g. 250 or 800 ml) of diluent. The suspension should then be carefully mixed in order to obtain the sample ready for analysis.

4.5.3. Stability of the prepared samples

After reconstitution of the freeze-dried material, the microorganism concentrations cannot be presumed to be stable for more than about an hour, even after cooling. The prepared sample should therefore be used for analysis within one hour.

At least some bacterial spores can be maintained for a considerably longer period of time, e.g. several weeks for *Clostridium perfringens*, to be used as qualitative controls.

4.5.4. Destruction of test material

4.5.4.1. Unopened vials containing test material

The microorganisms need to be killed prior to discarding the material. This can be done e.g. by auto-claving at 121 °C, for a sufficient time to ascertain that the entire content has reached that temperature (e.g. 50 minutes). An alternative is to hand in the vials to a facility specialized in the destruction of infectious material.

4.5.4.2. Opened and used vials

Rubber stoppers and glass vials that contain/has contained test material can be discarded in containers for infectious material, which are to be destroyed by a special facility. The aluminium cap can be discarded as metal waste.

4.5.4.3. Remains of prepared sample

Remains of the prepared sample should be autoclaved at 121 °C for at least 15 minutes, or treated in any other way ensuring the destruction of the remaining microorganisms, before they are discarded.

5. Instructions for a particular testing round

5.1. Time schedule

Dispatch of test material is usually 1–3 weeks ahead of the starting date for analyses in a PT round. Instructions for sample preparation, analysis and reporting are sent by e-mail latest the day after dispatch. The instructions also include the starting date and the final day to report the results.

5.2. Analyses

The analytical parameters included in each PT round are stated in the instructions, and can also be found on the webpage.

5.3. Other information

The instructions sent by e-mail may also contain information regarding particular conditions concerning the different analyses, such as the dilutions or volumes that should be tested or the methods that can be used.

6. A testing round

6.1. Participant activities

6.1.1. Registration/cancellation

Participants are expected to administer (register or cancel) their participation on the webpage. Participants can register for individual PT rounds, or as a **subscriber** for all or a specific series of PT rounds. Subscription means that the participant is automatically registered for all of the indicated upcoming PT rounds.

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.

6.1.2. 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.

6.1.3. Reporting method information

Participants are required to report method information on the webpage, for all analytical parameters. This is also a requirement to be able to report analytical results on the webpage. The reported method information should represent what was used to obtain the reported analytical results. It applies until further notice. It can be entered and adjusted at any time on the webpage, including after the reporting deadline for a PT round. The Swedish Food Agency does not make any changes to the stored method data, unless it is specifically requested by a participant.

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.

6.1.4. Reporting analytical results

Results must be reported as integers for the drinking water PT scheme, and as $\log_{10}$ transformed results for the food PT scheme. This is described in detail in the instructions for each PT round.

All results are saved in the database exactly as entered by the participant. There is no processing in between that can cause incorrect registration. The only exception to this is when quantitative results larger than 10 are reported for food analyses. Such results are assumed to be mistakenly reported as non-$\log_{10}$ transformed, and a $\log_{10}$ transformation is therefore automatically made.

Results must be reported on the webpage before the stipulated deadline. Results can be entered, checked and changed through the webpage 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. A confirmation e-mail with the entered results will in these cases be sent to the participant. 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 reporting webpage). With a few exceptions, incorrectly reported results are not corrected, but are considered part of the proficiency test.

6.2. PT provider activities

6.2.1. 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.

6.2.2. 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.

6.3. Possible sources of errors in a testing round

6.3.1. The test material

During filling of the vials, the amount of dispensed test material is systematically checked by sampling and weighing. After freeze-drying, all vials are checked for vacuum, which is necessary for the survival of the organisms. If more than 4 months have passed since this 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).

6.3.2. Dispatch and transport

Incorrect labelling of samples for participants is a possible risk. As described previously the vials are labelled and packaged in a way that minimises the risk of this happening.

Sending samples to the wrong participant address is possible, but is avoided by careful and continuous comparison of label addresses, participant numbers and sample numbers against each other. Addresses in the participants' database are also updated as soon as changes are received from the participants.

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.

