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

Designation: ***Dw 2022:A***

Date of production: 2022-02-02

Manufacturer: Swedish Food Agency (SFA), Sweden

Homogeneity: A separate document on our website (see below)

Expiry: **2 February 2024** kept at –55 °C at SFA. *Should be used within 1 year after delivery when kept at about –20 °C, but not beyond the expiry date.*

Bacteria strains included *(Table 1)*

Parameter	Target organisms	Strain no.	Reference method[1]
Coliform bacteria	*Escherichia coli* *Citrobacter freundii*	SLV-084 SLV-424	EN ISO 9308-1:2014
E. coli	*Escherichia coli*	SLV-084	EN ISO 9308-1:2014
Intestinal enterococci	*Enterococcus faecalis*	SLV-051	EN ISO 7899-2:2000
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	SLV-395	EN ISO 16266:2008
Clostridium perfringens	*Clostridium perfringens*	SLV-442	EN ISO 14189:2016
Culturable microorganisms 22 °C 3 days / 37 °C 2 days	Sum of bacteria **except** *C. perfringens*		EN ISO 6222:1999

[1] See references in INFORMATION about RM for Drinking water on www.livsmedelsverket.se/en/RM-micro

300 ml

Preparation of simulated water sample

The freeze-dried material in one vial is transferred to ***300 ml* ± *3 ml*** room tempered diluent or rinsing solution *according to enclosed instructions*. After thorough, intermittent (repeated with interruptions) mixing during at least 3 minutes (>10 minutes even better) the sample is ready for use.

Performance of the analyses

The analyses should be performed, in accordance with the methods of the laboratory. Initial control limits for specified volumes are given in Table 2. Other volumes and limits can be used by choice.

Mean values and control intervals

In Table 2 *"Guidance values"* from the SFA are given together with *initial control limits*, which should be used before a laboratory is able 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 material.

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 $3s_0$. Due to bias, the probabilities of an individual laboratory can be greater, but usually less than these values, because laboratory specific limits usually are smaller than the broad initial ones.

The control intervals are constructed with regard to different sources of variation (see Table 2, note 3). *The intervals are appropriate when the reference methods in Table 1 are used*. Variations due to the use of other methods are not included in the intervals*. The initial intervals may, however, still be used as *guidance* until the laboratory has constructed its own intervals. They are valid for single analytical values, not mean values. 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. Intervals can be calculated according to the document (pdf) CONTROL CHARTS – WATER on www.livsmedelsverket.se/en/RM-micro.

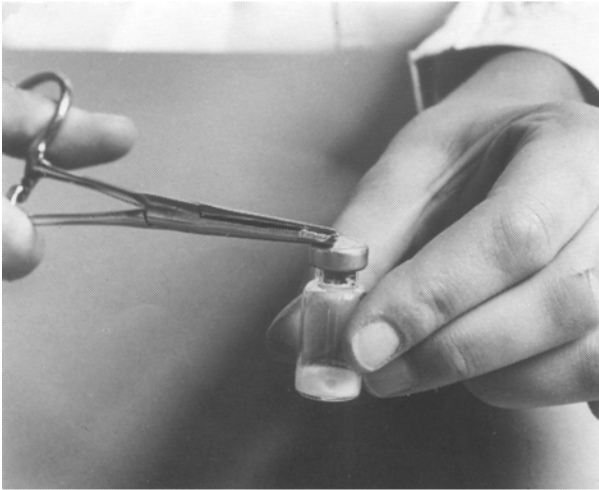
* For exceptions, see footnotes 4 and 5 beneath table 2

