Food Processing Machinery and Packaging Machinery

VDMA Documents
Food Processing Machinery and Packaging Machinery

Guidelines for the identification of positive and the exclusion of false positive and false negative findings in microbiological evaluation procedures

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This publication was compiled by the working group "Interface problems with aseptic systems" of the VDMA Packaging Machinery Department. It is available for download at http://www.VDMA.ORG/Publikationen - Branch: Nahrungsmittelmaschinen und Verpackungsmaschinen (Food Processing Machinery and Packaging Machinery). Search for "VDMA FPPMAA Documents".

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Introduction

This trade association publication provides information on the implementation and evaluation of microbiological evaluation procedures, which are used, for example, in sterile and standing tests during the commissioning of hygienic filling machines of VDMA hygiene classes IV and V.

1 Terms and definitions

Term	**Definition**	**Explanation**
Growth Promotion Test	Test performed to demonstrate that media will support microbial growth (PIC/S PI 007-6 2011)	Test to demonstrate that, in case of microbiological contamination, culture media allow sufficient growth at the end of the incubation period to detect the contamination (avoidance of false negative results) Growth promotion tests are required in pharmacopeias, for example. In PIC/S PI 007-6 (2011), for example, growth promotion tests are required to demonstrate low selectivity with regard to the expected germ spectrum and to detect contamination with low initial germ counts (e.g. 10-100 KbE or less).
Commercially sterile product	Product free of viable microorganisms and free of organisms that can multiply in the product under normal, nonrefrigerated storage and distribution conditions.	

2 General notes on laboratory and personnel

- Preparation of the agar plates, sampling and smearing of the samples should only be carried out by trained personnel.

  Note: Trained personnel should follow standard written instructions for correct sampling, e.g. ISO 707:2008 (IDF 50:2008) Milk & milk products -Guidance on sampling, see also DIN CEN ISO/TS 17728:2015-11 (D) Microbiology of the food chain -Sampling techniques for microbiological testing of food and feed samples (ISO/TS 17728:2015) and DIN EN ISO 19458 Water quality -Sampling for microbiological testing
- Sampling and testing should preferably be carried out in a laminar flow bench or biosafety cabinet in the laboratory
- Good laboratory practice (GLP), e.g. with regard to disinfection, order at the workplace and personal hygiene should be followed
- General conspicuous features such as packaging and product changes must be observed and documented.
- Pre-sterilized disposable materials are preferable to reusable materials in order to exclude contamination due to insufficient sterilization of the reusable materials.

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3 Notes on preincubation and sampling

- Sufficient pre-incubation of the product to be tested must be ensured to achieve an accumulation of potentially 1 CFU per container to a level detectable with the detection method used.

  Note: Pre-incubation conditions are product dependent and differ e.g. between high-acid and low-acid products

  Note: The required pre-incubation time should be aligned with the coldest point of the container to be incubated (pallet, tray, etc.).
- Secondary packaging should be removed outside the sampling room or laboratory.
- Sedimentation plates should be placed immediately adjacent to the sampling point and the spreading of the plates as a control. (plate open for 30 minutes)

  Note: The sedimentation plates may be required to exclude false positives
- The surface of the packaging to be opened, especially around the area to be opened, should be sufficiently decontaminated (sporicide if necessary)

  Note: This reduces the risk of contamination by surface micro-organisms and their persistent forms
- Samples should not be taken directly from the surface around the opened area to avoid false positive findings due to contaminants possibly introduced during the opening of the packaging.
- For sampling, the packages are opened with sterile tools (e.g. scalpel, scissors, soldering iron). The opening should not be carried out near sealing seams or opening aids, in order to be able to check the package integrity afterwards if necessary.

  Note: When opening over already existing openings of the packaging (e.g. lids), a subsequent check of the package integrity is not possible. Samples must be taken with a sterile object (e.g. inoculation loop, pipette).

4 Instructions for preparing the plates for smear and pour plate procedures and for incubating the samples

- The agar plates to be used should be dry (free of condensate, drops in the lid and plate rim) and sterile. To dry agar plates and test for sterility at the same time, they should be stored at room temperature for 12-24 hours before testing (or better 100% control of the culture media). This storage should preferably be under a laminar flow bench and bio-safety cabinet (< 200 KbE/m3 according to EU GMP Grade D).