6.3.3. Inaccuracies in the final report

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

7. Follow-up of analyses

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. Such third parties can demand that the participant maintains a certain quality level and takes measures when the quality is questioned. How the follow-up should be done is therefore the responsibility of the participant itself, or in cooperation with the third party.

The Swedish Food Agency does, however, strive to aid the participants as much as possible in their efforts to understand and correct potential errors.

The Swedish Food Agency also facilitates the follow-up by offering extra vials of the test material to participants that ask for it – for a limited time period – and provided that the stocks last. Each participant can request one extra vial per test mixture free of charge. Additional vials can be requested, but are subject to a charge.

8. Statistics and reporting

8.1. General

The statistical processing in the PT includes the following main steps:

- Numerical checking of the amount, concentrations, homogeneity and stability of the test material.
- Transformation of analytical results before statistical calculations, in order to obtain a normal distribution and a uniform variance within the range of results for the respective analysis. In the Food scheme, *log_{10} transformation* is made and in the Drinking water scheme, *square root transformation* is made.
- Identification of false and deviating analytical results (outliers), including statistical determination of mean values and standard deviations for all quantitative parameters.
- Compilation of the participants results in tables, along with summary statistics.
- Visualisation of the results for each relevant quantitative analysis in a histogram for the respective samples.
- Presentation of statistical evaluation and/or result distribution by method variants per sample for the analysis of a parameter.
- Visualisation of the standardised analytical results (z scores) in box plots, including a summary of the number of outliers and false results below each individual participant's box plot.

Results that – based on the knowledge of the test material – are obviously erroneous (e.g. undoubtedly false), are excluded without any statistical test (blunder removal).

8.2. Homogeneity and determination of concentration

8.2.1. Matters common to both schemes

8.2.1.1. General approach

Accredited analytical methods are used when determining the concentration and homogeneity of the test materials. Non-accredited methods may be used when a new parameter is tested or in certain special cases. This is noted when done.

Concentrations of the various microorganisms in a test material are determined in part to verify that the material possesses all the desired qualities, and in part to obtain reference values to be used when evaluating the participants' results. The determination of homogeneity is made based on the micro-organism concentrations in vials from a stratified design (directly after manufacture), or by randomly selected vials (subsequent stability tests).

The homogeneity of the freeze-dried test material is tested prior to dispatch to the participants, as packaging and shipment of the vials is not considered to interfere with the homogeneity. Aliquots from several vials are consecutively analysed by the same person. The relative variation, both *between* and *within* vials, varies depending on which parameter is analysed and on the concentration of the target microorganism. 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.

8.2.1.2. Exclusion of deviating results

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 test mixture will be approved.

8.2.1.3. ANOVA

A non-decisive analysis of variance (ANOVA) is included based on the first edition of an international protocol [8]. This was however mainly adapted for quantitative chemical analyses, were the results are often normally distributed, and therefore cannot be strictly applied for microbiological analyses. In later editions of the international protocol [9] and in other publications the calculations for determination of homogeneity have changed a bit. The new calculations are not considered applicable for the micro-biological activity described here, and are therefore not used.

ANOVA is therefore used only as a guidance during the homogeneity testing. It is performed on the results from the 10 vials with duplicate determinations [8, also described in reference 10]. ANOVA calculations are carried out on *square root transformed results* for the drinking water scheme and on log_{10} *transformed results* for the food scheme. These transformations are made to obtain uniform variances and to achieve an as good normal distribution as possible. An F-test is carried out to ascertain that the dispersion between vials is not markedly larger than within the vials.

8.2.1.4. "Index of dispersion" – check of randomness

The "Index of dispersion" is a test more suitable for microbiology, and is used to check that the obtained analytical results both *within* vials (I_1; 10 duplicate analyses) and *between* vials (I_2; 10 vials) do not differ markedly from what would be expected based on the appropriate Poisson distributions [11, 12]. In these tests, calculations are carried out on the original *non-transformed colony counts* from the sample volume chosen for counting. The test is dependent on the concentration in the sense that it is easier to obtain acceptance for randomness, i.e. no contradiction to the Poisson distribution, at low colony counts compared to at high.

8.2.1.5. Test of reproducibility

The test of reproducibility (T) [13] is independent of the microorganism concentration. It is therefore a necessary complement to the "Index of dispersion". The test is performed on the 10 *average values* or *sums* (both give the same results) from the two results obtained for each vial, expressed in log_{10} *units* to normalize the results in a general way.

