Livsmedelsverket
Swedish Food Agency

19 September 2022 Dnr 2021/03075

Science Division
Department of Biology

Reference material RM Food 2021:8

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 2021:8**
Date of production:	21 October, 2020
Manufacturer:	Swedish Food Agency, Sweden
Storage:	Keep dark at -18 °C or lower. (But not lower than -55 °C)
Expiry date:	30 June, 2023

Table 1. Microorganisms included in the material

Microorganism	Strain[1]	Hazard group[2]
Penicillium roqueforti	SLV-510	1
Cladosporium cladosporioides	SLV-488	1
Saccharomyces cerevisiae	SLV-375	1

[1] Strain identification no. at the Swedish Food Agency.

[2] Hazard group according to the Swedish Work Environment Authority (AFS 2018:4)

Quality control

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

Diluent

The recommended diluent for analysis of yeasts and moulds varies:

- NMKL 98:2008 stipulates the use of peptone water without salt as diluent, but also states that peptone water with salt can be used.
- ISO 21527-1:2008 and ISO 21527-1:2008 stipulates the use of peptone water without salt as diluent.
- With Petrifilm YM and Petrifilm RYM, both of these types of diluents (as well as others) are recommended.

The Swedish Food Agency uses peptone water (0.1 %) with NaCl (0.85 %) as diluent for the preparation and analysis of RM Food 2021:8. Laboratories are however advised to use the same diluent as they normally use for the analysis of yeasts and moulds.

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www.livsmedelsverket.se/en/RM-micro

VAT number
SE202100185001

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.

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 2015-2019 (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 2021:8

Analysis	Mean value[a]	s_0[b]	Control limits[c]				Method and medium for mean value	
			$-3s_0$	$-2s_0$	$+2s_0$	$+3s_0$	Method	Medium
Moulds	3.39	0.22	2.72	2.94	3.83	4.05	NMKL 98:2005	DG18/DRBC
Yeasts	3.36	0.19	2.79	2.98	3.74	3.93	NMKL 98:2005	DG18/DRBC

DG18: Dichloran glycerol 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 intial 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.

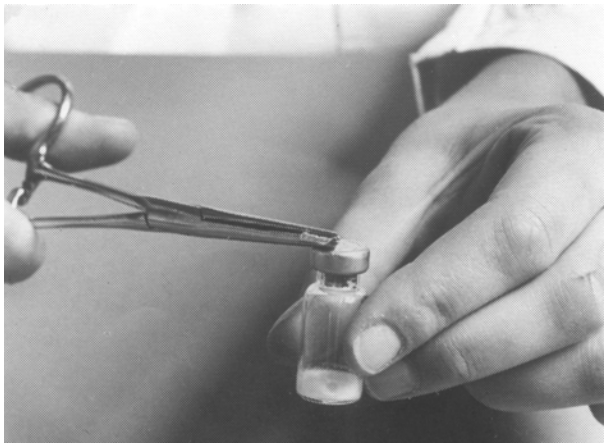
Instructions for the construction of control charts are available at our website:
www.livsmedelsverket.se/en/RM-micro

Information

www.livsmedelsverket.se/en/RM-micro

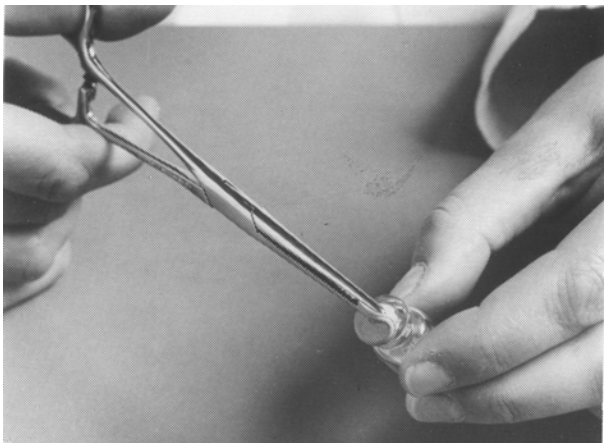
e-mail: RM-micro@slv.se

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



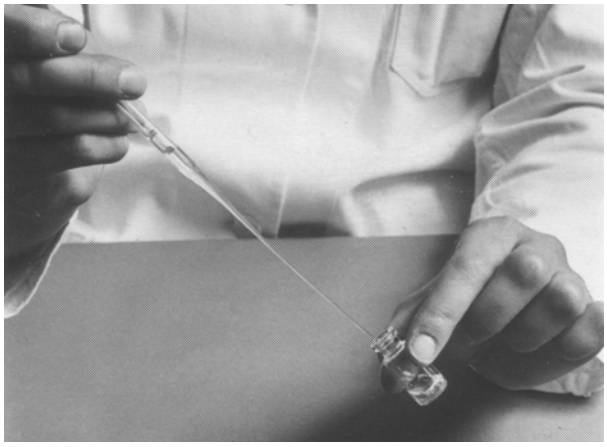
1. Twist the flap on the aluminium cap in the direction of the arrow.

2. Remove the aluminium cap.



3. Remove the rubber plug.

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

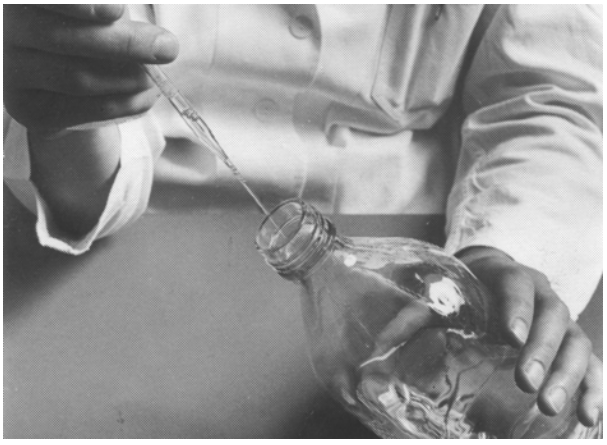


5. Add 1 ml diluent with a sterile pipette.

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 within 60 minutes.*

** From experience, for analysis of moulds more accurate results are often obtained if the final 104 ml sample is allowed to rest for 30 minutes (followed by a new mixing) prior to analysis.*