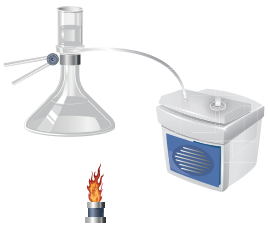
MICROBIOLOGICAL PROTOCOL

Quick microbiological protocol for filtration analysis on gelose medium.

Equipment preparation



- Clean and sterilise with alcohol the working table (preferably made of glass).
- Clean and sterilise filtration equipment with alcohol.
- Homogenise the sample by turning it upside down.
- Identify Petri plates (germ/batch/reading date…)
- Prepare the side equipment (filtration membranes, pliers, sterile disposable pipettes...).

Filtration

In order to have a good idea of the population, we suggest to make 2 routine filtrations, one with 100 µL of wine, the other with 10 mL. Make your experiments close to the Busen burner (15-20 cm away from the burner).

100 µL

First pour distilled water then the 100 µL of wine in order to have a correct repartition of the wine sample on the whole membrane surface.

Necessary equipment:

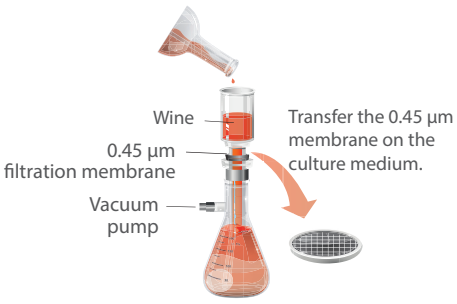
- Sterile membranes: diameter 47 mm and porosity 0.45 µm.
- Sterile distilled water.
- 1 mL sterile pipettes.

10 mL

Pour directly the volume of wine on the membrane.

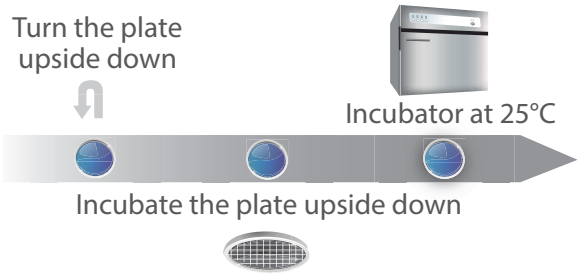
Necessary equipment:

- Sterile membranes: diameter 47 mm and porosity 0.45 µm.
- 10 mL sterile pipettes.



Incubation

Culture conditions must be adapted according the microorganisms we want to count.

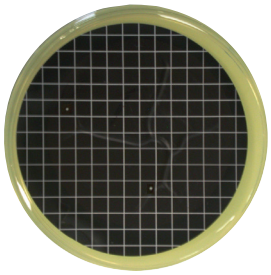


Selective culture medium	Incubation time at 25°C
MIL-LEV	2 days
MIL-LM	2-5 days
MIL-BRETT	7 days
MIL-BA	6 days
MIL-BT	12 days
MIL-BL	12 days under anaerobiosis condions*
MIL-FT	12 days

** Necessary equipment for anaerobiosis: Anaerobic jar + Anaerobiosis kit + indicators of anaerobiosis.*

Counting

Counting example on MIL-BRETT:

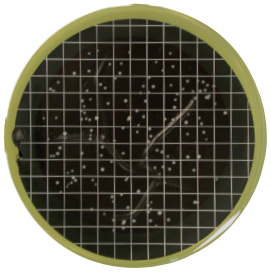


100 µL

Calculation detail:

2 UFC* in 100 µL = 20 CFU* in 1 mL

→ 2.10E1 CFU*/mL



10 mL

Calculation detail:

150 CFU* in 10 mL = 15 CFU* in 1 mL

→ 1.5.10E1 CFU*/mL

Final result:

Arithmetic average of both dilutions.

→ 1.75.10E1 CFU*/mL

** Colonies Forming Unit*

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