8.2.2. The Drinking water scheme

8.2.2.1. Prerequisites and assumptions

Quantitative analytical results, concentration determinations as well as the participant's results, are obtained by manual counting of colonies. A result may be derived from one or more different volumes of a sample (dissolved test material) but is in general converted into a pre-set analytical volume of the sample. Colony counts from different aliquots of the undiluted sample are generally considered to be Poisson distributed. Since dilutions are never made, the Poisson distribution is regarded to be approximately valid, even for the converted results. Strictly however, it is only valid for the test volumes that have been analysed. In a Poisson distribution, the variance is numerically equal to the average number of colonies.

As a consequence of assuming a valid Poisson distribution, a square root transformation is, based on theory [12], used to approximate a normal distribution. This is supported by studies from the early years of the scheme, where in most cases the participants' results had the best normal distribution when they were square root transformed, compared to log-transformed or not transformed at all.

The analytical results are used square root transformed in determinations of concentrations, in the ANOVA and in detection of outliers, where a reasonable normal distribution is a prerequisite. In tests based on Poisson-distributed results, the results are instead used without transformation.

Depending on the testing round and the analytical parameter, the number of quantitative analytical results varies between approximately 40 and 100.

8.2.2.2. Initial control of concentration

As close as possible to the freeze-drying of a test mixture, 5 vials, randomly picked at different stages of the filling process (beginning, middle and end), are analysed. The content of each vial is dissolved in a fixed volume of diluent, and single analyses are performed from relevant sample volumes. This initial check of concentration is done to get an early indication on whether the test mixture appears to be acceptable, and if it can be assumed to be homogeneous with respect to the various organisms included. It is also done to decide which sample volume to use for each parameter in the final determination of concentration and homogeneity.

8.2.2.3. Final determination of concentration and homogeneity test

Before dispatch of the samples, 10 vials per test mixture from the various stages of dispensation process (stratified sampling) are tested with respect to homogeneity and concentrations. The test items are prepared in the same was as for the initial concentration check. Each sample is analysed in duplicate, and the analyses are performed on all 10 vials during the same day under repeatability conditions. The duplicate aliquots from the 10 vials cannot be analysed in a random order, since unwanted changes in the concentration of organisms could occur in the sample suspension if too much time elapses before analysis. The two aliquots from the same vial are therefore analysed within a relatively short period of time, first aliquot one on the various media and thereafter aliquot two. This could potentially lead to under-dispersion. Only one specified sample volume, based on the initial concentration check, is used for each parameter. A global average and a coefficient of variation (CV) are calculated from the 10 mean values (each from 2 determinations) per analysis.

8.2.2.4. Criteria for homogeneity

The three statistical analyses described above are performed on the results from the 10 vials. ANOVA and "Index of dispersion" within vials (I_1) are used as a *guidance*, to understand the contribution of randomness in the results, whereas "Index of dispersion" between vials (I_2) and the test of reproducibility (T) are used to *determine* homogeneity.

Empirically verified limits of I_2 and T are used to determine homogeneity. Test items are considered to be homogenous when $I_2 \leq 2.0$ and/or $T \leq 2.0$, i.e. when T and I_2 *do not simultaneously* exceed the limits of 2.0.

For production of test items before 2015, the guideline for homogeneity to be acceptable was that the coefficient of variation (CV) should not exceed 25 % when the average content was at least 10 cfu per analysed sample volume. With a lower cfu average (<10 cfu; often poor normal distribution even after transformation), a CV higher than 25 % was accepted if the distribution of the colony counts was as expected, based on the interpretation of the test "Index of dispersion" (with 95 % confidence).

8.2.3. The Food scheme

8.2.3.1. Prerequisites and assumptions

Quantitative analytical results are obtained by manual counting of colonies. Colony counts are thereafter in general converted to a pre-set analytical volume, taking into account the dilution factors. Colony counts obtained from aliquots of the same dilution in a dilution series are in general considered to be Poisson distributed. Since colony counts from several dilutions are often recorded, and since conversion is made back to a specified volume or weight of the original sample, results cannot fully be considered to strictly follow a Poisson distribution. The variance increases more than what is assumed in the Poisson distribution and therefore a log-normal distribution is used as an approximation. The usage of common logarithms ($\log_{10}$) is also the practice when it comes to microbiological food analyses.

