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L'EXPERTISE ANALYTIQUE

MICROBIOLOGICAL ANALYSIS

Recommendations for carrying-out microbiological analysis using culture mediums.

Recommendations for the choice of filtration materials

For the filtration we recommend using:

- Cellulose ester membrane filters of 47 mm diameter, with hydrophobic surfaces, gridded, 0.45µm pores, black colour.
- Filtration system + vacuum pump.

Suggestions for carrying out the filtration

1) Sterilise all of the filtration system with alcohol and by passing through a Bunsen burner flame.
2) Take the membrane filter out of its individual sterile packaging using tweezers which have been passed through the flame and cooled.
3) Place the membrane in the middle of the base of the filtration support, with the grid facing-up.
4) Fix the funnel onto the filter support using an appropriate clamp.
5) Pour a few milliliters of sterile water so that the membrane sticks well to the filter support, allow this to flow through entirely.
6) Homogenise the sample well and pour in the quantity (500 mL to 10 mL*) to be filtered. You must always filter a known volume of the sample.
7) Switch on the pump to carry out filtration.
8) After filtration, break the vacuum carefully in order to avoid reflux.
9) Take off the funnel and grab the membrane using tweezers (sterilised by the flame and cooled) and place the membrane on the culture medium, in the petri dish, grids facing upwards.
10) Avoid the formation of air pockets between the membrane and medium (this will inhibit a good contact with the medium thus allowing growth of other microorganisms).
11) Turn the dish upside-down (membrane facing downwards) and incubate during the prescribed time and under the necessary culture conditions depending on the micro-organism to be detected.

**if you wish to filter a smaller volume (< 10mL), add some sterile water or sterile peptone waterbeforehand.*

Recommendations for choosing the volume to filter and carrying out the dilutions

In order to be able to count the microorganisms, the number of colonies after culture should be between 20 and 200 UFC (colony forming units) per dish (for dishes of 55mm diameter).

Depending on the stage of development of the wine or matrix (fermenting or non-fermenting must, fermenting or non-fermenting wine, maturing wine or after bottling) the number of microorganisms varies and the volume of wine used must thus be adapted.

For matrices with a low microbial population, one may increase the volume filtered.

For matrices with a high microbial population, one or more dilutions are necessary.

The dilution factor depends on the estimated quantity of microorganisms in the sample.

The dilutions must be carried out in sterile conditions (using sterile pipettes and tubes as well as sterile distilled water or peptone water).

To carry out a dilution of a factor of 10, it is recommended to place 1 mL of the matrix to be analysed in 9 mL of sterile liquid (distilled water or peptone water). This 10 mL may then be filtered onto a membrane as indicated in part 2.

Culture conditions

The culture conditions must be adapted depending on the mircroorganisms you wish to count.

Yeasts Molds	Yeasts	Yeasts *Brettanomyces*	Acetic bacteria	Lactic bacteria	Total bacteria	Total flora
25°C (constant temperature = in incubator)						
2 - 5 days	2 days	7 days	6 days	12 days	12 days	12 days
Aerobic	Aerobic	Aerobic	Aerobic	Anaerobic*	Aerobic	Aerobic

** Use anaerobic kits.*

The dishes should be kept upside-down throughout incubation.

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