  Note: Condensate and droplets can lead to false positive results
- Low selectivity: Incubation conditions and culture media of the agar plate should be of low selectivity to cover the whole bacterial spectrum to be tested. Depending on the application, anaerobic incubation (e.g. when testing for microaerophilic lactic acid bacteria) should also be considered.
- Sufficient sensitivity: Incubation conditions and culture media of the agar plate should ensure sufficient sensitivity so that, for example, even pre-damaged bacteria show sufficient growth. If necessary, a growth promotion test should be performed.
- For **smear procedures** with inoculation loops, no more than 4 parallel smears should be taken per agar plate. The smears should be taken either in a straight line or in loops in appropriate quadrants at least 1 cm from the edge of the plate (see Fig. 1).

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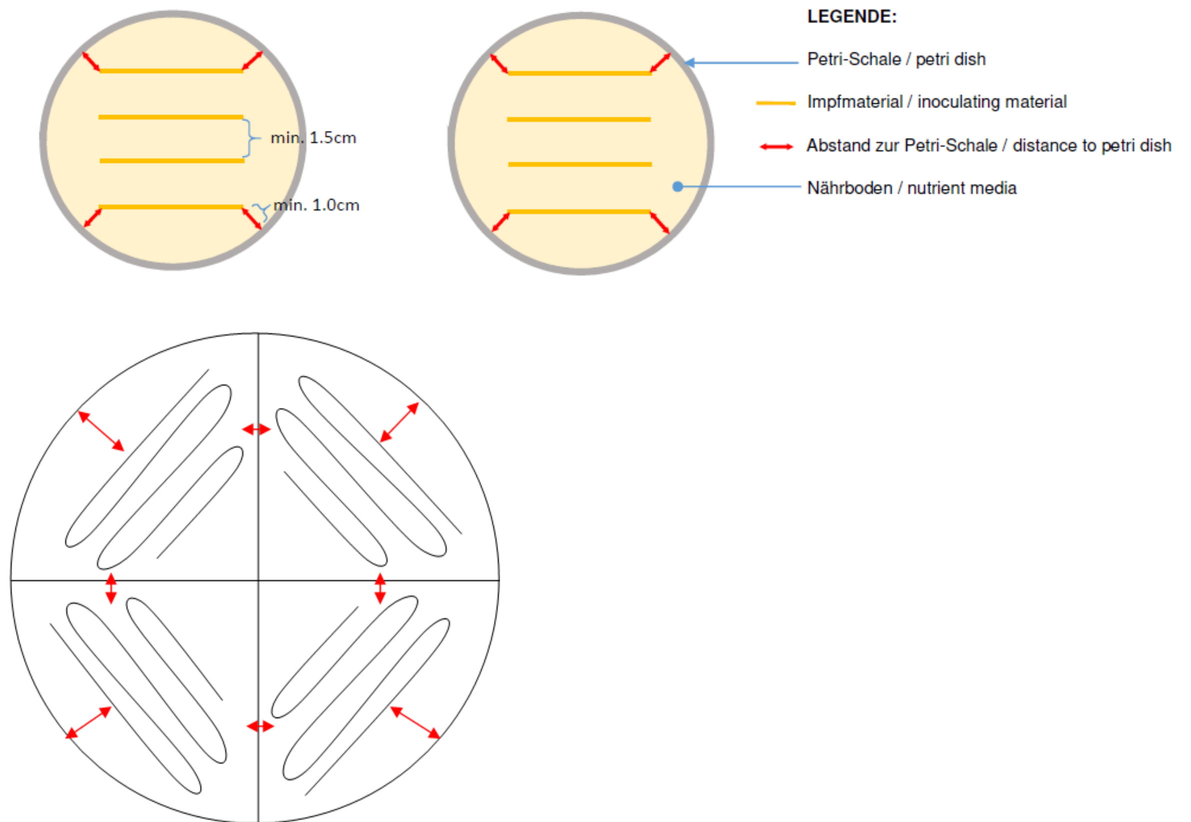