The final calculated analytical results are therefore expressed as $\log_{10}$ results and are considered more or less normally distributed. Log-transformed results are used for calculations of concentrations and for assessment of homogeneity (except for I_1 and I_2, where original counts are used).

Depending on the testing round and the analytical parameter, the number of quantitative analytical results varies between approximately 40 and 200.

8.2.3.2. Initial check of concentrations

After the freeze-drying of a test mixture, a single vial is tested to determine the organism composition and concentrations. The content of the vial is dissolved in a fixed volume of diluent, which is then referred to as the zero dilution. Single analyses are made from various dilutions. The initial check of concentration is used to obtain an indication on whether the test mixture is acceptable regarding the different included organisms, and also to decide which dilution to use for each analysis in the final determination of concentration and test of homogeneity.

8.2.3.3. Final determination of concentration and homogeneity test, quantitative analysis

Before dispatch of a test material, 10 vials from the various stages of dispensation process (stratified sampling) are analysed by one person on the same occasion, and under repeatability conditions. The test material is prepared in the same way as the initial concentration control, and the most suitable dilution to analyse is based on the results from the initial concentration check. The 10 samples are analysed either manually or with a spiral plating equipment. Two dilution series are made from each vial, and one Petri dish per series is used for each analytical parameter. The two dilution series from each vial are not analysed in a random order, since unwanted changes in the microorganism concentrations could occur in the dissolved and diluted sample if too much time elapses before analysis. The two dilution series from a vial are instead analysed within a relatively short period of time; first all media from one series and thereafter all media from the second series. This could potentially lead to some under-dispersion. An average result is calculated for the two Petri dishes from each vial. The mean value of these 10 average results *$\log_{10}$ transformed*, and converted back to the original volume using the dilution factor, constitutes the final concentration. The standard deviation for these 10 results, expressed as $\log_{10}$ values, is also calculated.

8.2.3.4. Final determination of concentration and homogeneity test, qualitative analysis

To determine the concentrations of target organisms in *qualitative* analyses separate vials containing a pure culture, "special vials", are freeze-dried in parallel with the vials containing the main test mixture. The volume ratios used in the manufacture of the “special vials” are identical to those in the test mixture; the same final concentration of microorganisms are therefore expected in both. Initial control of

concentrations, as well as final determination of concentration and test for homogeneity, is performed on 1 and 10 vials, respectively, in the same way as for the main test mixture. The mean value per vial is therefore considered to reflect the organism concentration in the main test mixture.

Target organisms for qualitative analyses may also at times be quantified directly from the test mixture by direct inoculation on selective agar media. However, due to stress and competition, this may lead to a lower colony recovery than the actual concentration in the vials. In such a case the dispersion will also usually become larger.

8.2.3.5. Criteria for homogeneity

Homogeneity is determined in the same way as for the drinking water scheme, but with different limits for I_2 and T. For the food scheme, test items are considered to be homogenous when $I_2 \leq 2.0$ and/or $T \leq 2.6$, i.e. when I_2 and *T do not simultaneously* exceed the limits of 2.0 and 2.6, respectively.

For qualitative analyses, the same criteria prevail and the target organisms must be detected from all the 10 vials containing the mixed cultures.

For production of test items before 2015, the criteria of homogeneity for quantitative analyses according to Peterz, 1992 [14] was used. The range for the $\log_{10}$ transformed values of the mean result of the 10 vials should not exceed 0.5 $\log_{10}$ units and the standard deviation should be <0.15 $\log_{10}$ units. In the original test of Peterz results from only 5 vials were used.

A value of 2.0 for *T* was desirable when *T* was decided to be used as a measure in the Netherlands [13], but in practice a value of *T* = 3.0 were used. Here the value *T* = 2.6 is chosen for food analyses because it corresponds exactly to the standard deviation of 0.15 $\log_{10}$ units that was previously used. For drinking water analyses the desired value *T* = 2.0 is used based on empirical checks. This difference is justified by the fact that for food analyses more divergent high values are obtained (in theory) that become normalized after $\log_{10}$ transformations in comparison with results from drinking water analyses which are normalized by square-roots.

