

Science Division
 Department of Biology

Reference material RM Food 2021:12

This reference material (RM) is designed for internal quality control of analytical work at food microbiology laboratories. After reconstitution, the test material can be used for control of quantitative food microbiology analyses, as well as for direct or indirect quality control of microbiological media.

Designation: RM Food 2021:12
Date of production: 11 March, 2021
Manufacturer: Swedish Food Agency, Sweden
Storage: Keep dark at -18 °C or lower. (But not lower than -55 °C)
Expiry date: 31 August, 2022

Table 1. Microorganisms included in the material

Microorganism	Strain*
<i>Klebsiella oxytoca</i>	SLV-089
<i>Escherichia coli</i>	SLV-082
<i>Staphylococcus aureus</i>	SLV-350
<i>Clostridium perfringens</i>	SLV-442
<i>Enterococcus faecalis</i>	SLV-051
<i>Bacillus cereus</i>	SLV-556
<i>Candida</i> sp.	SLV-052

* Internal strain identification number, Swedish Food Agency

Quality control

The reference material is tested for homogeneity and stability at regular intervals at the Swedish Food Agency.

Preparation of sample

Reconstitute the vial content according to the instructions on the last page.

Please note that the final 104 ml corresponds to the undiluted sample to be analysed.

The Swedish Food Agency uses peptone water (0.1 %) with NaCl (0.85 %) as diluent.

Analyses

The analyses should be performed in accordance with the methods used by the individual laboratory.

Initial control limits

Table 2 shows the *initial control limits* for each analysis. They can be used as *provisional intervals* until a laboratory has determined its own control limits. The limits indicate where a single result at a laboratory is likely to be obtained. For the individual laboratory, it is here important to note:

- The mean values in Table 2 are from *initial determinations of the concentration* at the Swedish Food Agency. The values do not take in consideration measurement uncertainty in these initial analyses.
- The results of an individual laboratory may be higher or lower than the mean values provided in Table 2, due to random variations between laboratories, methods, media and technicians.
- The standard deviations (s_0) in Table 2 are calculated from measures of dispersion, obtained from external laboratories' results for the respective parameters in analyses of similar materials in proficiency testing organised by the Swedish Food Agency 2015-2021 (s_{PT}). When available, measures of dispersion from an earlier study of similar materials at external laboratories (s_{lab}) have been pooled with s_{PT} to obtain s_0 .
- The standard deviations s_0 are used to calculate *initial limits of warning and action* ($2s_0$ and $3s_0$, respectively) in Table 2.
- Since the s_0 in Table 2 originate from different freeze-dried test materials, methods, laboratories, technicians and dates for analysis, they should *in general* be larger than the standard deviations obtained at an individual laboratory.

Table 2. Mean values and initial control limits for RM Food 2021:12

Analysis	Mean value ^a	s_0^b	Control limits ^c				Method and medium for mean value	
			-3 s_0	-2 s_0	+2 s_0	+3 s_0	Method	Medium
Aerobic microorg., 30 °C	6.01	0.17	5.51	5.67	6.34	6.51	NMKL 86:2013	PCA
Contaminating microorg.	5.84	0.29	4.98	5.27	6.41	6.70	ISO 13559:2002	SFA
Lactic acid bacteria	5.85	0.20	5.26	5.46	6.25	6.44	NMKL 140:2007	MRS-aB
Coliform bacteria, 37 °C	4.93	0.25	4.19	4.44	5.43	5.68	NMKL 44:2004	VRB
Coliform bacteria, 44 °C	4.43	0.23	3.75	3.98	4.89	5.12	NMKL 125:2005	TSA/VRB
Enterobacteriaceae	4.98	0.23	4.29	4.52	5.44	5.67	NMKL 144:2005	VRBG
<i>Escherichia coli</i>	4.43	0.22	3.76	3.99	4.88	5.11	NMKL 125:2005	TSA/VRB
Anaerobic sulph. red. bact	3.73	0.27	2.93	3.20	4.27	4.53	NMKL 56: 2015	ISA
<i>Clostridium perfringens</i>	3.51	0.20	2.90	3.10	3.92	4.12	NMKL 95:2009	TSC
Coagulase pos. staphylococci	4.68	0.13	4.29	4.42	4.94	5.07	NMKL 66:2009	RPFA
Enterococci	5.85	0.14	5.44	5.57	6.12	6.25	NMKL 68:2011	ENT
Presumptive <i>Bacillus cereus</i>	4.55	0.23	3.86	4.09	5.01	5.24	NMKL 67:2010	BA
Yeasts	4.50	0.19	3.93	4.12	4.88	5.07	NMKL 98:2005	DRBC

