

**DETERMINATION OF TOTAL AEROBIC BACTERIA, FUNGI AND
YEAST COUNTS, IN SOLUBLE OIL EMULSIONS**

Application

The methods are used to determine the aerobic bacteria, fungi and yeast counts in used soluble oil emulsions.

Apparatus and Materials Required

Method A - Precise Method - (Total Aerobic Bacteria Count only)

Incubator

Capable of maintaining a temperature of 37 +/- 1 °C.

Autoclave or Pressure Cooker

Capable of achieving a pressure of 100 kN/m² at 121 °C.

Test Tubes

150 mm long x 12.5 mm diameter - lipless - sterile.

Dropping Pipettes

Graduated and sterile. Capacity 1 mL and 10 mL. Preferably glass throw away type.

Petri Dishes

Sterile (preferably plastic - throw away type).

Nutrient Agar

Prepared by dissolving 28 g of nutrient agar in 1 liter of distilled water.

Source: Oxiod Ltd.

Sterile Water

Dettol

2 % solution

Aluminum Foil

Date	Action	Revisions
2000 08 30	Revised	Editorial – no technical change A. Cockman
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Cotton Wool or Aluminum Caps

For test tube seals.

Formaldehyde

40 % aqueous solution.

Bunsen Burner

Laboratory Oven

Capable of maintaining a temperature of 180 +/- 2 °C.

Rotary Test Tube Mixer

Steam Bath or Incubator

Conditioning and Test Conditions

All test values indicated herein are based on material conditioned in a controlled atmosphere of 23 +/- 2 °C and 50 +/- 5 % relative humidity for not less than 24 h prior to testing and tested under the same conditions unless otherwise specified.

Procedure

A. Sterilization of Apparatus

Note: All apparatus must be sterilized before use and used only once during the determination. After use and before re-sterilization, soak the apparatus in 2 % dettol to prevent laboratory contamination.

1. Sterilize test tubes and pipettes by wrapping in the aluminum foil and place in the oven for 1 h at 180 +/- 2 °C. Start oven off from cold.
2. After sterilization, the apparatus should be kept in the aluminum foil until required for use. A small plug of cotton wool must be placed in the mouth piece prior to sterilization to prevent oral contamination.
3. Sterilize the nutrient agar by placing in a loosely topped bottle and place in the autoclave or pressure cooker at 100 kN/m² at 121 °C for 15 minutes.
4. If glass Petri dishes are used, sterilize by placing in the oven at 180 °C for 1 hour and then store in sealed containers. It is advisable to use sterile plastic dishes as they can be discarded after each determination.