8.2.4. Measurement uncertainty for the final determination of concentration and homogeneity

When a manufactured material has been assessed as homogenous (i.e. the dispersion is not significantly larger between vials than within vials) the individual analyses are regarded as 10×2 (20) *independent analyses*. The pooled standard deviation from the ANOVA is therefore used as the measurement uncertainty for the concentration that was determined during the test of homogeneity.

In these determinations, transformed analytical results are used in the same way as in the ANOVAs, i.e. *square root transformed* values are used for drinking water analyses and log_{10} values are used for food analyses.

8.3. Stability

Not all test mixtures with freeze-dried microorganism are tested for stability. However, several weeks often pass between the initial concentration check and the homogeneity tests. To a certain degree, this facilitates the detection of unexpected changes in the concentrations (e.g. due to loss of vacuum in the vials). Mainly however, the Swedish Food Agency relies on decades of experience from working with freeze-dried materials, including continuous follow-ups for several years of a large number of similarly manufactured reference materials (RM)*.

* Stability tests for RM have been carried out on a regular basis during at least 2 years for materials stored at −55 °C (−65 °C before 2012) and in many cases at −20 °C. Analyses have been performed after about 1, 6, 12 18 and 24 months after production. In addition, in doubtful cases, the material has been further analysed. Follow-ups are often made in stable samples mixture for up to 36 and 48 months. In order to

detect changes better, the results are plotted over time for each analytical parameter and reference material for the temperature it was kept at.

Some test mixtures are used in more than one PT round. When vials from such test mixtures are used again in a subsequent PT round, and more than 6 months has passed since the last determination of homogeneity, a new combined stability check of the concentration and homogeneity is performed. Such a stability control is performed in the same way as the initial determination of 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 mixture is either not used, or the affected individual parameter(s) is excluded from evaluation in the PT.

8.4. False positive and false negative results in a testing round

8.4.1. False positive and false negative 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 a lot depending on which parameter is analysed, the composition of the sample and the degree of difficulty, e.g. concentration and/or background flora.

8.4.1.1. Additional considerations for the drinking water scheme

When the average concentration of a target organism is high, a zero result is obviously false and no outlier test is necessary to confirm this.

When the average concentration of a target organism is not high but not very low, zero results that are identified as outliers are defined as false negative results. Zero results that are not identified as outliers when the average for the parameter is >15 cfu/ml are considered as potentially false negative. In the annex in the final report they receive the remark "False negative?".

When the average concentration of a target organism is very low (most often <10 cfu/ml), zero results are often obtained randomly. In these cases they are usually not identified as outliers and, thus, not as false negative results.

8.5. Statistics for a testing round

8.5.1. General

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 on the webpage or in the report.

8.5.2. Mean value, standard deviation and assigned value

A robust statistical approach is used to determine the mean value and standard deviation. Algorithm A with iterated scale as described in ISO 13528:2022 [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:

- Microbiological quantitative results are strongly dependent on the method, and therefore a "true value" strictly does not exist. Rather, the true value is to some extent a question of definition. Results obtained with a certain method by one or several expert laboratories are not necessarily more correct than those obtained with another method by a participant.
- Different brands or batches of dehydrated culturing media – manufactured according to a relevant method standard, and properly evaluated – 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}, which usually varies from round to round for a parameter, is to use a *fixed* standard deviation for each parameter. This could for example consist of an average value for the standard deviation, based on previous suitable PT rounds. With such a procedure z scores can also be directly comparable from one round to another. However a prerequisite for this to be relevant, is that the test materials of different testing rounds have the same degree of difficulty. If not, a more difficult test mixture – with a larger dispersion of the participants' results – would consequently have a larger number of z scores outside the acceptance limits. As this would in essence be caused by the manufacturer of the test material, it can be argued it is something that should not affect the outcome for an individual participant.

The difficulty to obtain a correct result varies with the test material, both within and between testing rounds. This is true even for the same analytical parameter. The Swedish Food Agency has therefore chosen to use a standard deviation that varies with the difficulty of the test material, i.e. the s_{PT} for the current test material. In general, this leads to about the same proportion of "high" z scores for results within the accepted limits (i.e. without outliers) in all testing rounds. Added to this are a varying number of extreme "z scores" for the outliers (that are not included in the calculations of mean value and standard deviation). The total number of extreme z scores may therefore vary more or less independently between rounds.