Fig. 1: Recommended smear patterns

Source: VDMA Food Processing Machinery and Packaging Machinery

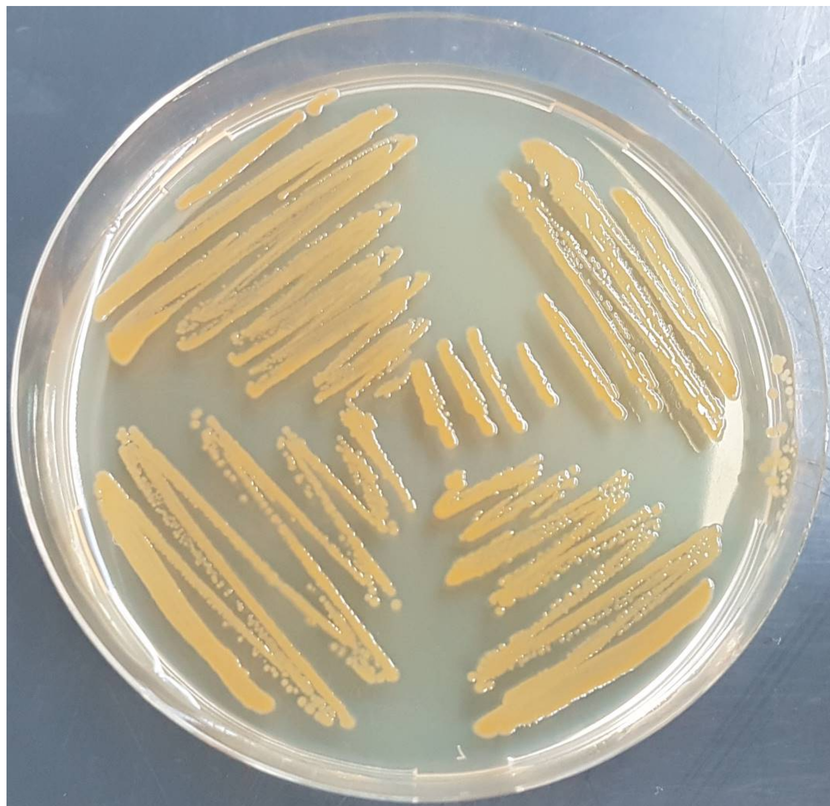

Fig. 2: Example of insufficient spacing between smears

Source: VDMA Food Processing Machinery and Packaging Machinery

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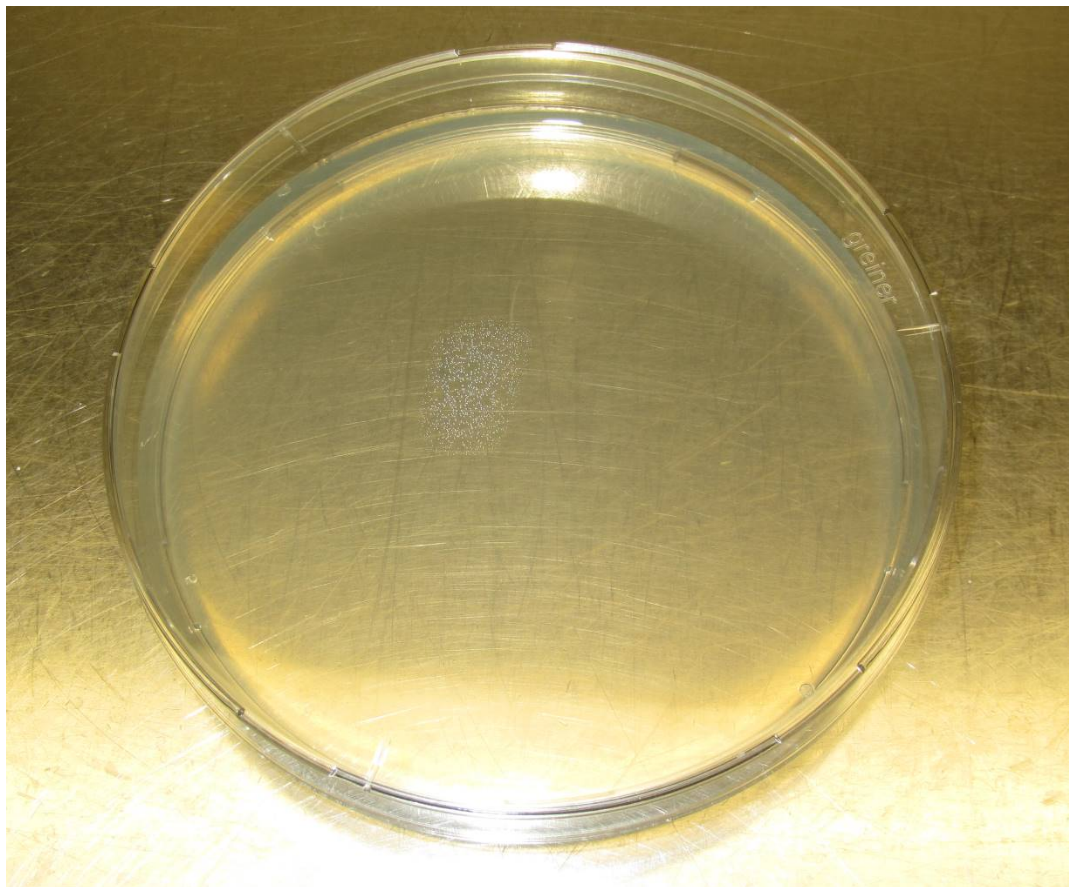
- In the case of **pour-plate methods**, 1 ml of the sample to be analysed is taken with a pipette and placed in the sterile, empty petri dish (by derogation, in the case of milk and milk products or other turbid samples, not more than 0,1 ml of the sample is placed in the petri dish to avoid excessive clouding of the culture medium and to allow visual evaluation of bacterial growth). The petri dish is then filled with approximately 12-15 ml of culture medium.

