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Biology Department
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Reference material (RM) for drinking water microbiology (Dw)

General

Field of application

The reference material can be used for:

- regular checks, or when needed, of the microbiological analyses done by the laboratory, i.e. check that no disturbance has occurred that affects the results of the analyses ("the results should be under control")
- comparisons of different batches of media, membrane filters and so on
- comparisons between results from different analysts (e.g. training)

The material is freeze-dried and adapted to microbiological drinking water analyses. A simulated water sample is obtained by dissolving the material in liquid. The bacteria in sample type A make it possible to check the drinking water analyses routinely performed, e.g. according to European Union directive. Some target ogranisms, specific to certain countries, can be tested by sample type B and C. The target groups the material are aimed for are described in Table 1.

How the material should be used

Intervals describing within which limits the results initially should fall are obtained by *the reference methods found in Table 1*. The interval limits are given with the samples. These initial intervals are broad since variation *between* laboratories and between six dates during one year are included.

Laboratories are asked to construct their own intervals after some analyses. The new intervals should be narrower, since the analyses are *within* one laboratory. An individual laboratory may, due to a specific cause (method, medium, time, temperature), get a biased mean compared to the reference value.

Laboratories using **other methods** than the reference methods **can also use the reference material**. The limits given can then be used as guide values until limits of their own are obtained.

The materials are mainly intended for quantification of the primary steps in analyses by membrane filtration (counts of cfu on filters) and the number of culturable microorganisms (pour plate technique). Ordinary confirmation steps can still usually be performed from the filters without any problem.

Homogeneity in the material and control charts

Documents with calculations of homogeneity and construction of control charts are available on the website www.livsmedelsverket.se/en/RM-micro.

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Table 1 *Organisms (strains), analyses and reference methods used*

Material A *Escherichia coli*
Citrobacter freundii
Enterococcus faecalis
Pseudomonas aeruginosa
Clostridium perfringens

Target group for analysis	Reference method	Comments
Coliform bacteria	EN ISO 9308-1:2014	37 °C
E. coli	EN ISO 9308-1:2014	37 °C
Thermotolerant coliform bacteria	SS 028167	44 °C
Intestinal enterococci	EN ISO 7899-2	37 °C
Pseudomonas aeruginosa	EN ISO 16266	37 °C
Clostridium perfringens	EN ISO 14189:2016	44 °C
Culturable microorganisms 37 °C, 2 d	EN ISO 6222	37 °C
Culturable microorganisms 22 °C, 3 d	EN ISO 6222	22 °C

Material B *Cladosporium cladosporoides*
Saccharomyces cerevisiae
*Streptomyces sp. (griseu*s group)

Target group for analysis	Reference method	Comments
Microfungi in water (Moulds)	SS 02 81 92	25 °C, 7 days; Rose bengal agar with chloramphenicol and chlortetracycline
Microfungi in water (Yeasts)	SS 02 81 92	25 °C, 7 days; Rose bengal agar with chloramphenicol and chlortetracycline
Actinomycetes in water	SS 02 82 12	25 °C, 7 days, black filters

Material C *Sphingomonas sp.*

Target group for analysis	Reference method	Comments
Slow growing bacteria *	SS-EN ISO 6222, modified	22 °C, 7 days, only bacteria

* This term is used in the Swedish legislation. Those bacteria should be incubated for 7 days but otherwise analysed in concordance with .the standard EN ISO 6222 “Culturable microorganisms” except that only bacteria should be counted. Probably it differs between laboratories whether they include other microorganisms (e.g. yeast) or bacteria only when reading the plates.

Ordering and deliveery

Orders should preferentially be made on-line on our website www.livsmedelsverket.se/en/RM-micro. If facing problem the corresponding order could be sent by e-mail.

Vials from the latest batch will be delivered, if nothing else is stated. The expiry date for the material is set to 1 year after delivery when stored at ordinary freezing temperature, but as most to 2 years after the production date. Before delivery the material is stored at low temperature (currently –55 °C). Often the material is still fit for purpose longer than 2 years. The expiry date may then be postponed after relevant checks.

Instructions for use, including sample preparation, are sent *together with* the vials. A document describing construction of control charts are available on the website. An invoice is sent *in connection with the consignment*.

Questions and ordering

For special ordering and questions about the consignments, as well as about analyses and results, you are asked to use the e-mail address:

RM-micro@slv.se

References *

EN ISO 14189:2016. Water quality – Enumeration of *Clostridium perfringens* – Method using membrane filtration (ISO 14189:2013), European Committee for Standardization, Brussels.

EN ISO 16266. Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration (ISO 16266:2006), European Committee for Standardization, Brussels.

EN ISO 6222. Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium (ISO 6222:1999), European Committee for Standardization, Brussels.

EN ISO 7899-2. Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method (ISO 7899-2:2000), European Committee for Standardization, Brussels.

EN ISO 9308-1:2014. Water quality – Enumeration of *Escherichia coli* and coliform bacteria– Part 1: Membrane filtration method for waters with low bacterial background flora (ISO 9308-1:2014), European Committee for Standardization, Brussels.

SS 02 81 67. Water quality – Coliform bacteria, thermotolerant coliform bacteria and *Escherichia coli* in water – Determination with membrane

filtration method (MF), Ed. 2 1996, Swedish Standards Institute, Stockholm, Sweden *(Used as reference method for thermotolerant coliform bacteria)*.

SS 02 81 92 Water quality – Microfungi in water – Quantitative determination by the membrane filter method *(in Swedish)*, Ed. 1 1989, Swedish Standards Institute, Stockholm, Sweden.

SS 02 82 12 Water quality – Actinomycetes in water – Quantitative determination by the membrane filter method *(in Swedish)*, Ed. 1 1994, Swedish Standards Institute, Stockholm, Sweden.

* The Swedish Food Agency has used the Swedish translation (SS-prefix) of a standard when available.