Livsmedelsverket
Swedish Food Agency

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

Designation: ***Dw 2019:C***

Date of production: 2019-09-02

Manufacturer: Swedish Food Agency (SFA), Sweden

Homogeneity: A separate document (pdf) on our website

Expiry: **31 March 2022** when kept at –55 °C at SFA.
Should be used within 1 year after delivery when kept at about –20 °C, but not beyond expiry date.

Table 1 ***Bacteria strains included***

Species name	Strain	Parameter	Reference method [1]
Sphingomonas sp.	SLV-547	Slow growing bacteria 7 days ("Swedish analysis")	EN ISO 6222:1999, modified

1 See references in INFORMATION about RM for drinking water on our website www.livsmedelsverket.se/en/RM-micro

Preparation of simulated water sample

200 ml

The freeze-dried material form one vial is transferred to ***200 ml ± 2 ml*** room tempered diluent or rinsing solution according to *the enclosed instructions*. After thorough, intermittent (repeated with interruptions) mixing the sample is ready for use. **Other volume of diluent is possible to use after your own choice, e.g. 300 or 150 ml, which corresponds to an average of 41 and 81 cfu/ml, respectively, instead of 61 cfu/ml for YeA.**

Performance of the analysis

The analysis should be performed, in accordance with the methods of the laboratory, by using appropriate volumes. Tentative control limits for 1 ml are given in Table 2. Other volumes can be used by choice.

Mean values and control intervals

In Table 2 the "guidance values" from the NFA are given together with initial control limits, which can be used by a laboratory before it has a number of analyses to construct its own intervals. The limits $\pm 2s_0$ and $\pm 3s_0$ are used as the limits 2-sigma and 3-sigma, respectively. s_0 is the standard deviation for the analysis.

Usually, there are systematic differences (bias) between laboratories giving somewhat different mean values and measures of dispersion – i.e. leading to different intervals. The probability of results of all laboratories falling beyond the control limits of Table 2 is approximately 5% for $2s_0$ and 0.3% for $3s_0$.

The initial control intervals have partly been calculated from variation for analyses from reference material type A, used for many years (see Table 2, note 3). Corresponding challenge tests have not been performed with type C material. *Hence,* the intervals are regarded as tentative and *are appropriate when the reference method in Table 1 is used*. The intervals are valid for single analytical values, not mean values. They may be used as *guidance* until the laboratory has constructed its **own intervals**.

Laboratory specific limits for an analysis are usually smaller than the broad initial interval but the position differs depending on the bias.

If other volume than 200 ml of diluent or 1 ml test portion is used you have to construct your individual control limits as soon as possible.

Results from all analysts routinely performing the analysis should be included for *construction of control charts*. The design (no. of results per vial) should be the same every time. For example of calculations, see the document CONTROL CHARTS – WATER on the website www.livsmedelsverket.se/en/RM-micro.

As known for a couple of years, not all laboratories obtain full recovery of the strain in the reference material by use of the medium prescribed in the standard EN ISO 6222 – and probably also not of strains in natural samples.

The recovery is – at least partly – connected to media batches and how they are handled. It seems to vary between media lots but sometimes also between specific finished media batches. To check recovery is currently best done by use of the medium R2A agar, which gives more stable results.

If you otherwise obtain results systematically different from the intervals given, you must as soon as possible construct your own intervals for use.

Please, inform us regarding strange recovery at: RM-micro@slv.se.

Table 2 ***Mean values and initial control limits for RM Dw 2019:C***

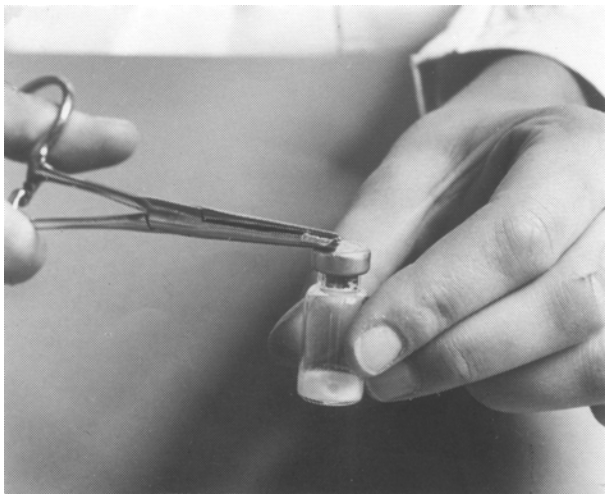
Parameter [1]	**Volume of analysis**	**Mean values** [2]	**Control limits** [3]			
	(*ml*)	(*cfu*)	$-3s_0$	$-2s_0$	$+2s_0$	$+3s_0$
Slow growing bacteria 22 °C, 7 d (Yeast extract Agar, YeA)	**1**	61	27	36	92	110
Slow growing bacteria 22 °C, 7 d (R2A agar)	**1**	90	47	60	127	148

1 Pour plate method for slow growing bacteria.

2 Retransformed values of square-root transformed results from 10 vials in duplicate 5.5 weeks after freeze-drying; cfu = colony forming units.