  Note: The temperature of the nutrient medium must not exceed 50 °C during pouring; at temperatures above 50 °C a temperature-related, germ reducing effect could falsify the test result

  After the nutrient medium has been added, the petri dish is immediately reclosed and the sample and the nutrient medium are mixed together homogeneously by carefully pivoting the petri dish on the support several times.

  Note: Care must be taken to ensure that the culture medium does not wet the rim and lid of the dish.



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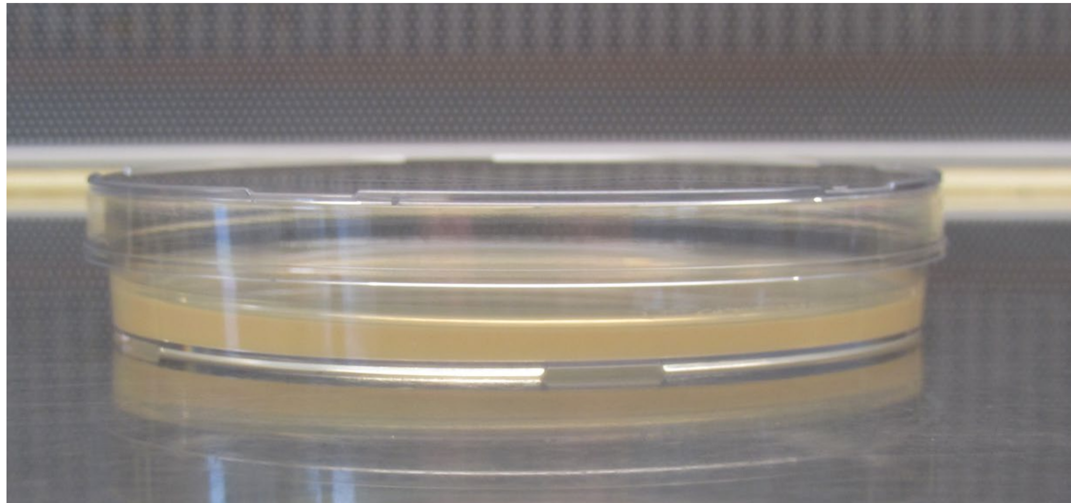

Fig. 3: Correct pour-plate

Source: VDMA Food Processing Machinery and Packaging Machinery

After the nutrient medium has solidified, the pour-plates are incubated overhead in the incubator according to the specifications and evaluated after the specified incubation period.

In the case of quantitative evaluations, counting is only possible if the culture medium is not too dense with colonies. Generally, pour-plates are counted if the colony count (CFU) is between 20 and 300 colonies per plate.

5 Notes on evaluation

5.1 Assessment of completely overgrown smears

- A completely overgrown smear (mono- or mixed flora) is a sure indication of a truly positive result.
- Single colonies of a different colony morphology type next to the smear or distributed over the plate are a strong indication of secondary contamination (comparison with sedimentation plates, species identification may provide further information on the cause of secondary contamination).

5.2 Assessment of sparsely vegetated smears

- Sparsely overgrown smears (few colonies) with no other colonies outside the smear:
    - Indication of inadequate pre-incubation or very slow/low growth of the contaminant (re-inoculation test to test the growth rate is advisable) or
    - Indication of too high selectivity of the nutrient medium (e.g. low growth of product-damaging, acidophilic microorganisms when using agar plates with neutral pH) (adaptation of the nutrient medium is advisable).
    - If necessary, also consider anaerobic incubation (e.g. microaerophilic micro-acid bacteria).
- Few colonies also outside the sparsely vegetated smear:
    - Test whether they are the same or different colonies (species): Same colonies indicate the same source, different colonies indicate different sources (comparison with sedimentation plates).
    - Microbiological contamination pressure from the environment (e.g. heavily contaminated outside of the package) must be taken into account and if possible eliminated.
    - Adjust the incubation time and temperature to the germ spectrum to be tested.