In this way the z scores are more accurate for the performance over time as well, since a compensation for the degree of difficulty is made. Use of the participants' standard deviations is therefore considered to be the most appropriate way to make comparisons of the microbiological analyses within the frame of the program.

8.5.3. 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 13528:2022, 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.

Outliers can also be objectively identified in other ways, for example with a Grubbs test. This test is mainly suited for identifying small number of outliers, which is not at always the case with microbiological proficiency testing. An alternative approach based on the Grubbs test may however still in certain circumstances be used as the base for statistical analysis in the food and drinking water schemes. In these cases, a Grubbs test modified by Kelly [15, 16], with a 1 % level set as the risk to erroneously identify a result as an outlier is used. The test requires

that the results are normally distributed. The best possible normal distribution if facilitated by transforming the results before processing; square root transformation for drinking water results and $\log_{10}$ transformation for food results. The test is thus used even when the transformed results are not fully normal distributed, and the assumption of normal distribution is not fulfilled. The identified outliers are excluded before calculations of medians, mean values and measures of dispersion for the various analytical parameters. However, z scores are calculated also for the outliers, using the same mean value and standard deviations for a parameter as for ordinary z scores.

8.5.4. 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 13528:2022 [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:

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

8.5.5. 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.

8.5.6. Other statistical measures

8.5.6.1. Mean, standard deviation and median

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.

8.5.7. Statistical exceptions

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.

8.5.8. 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

8.6. Reports from a testing round

8.6.1. Preliminary report

A preliminary report is published within two weeks after the reporting deadline. In connection with this, preliminary statistical measures as shown on the webpage, for at least two weeks. Since the preliminary statistics are subject to change, they are not always shown during the final processing of the results.

The preliminary report consists of a pdf document with comments, which should be read alongside the preliminary results on the webpage. The report contains a short description of the sample content, and a description of how to interpret the results with respect to the preliminary limits of acceptance. The report may also contain an annex with photos that show the outcome and appearance of the colonies on various selective media.

8.6.2. Final report

8.6.2.1. General

A final report is published within two months after the reporting deadline. The report contains comments and discussions on the results for the different parameters, as well as general discussions on the outcome

and performances of the parameters and methods. Special attention is given to deviating results, and to instances where an analytical parameter was difficult or impossible to evaluate in a certain sample.

The report is available as a pdf-document on the webpage. It is also available on the public webpage of the Swedish Food Agency (https://www.livsmedelsverket.se/en/pt-micro).

8.6.2.2. Histograms

The results for parameters with quantitative non-zero results are present 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 also displayed in tables.

Histograms in drinking water reports are based on the original colony counts and histograms in reports food are based on $\log_{10}$ results. Examples of histograms are shown in figures 1 and 2. An asterisk indicates that the results are outside the adjacent horizontal axis limit.

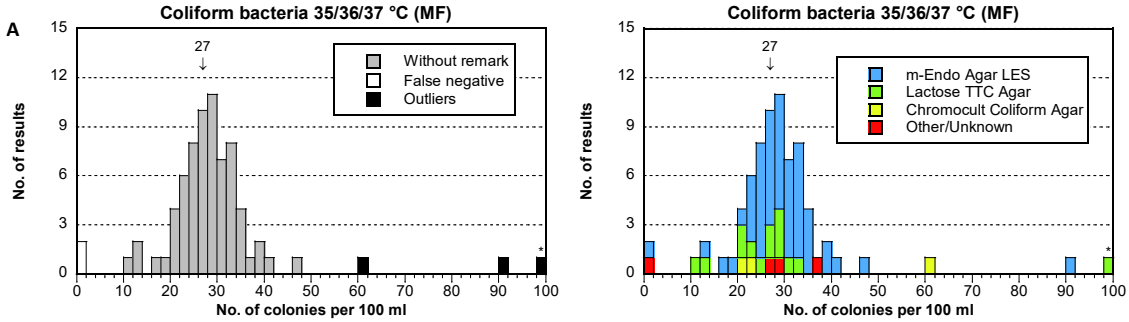

Figure 1. Examples of histograms for a drinking water analysis.