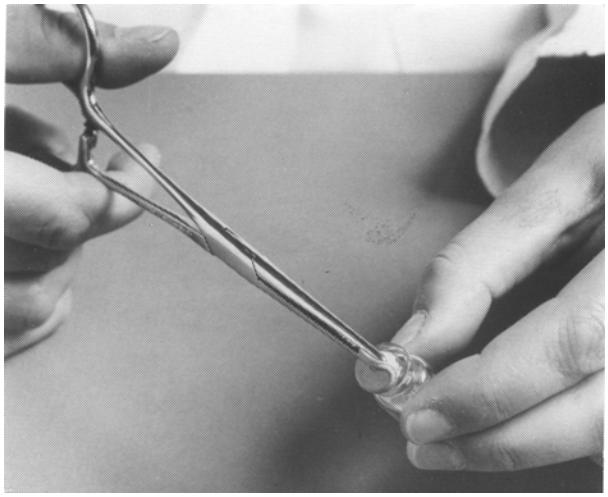
3 Based on the internal reproducibility dispersion at Livsmedelsverket (because of abnormally low repeatability dispersion in the homogeneity test) and on dispersions from reference material type A during one year before the first launching (six parameters analysed six times independently by 16 laboratories). The intervals are asymmetrical around the mean value, since they have been obtained by retransformation after calculation with square-root transformed results. Cfu at $\pm 2s_0$ is **limits of warning** and $\pm 3s_0$ **limits for action** in the control chart.

Sample preparation of freeze-dried cultures in glass vial (RM)

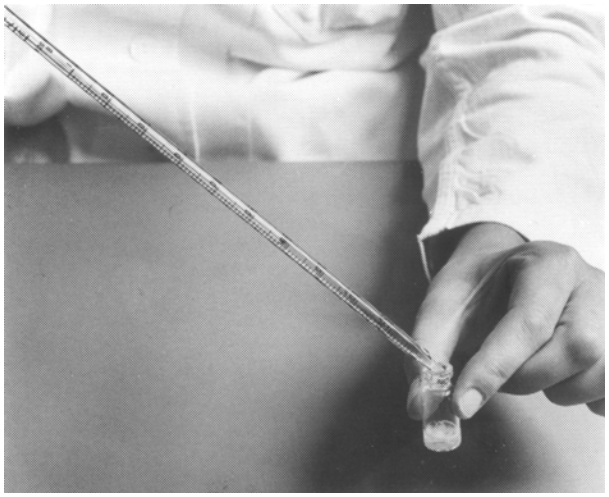


1. Twist the flap on the upper side of the aluminium cap in the direction of the arrow. Use a pair of forceps, tweezers or similar.

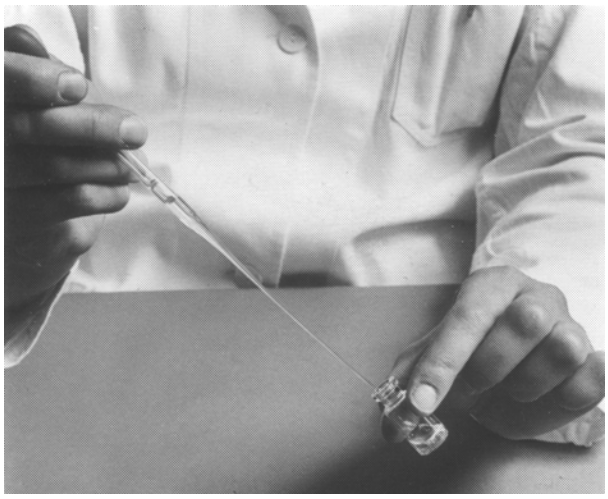
2. Remove the aluminium cap.



3. Remove the rubber plug. Carefully burn off the opening of the vial over a gas flame.



4. Add 1 ml of diluent.



5. Loosen the suspension using a sterile pasteur pipette. Transfer the suspension to a sterile ½ – 1 liter bottle containing **correct volume** (± 1%) **room tempered** diluent.

6. Repeat steps 4 and 5 three more times with the same pasteur pipette. Rinse the walls of the vial carefully. After thorough intermittent mixing, for a minimum of 3 minutes (even better >10 min), the sample is ready for analysis. Perform the analyses within 60 min.