5.3 Notes on the evaluation of results and limitation of causes of contamination

Commercially sterile products may contain germs that cannot multiply in the product under normal, non-refrigerated storage and distribution conditions. For example, commercially sterile beverages with a low pH value may contain germs that remain inactive in the anaerobic environment and with inhibitors contained in the product (e.g. hops or citrus oils) and do not cause any organoleptic changes to the product. However, these germs can grow on nutrient media and lead to false positive laboratory results, as nutrient media have a pH > 4.2 (below which the agar no longer becomes solid), do not contain inhibitors and are usually incubated aerobically.

In order to exclude such false positives, it is advisable to deliberately contaminate a sterile sample of the product by inoculating the colonies back from the culture medium. If, under the specified incubation conditions, growth appears in the product (turbidity, gas formation, sensory changes, coagulation, recognizable growth due to other changes), it can be assumed that the positive result was due to contamination of the product with the harmful germ and that the result is indeed positive.

Literature:

PIC/S Validation of Aseptic Processes PI007-6, 2011
Downloadable from www.picscheme.org

FDA (2014) Pharmaceutical Microbiology Manual
Downloadable from: https://www.fda.gov/downloads/

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Appendix I (informative)
Examples Causes of false positive findings

Fig. 4: Condensate in the lid
Source: VDMA Food Processing Machinery and Packaging Machinery

Fig. 5: Secondary contamination caused by condensate
Source: VDMA Food Processing Machinery and Packaging Machinery

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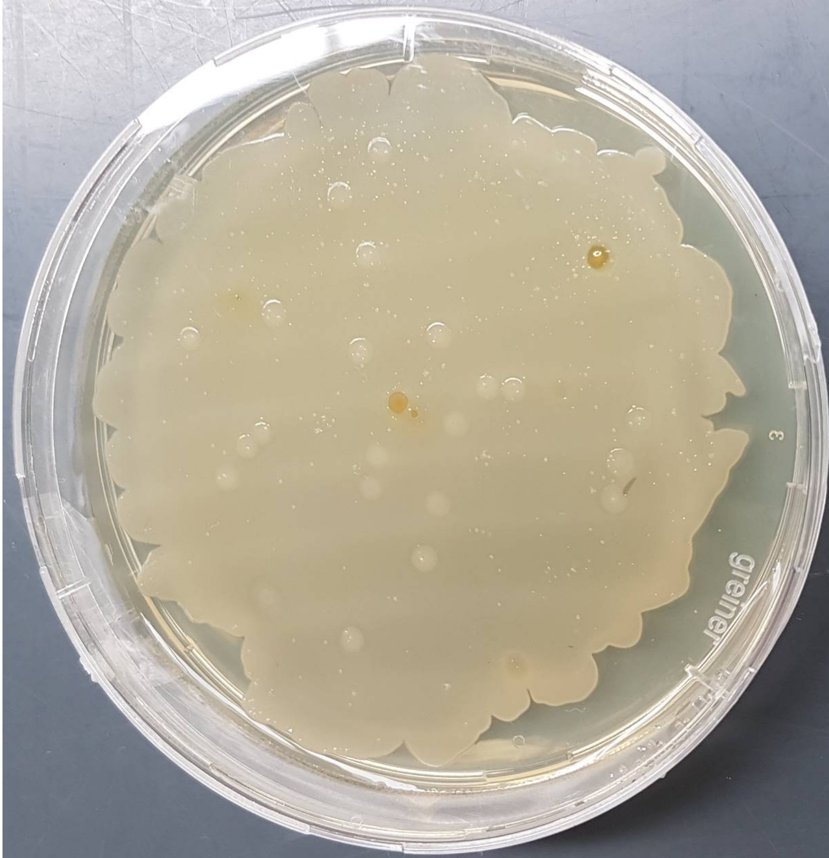

Fig. 6: Secondary contamination due to moisture; plate cannot be evaluated

Source: VDMA Food Processing Machinery and Packaging Machinery

Fig. 7: Growth at the edge of the filter in membrane filtration: use of reusable material with insufficient sterilisation