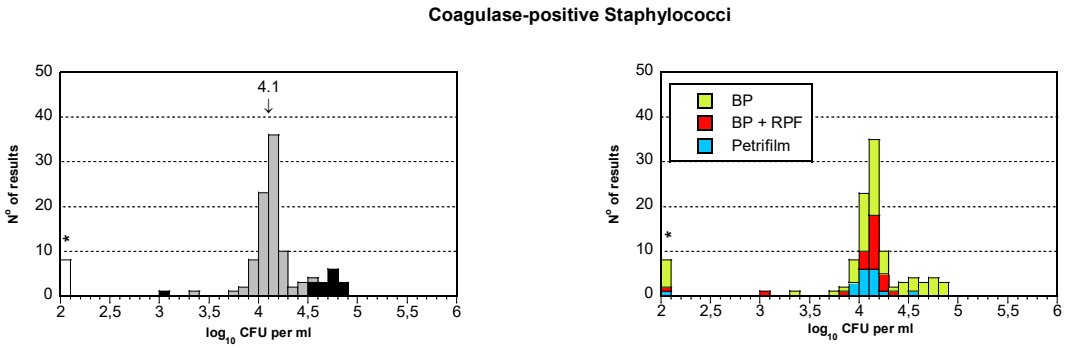

Figure 2. Examples of histograms for a food analysis.

8.6.2.3. Box plots

Z scores are visually summarised in box plots, in order to assist in the evaluation of the participants' overall performance (Figure 3).

Each box plot is based on the *z* scores of an individual participant. It illustrates how the participant's *z* scores are situated as a group in relation to the common, "true", mean value zero. The median value of the participant's *z* scores is illustrated by a solid horizontal line in the box. The box itself consists of 25 % of the *z* scores above the median and 25 % of the *z* scores below the median. The remaining upper

25 % and lower 25 % of the results are represented by vertical lines and circles. A *z* score is represented by a circle when it deviates to a certain degree* in relation to the extent of the box.

* < [lowest value in the box −1.5 × (highest value in the box− lowest value in the box)] or
> [highest value in the box + 1.5 × (highest value in the box − lowest value in the box)]

Z scores higher than +4 or lower than –4 are positioned at the limits +4 and –4 of the y-axis of the plots, respectively.

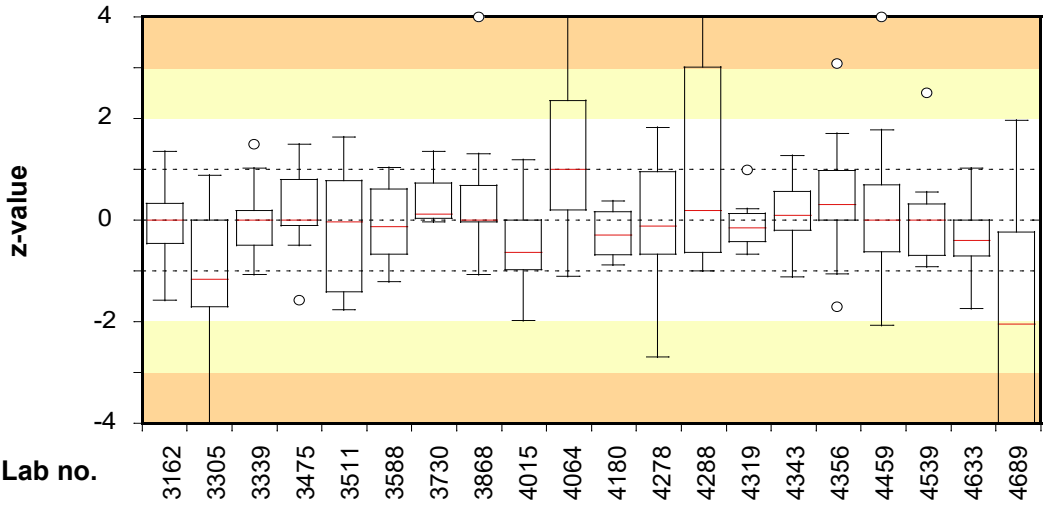

Figure 3. Example of a graph with 20 box plots.

