Pour Plate Method: Best Practices

Best Practices for Pour Plate Method

- According to a consensus of views, it is recommended to keep the molten agar in the water bath for no more than 3 to 4 hours; do not pour it until it has cooled to <50°C (preferably 44°C to 46°C).
- The agar should not be melted more than one time.
- If it is necessary to dilute the suspension, use phosphate buffer pH 7.2.
- To decrease the risk of contamination, pour plates in a laminar-airflow cabinet. To decrease the risk of contamination when pouring multiple plates, flame the mouth of the flask before moving on to the next plate.
- The FDA BAM recommends filling plates with 12 ml to 15 ml of agar. The USP recommends a fill of 15 ml to 20 ml of agar.
- Some microorganism species may recover better on spread plates than pour plates. If this is the case, it is recommended to verify counts with a spread plate.
- Incubate most bacterial species for 48 to 72 hours. Note: Incubate *Candida albicans* and *Aspergillus brasiliensis* for 3 to 5 days.
- Some microorganism colonies, such as *Pseudomonas aeruginosa*, are very small. Count the colonies with the aid of an illuminated colony counter or magnifying glass.
- If determining the number of CFU per ml use the formula below.

$$\text{Number of CFU per ml} = \text{\# of Colonies} \times \text{Dilution factor} \times \frac{1}{\text{Aliquot}}$$

- Recovery will be lower on selective agar. If selective agar is used, test non-selective agar in parallel using the same microorganism suspension. A higher CFU concentration for the selective agar may be necessary.
- The value obtained when performing tests may differ from the mean assay value. Note: Microbiologics uses non-selective Tryptic Soy Agar when testing most microorganisms.

References

International Standard for Organization. 2009-02-01. Second Edition: Microbiology of food and animal feeding stuffs – Guidelines on preparations and production of culture media. Part 1

Maturin, L. and Peeler, J. T. 2001. FDA Bacteriological Analytical Manual. Chapter 3, Aerobic Plate Count.

Standard Methods for the Examination of Dairy Products. 2004. 17th Edition

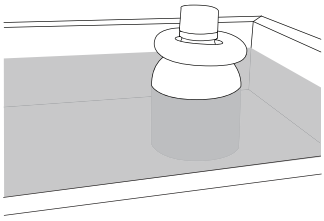
Standard Methods for the Examination of Water and Wastewater. 2005. 9020, Quality Assurance/Quality Control.

United States Pharmacopeia. 2013. <61>, Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests.

ILLUSTRATED INSTRUCTIONS

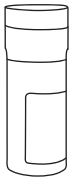
Instructions for performing the Pour Plate Method

1



Place your molten agar in a circulating water bath set at <50°C, preferably 44°C to 46°C.

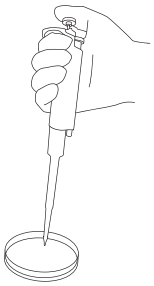
2



Prepare microorganism suspension according to protocol.

3

Pipette inoculum onto the bottom of the petri dish. Test each suspension in duplicate.



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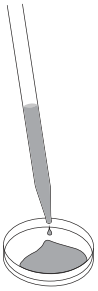
In accordance with your standards, pour 12-20 ml of molten agar over the inoculum.



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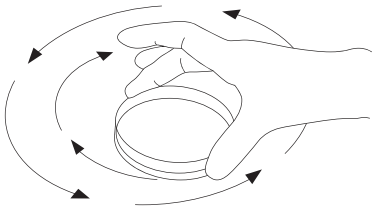
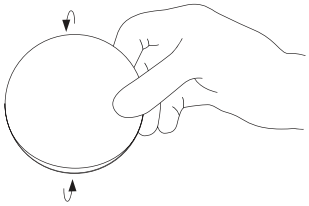


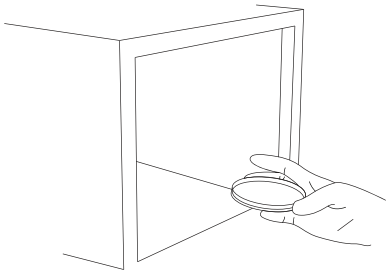
Thoroughly mix the organisms throughout the agar by gently swirling the petri dish by alternate rotation.

6



After the agar has hardened, invert the plates.

7



Incubate.

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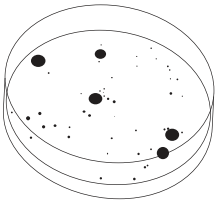

Count the colonies. Colonies under the agar surface will be small.