Source: VDMA Food Processing Machinery and Packaging Machinery

Example pictures of pour-plates

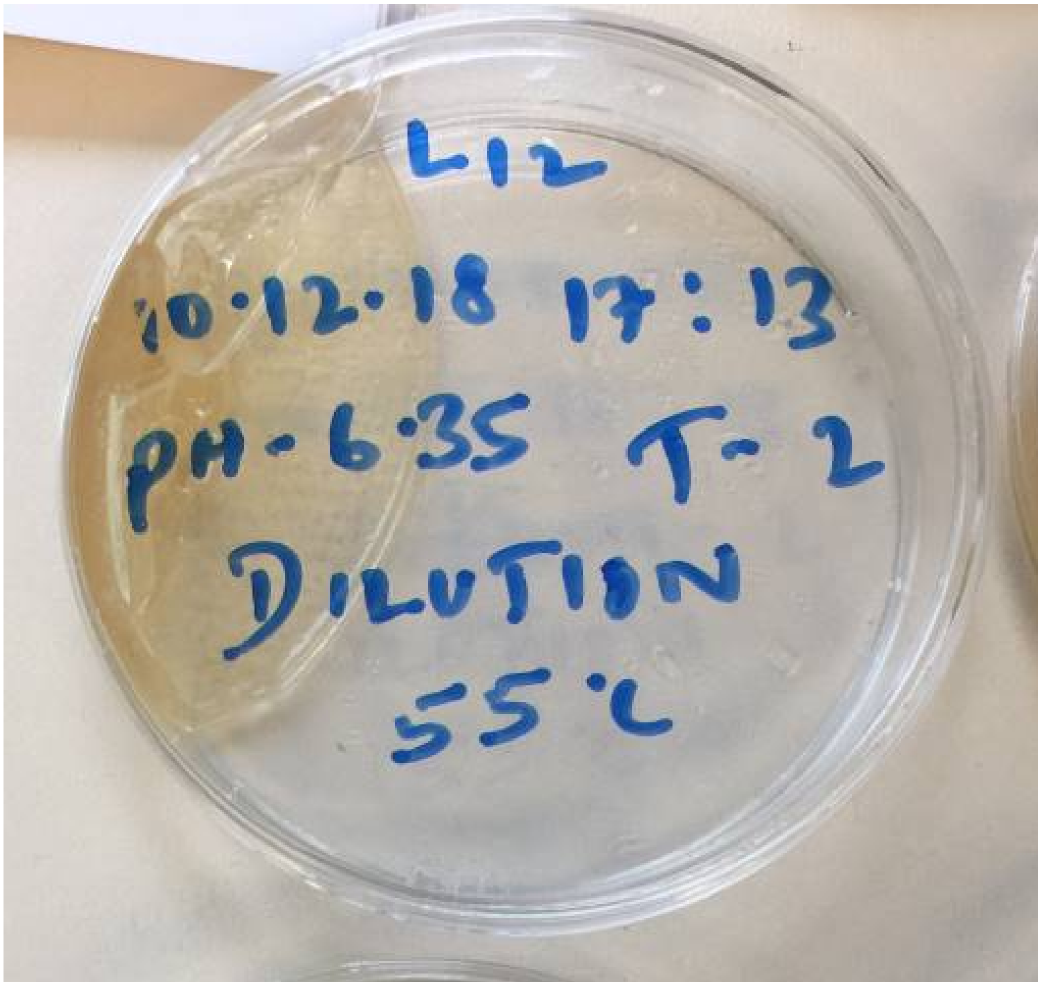

Abb. 8: Culture medium dissolved from plate; not evaluable
Source: VDMA Food Processing Machinery and Packaging Machinery

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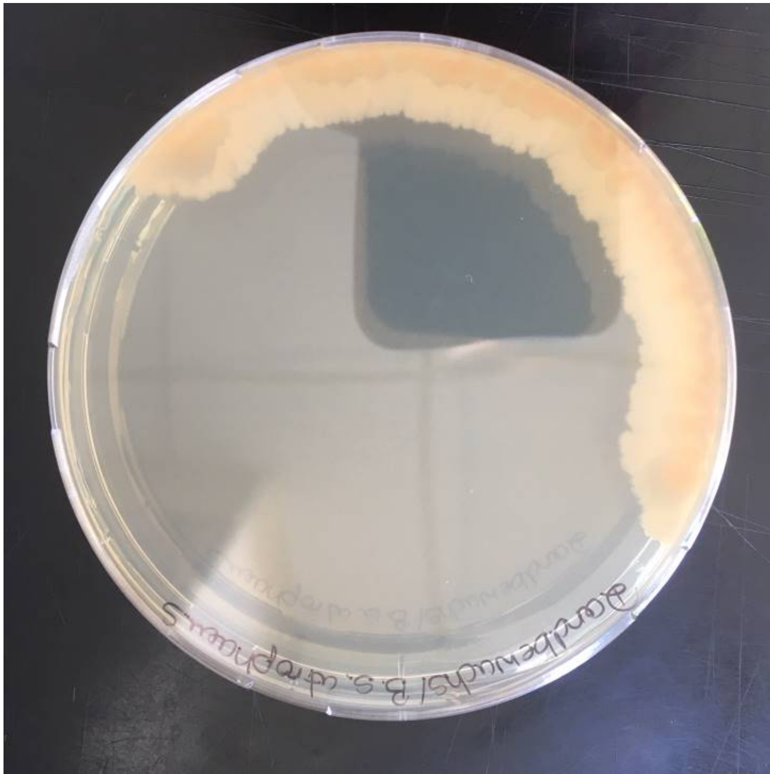