PCA: Plate Count Agar, SFA: Sugar-Free Agar, MRS-aB: de Man, Rogosa and Sharpe-agar with amphotericin, VRB: Violet Red Bile agar, TSA: Tryptone Soy Agar, VRBG: Violet Red Bile Glucose agar, ISA: Iron sulphite agar, TSC: Tryptose Sulphite Cycloserine agar, RPFA: Rabbit Plasma Fibrinogen Agar, ENT: Slanetz & Bartley *Enterococcus* agar, BA: Blood Agar, DRBC: Dichloran Rose Bengal Chloramphenicol agar

^a Mean value (\log_{10} cfu/ml) from analysis of 10 vials at the Swedish Food Agency.

^b In general s_0 = pooled standard deviation (s_{PT}) from laboratories' results for the respective analysis in the Swedish Food Agency's proficiency testing on similar materials during 2015-2021. When available, standard deviations from an earlier study by external laboratories on similar materials (s_{lab}) have been pooled with s_{PT} to estimate s_0 .

^c Initial control limits (\log_{10} cfu/ml) based on initial determination of the concentration at the Swedish Food Agency, and s_0 .

Control charts

Normally, there are systematic differences (bias) between the results from different laboratories. Laboratories are therefore encouraged to as soon as possible construct their own control charts, with their own laboratory-specific control intervals. Such charts can be designed according to the instructions provided at our website (below). This is especially important if the volume used for reconstitution, the sample volume or the method for analysis differs from the recommendations in these instructions. Results from all persons that normally perform the analysis should be included in the control charts. The design (the number of results per vial) should also be the same every time.

Instructions for the construction of control charts are available at our website:

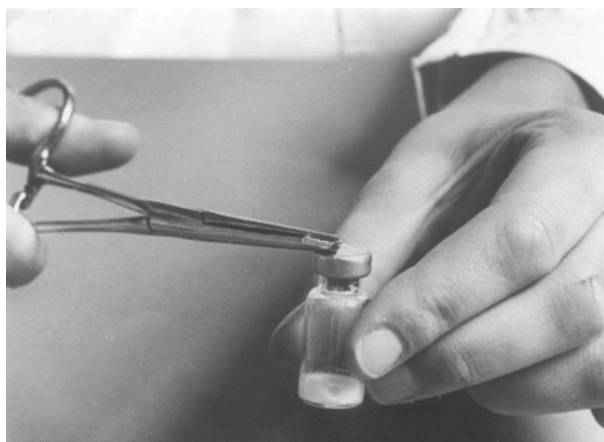
www.livsmedelsverket.se/en/RM-micro.

Information

www.livsmedelsverket.se/en/RM-micro

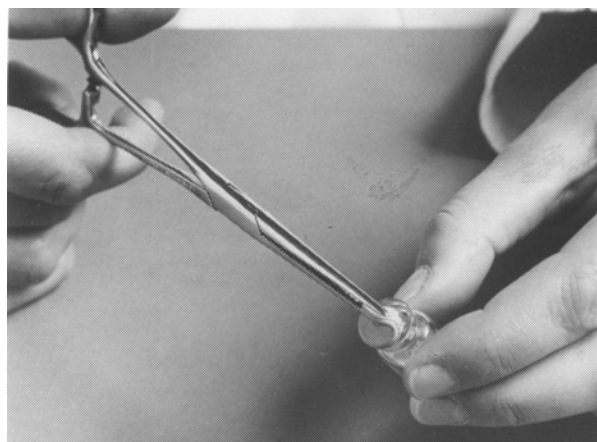
e-mail: RM-micro@slv.se

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



1. Twist the flap on the aluminium cap in the direction of the arrow.

2. Remove the aluminium cap.



3. Remove the rubber plug.

4. Carefully burn the opening of the vial over a gas flame.



5. Add 1 ml diluent with a sterile pipette.

6. Let the content dissolve (1-5 minutes).

7. Using a sterile Pasteur pipette, transfer the suspension to a sterile bottle containing 100 ml room temperature diluent.

8. Add another 1 ml and carefully rinse the walls of the vial with the Pasteur pipette.



9. Transfer the suspension to the bottle containing 100 ml diluent.

10. Repeat steps 8 and 9 two more times with the same Pasteur pipette.

11. After thorough intermittent mixing, the 104 ml sample is ready for analysis.

12. Perform the analyses immediately.*

** For the majority of analyses, the final 104 ml sample is normally viable for up to 60 minutes. From experience however, lower results are often obtained—especially for Gram-negative microorganisms—when the time between sample preparation and analysis is too long. As a general rule, it is therefore recommended to begin the analysis immediately after sample preparation.*