Table 2 *Mean values and initial control limits for RM Dw 2022:A*

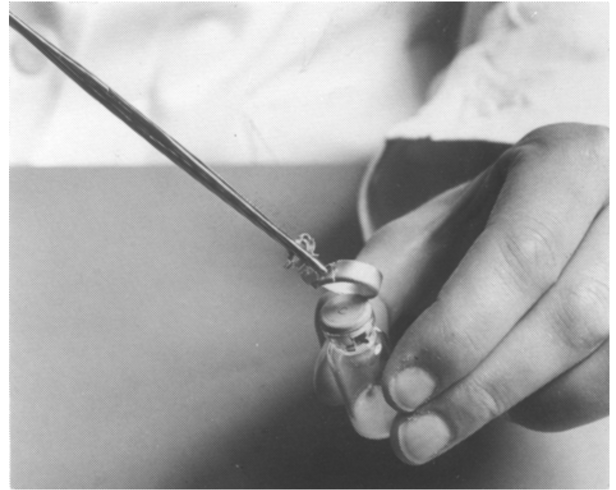
Parameter [1]	**Volume of analysis**	**Mean-values**[2]	**Control limits**[3]			
	(*ml*)	(*cfu*)	$-3s_0$	$-2s_0$	$+2s_0$	$+3s_0$
Coliform bacteria, 37 °C [4]	5	66	39	47	88	100
E. coli, 37 °C [4]	5	50	26	33	70	82
E. coli, 44 °C [5]	5	44	22	28	63	74
Intestinal enterococci	5	116	69	84	155	176
Pseudomonas aeruginosa	**2**	40	19	26	58	69
Clostridium perfringens, 44 °C	5	32	12	17	50	61
Culturable microorg. 37 °C, 2 d [6]	1	50	27	34	69	80
Culturable microorg. 22 °C, 3 d [6]	1	50	27	34	69	80

1. Pour plate method for culturable microorganisms, membrane filtration method (MF) for the others. The values are given for presumptive colonies before confirmation but should remain the same after confirmation.
2. Retransformed values of square-root transformed results from 10 vials in duplicate 4 weeks after freeze-drying; cfu = colony forming units.
3. The control limits include natural random variation in the material, variation between vials, variation between days of analysis as well as variation between laboratories. The intervals are asymmetrical around the mean values, 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. Results from a one year long (6 different dates) challenge study at the SFA together with results from 16 representative laboratories have been used.
4. Average from analyses with Chromocult Coliform agar and LES Endo agar incubated at 37 °C.
5. Results for m-FC agar incubated at 44 °C, without pre-incubation at lower temperature.
6. The results for Culturable microorganisms at 37 and 22 °C are pooled.

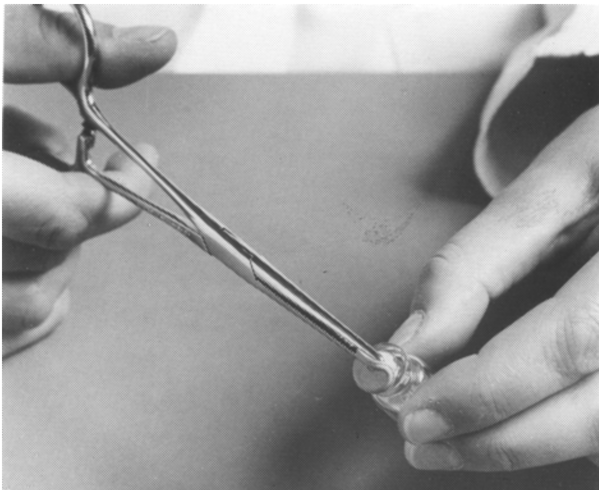
Sample preparation of freeze-dried cultures in glass vial (RM)



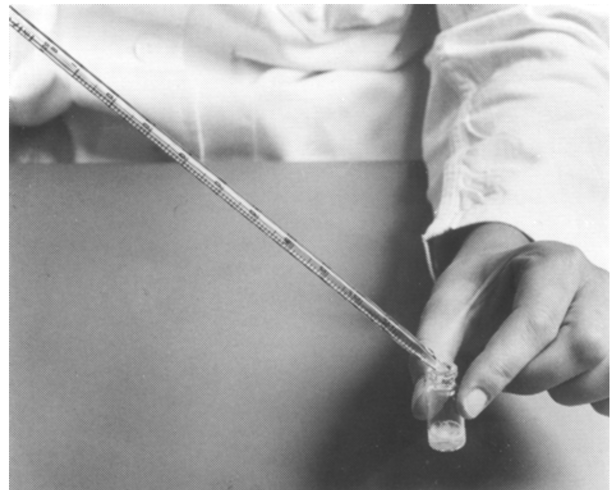
1. Twist the flap on the upper side of the aluminium cap in the direction of the arrow. Use a pair of forceps, tweezers or similar.



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.