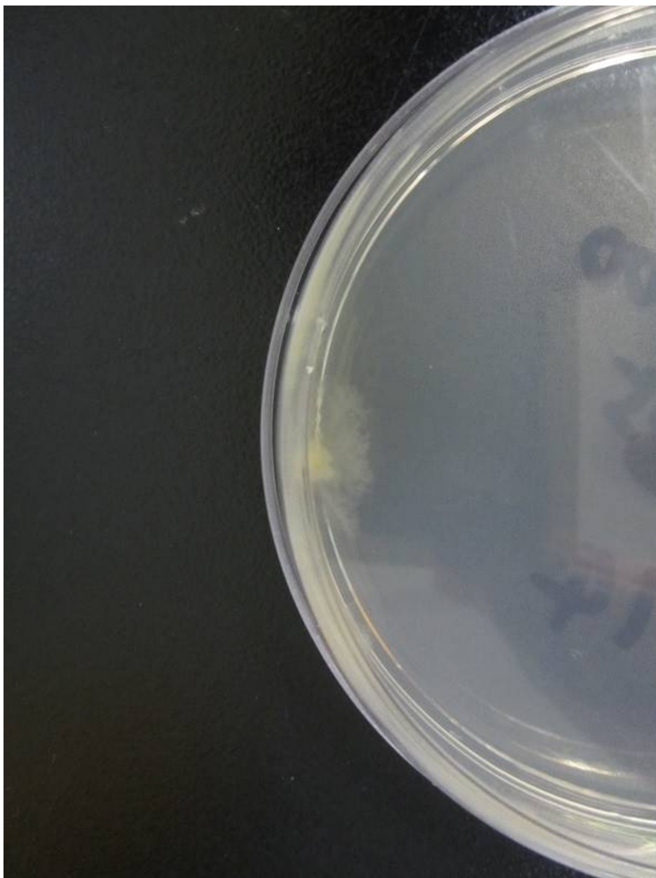

Fig. 9: Edge growth on pour plates
Source: VDMA Food Processing Machinery and Packaging Machinery

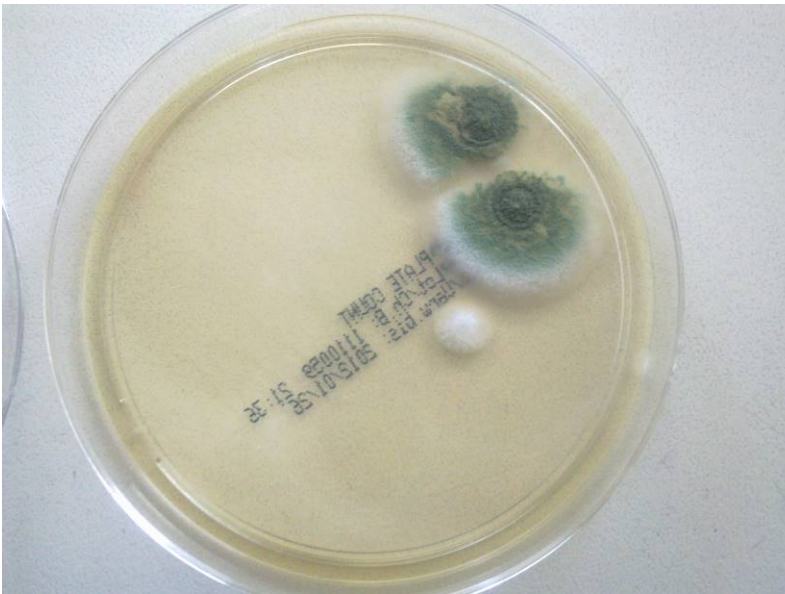

Fig. 10: Air contamination on pour plates
Source: VDMA Food Processing Machinery and Packaging Machinery