8.6.2.4. Quality control results

For both schemes, results from the final determination of concentration and the homogeneity test – or the most recent stability/homogeneity check – are presented in a table.

8.6.2.5. Annexes

Two annexes are provided at the end of the report. Annex 1 contains a table with the reported results of all participants. In it, outliers and false results are highlighted. The table also contains various statistical measures at the end. Annex 2 contains a table with the calculated *z* scores for each participant. It is intended to assist in the follow-up for the individual participants. The report may also contain an annex with photos that show the outcome on various selective media.

8.7. Assessing the performance of an individual participant

The performance of an individual participant is not directly assessed in the final report; it is thus up to each participant to follow-up and interpret their outcome and performance. The report does however provide the basic criteria for assessment. These are foremost the number of false negative and false positive results, together with the number of outliers or the numbers of deviating z scores. The box plot combined with the numbers of false results and outliers in the table below the plot is the easiest way for an accreditation body, or other interested parties, to get an overview of the performance of an individual participant in a PT round.

9. Confidentiality and user identity

9.1. Confidential participant number and password

Each participant is given a unique participant number, which is valid for the schemes where the participant is registered. The participant number is treated as confidential by the Swedish Food Agency, and it is never given to a third party, except after permission by the participant.

In addition to the participant number, all participants are also given a confidential password when registering. It is treated in the same way as the participant number by the Swedish Food Agency.

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

9.2. Usage of participant number and password

The participant number and password are used to identify participants in connection with PT participation:

- The participant number and password are used for login on the webpage, when reporting results and administering participation in PT rounds.
- Participants are required to provide the participant number – or otherwise in a suitable way prove their identity – when communicating with the Swedish Food Agency regarding a scheme.
- Participant numbers are used in annexes the PT reports, to anonymously display the results of individual participants.
- The participant number and password are printed on documents that are sent together with the test items in the package for a round.

9.3. Changing the laboratory number and password

The participant number and/or password may be changed in order to minimise the risk of unwarranted usage, e.g. upon staff turnover. The password and the participant number 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.

10. Filing

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.

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.

11. Comments from participants and remarks

11.1. Policy

Remarks in the form of deviations and complaints 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. Comments and suggestions for improvements are taken into consideration and dealt with in an appropriate way.

11.2. Complaints and deviations

Remarks within the PT schemes are divided into **complaints** and **deviations**.

Complaint*:* when a participant or other interested party is dissatisfied with a service or a product.

Deviation*:* when written procedures are not followed or when requirements or agreements are not fulfilled.

These definitions and the procedures for managing the complaints and deviations are described in general terms in a general instruction [17] and more specifically in the internal procedures of the program.

Complaints and deviations, as well as the corrective and preventive measures taken, are documented in a database according to internal procedures at the Swedish Food Agency. If the issue is more general it will also be managed within the case and document management system at the Swedish Food Agency.

11.3. Suggestions for improvement

Suggestions for improvement – including suggestions for preventive actions – are also documented. The procedures for managing these suggestions are described in general terms in a general instruction [17] and more specifically in the internal procedure of the program.

12. Conditions and obligations

The general conditions for participation and the obligations of the participants and the Swedish Food Agency are stated on the webpage (www2.slv.se/absint).

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

12.1. Extract of the general conditions of participation

12.1.1. 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.
- Laboratories with internet access, and that are able to use the webpage of the program.

12.1.2. 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.

12.1.3. Fee

- An invoice is sent for the PT round(s) the participant has participated in.
- The fee shall be paid within the time period stipulated (normally within 30 days after print-out of the invoice).

12.2. Other obligations of participating laboratories

- To visit the program webpage and actively register/unregister for participation in the PT rounds.
- To report results according to the written instructions.

12.3. 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.

12.4. 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 schemes run by the Swedish Food Agency.

13. Participation costs

Current prices for participation in the respective PT rounds are stated on the webpage. The Swedish Food Agency reserves the right to change the prices if necessary, in order to be able to continue the activity based on the set requirements.

The fee for participation is paid after invoice. The prices are stated in Swedish crowns (SEK) as well as USD ($) and Euro (€) and payment can be made in any of these currencies.

14. This protocol

This protocol will be revised when important modifications or organisational changes are made. Participants will be notified whenever a new edition is available.

15. References

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