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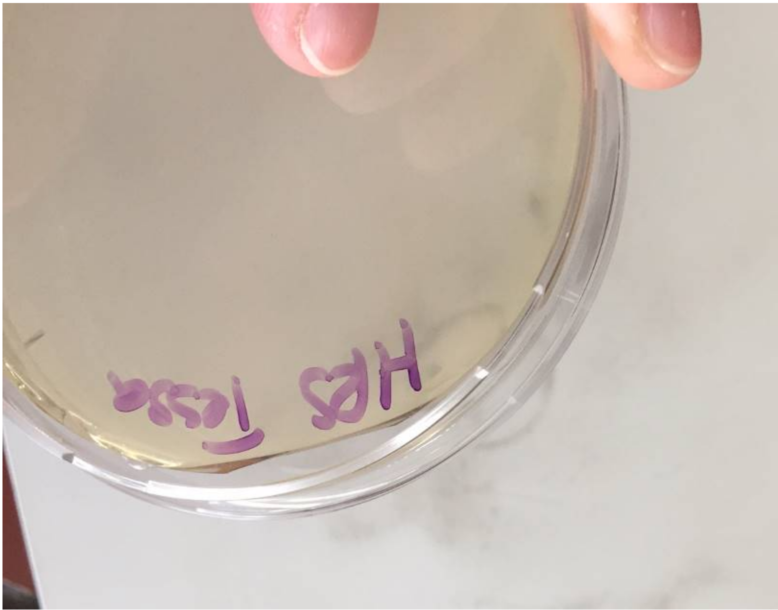

Fig. 11: Contamination with HRS (Pin-Point Colonies, difficult to detect)
HRS: Heat resistent sporeformers
Source: VDMA Food Processing Machinery and Packaging Machinery

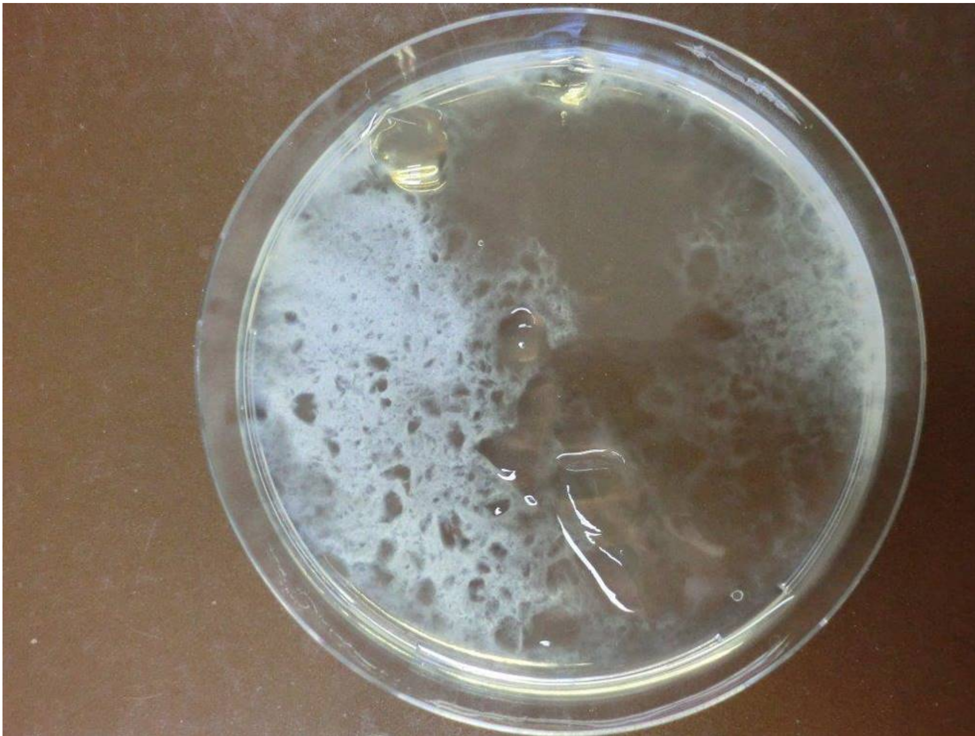

Fig. 12: Agar lumps during pouring, inhomogeneous distribution, visual evaluation difficult
Source: VDMA Food Processing Machinery and Packaging Machinery