Determination of the aerobic colony count in food

1. Introduction

The determination of the germ count, the number of cultivable microorganisms in a product, is a useful addition to the research into the overall condition of that product. A germ count may say something about the freshness of a certain food. The total germ count however, says little or nothing about the product's possible harmfulness to health. The aerobic colony count of a product is nothing more than the number of cultivable bacteria per gram of solid food. The number of microorganisms food contains can be a measure of the hygienic condition, especially when it contains so called indicator organisms (like *Enterococci and/or* faecal *E. coli*).

To get an accurate picture of the number of microorganisms in food, an extraction of the food sample is made in sterile liquid. This extraction then will be decimally diluted (see figure), dispensed in sterile petri dishes and mixed with sterilized culture medium. This culture medium consists of agar (a jelly-like substance obtained from algae) as a binding agent and nutrients to support the growth of micro-organisms. When incubated at 37°C for 48 hours the now visible bacterial colonies can be counted.

2. Materials

- Cooled / uncooled minced meat
- Weighing scale and paper
- Spatula
- Disinfectant wipes
- Micropipette
- 1 box of sterile pipette tips
- Bunsen burner and matches
- Permanent marker
- 1 Erlenmeyer flask which contains 50 ml of sterile diluent*
- 5 Test-tubes which contain 4,5 ml sterile diluent* each
- 6 Sterile petri-dishes (empty)
- 1 Erlenmeyer flask containing sterilized PCA (Plate Count Agar)

* diluent used = 0,9% NaCl

3. Execution and method

<u>Sample extraction</u>

- Put a weighing paper on the scale and press 'Tare'
- Take a disinfectant wipe and clean the spatula
- Weigh out 5 grams of minced meat on the weighing paper
- Open the Erlenmeyer flask with diluent and transfer the meat into the flask, close the flask again (To prevent contamination: make sure to hold the cap and put it back on the flask as soon as possible)
- Mix well and put the mixture on a rocking platform for 10 minutes.
- Meanwhile: leaving the petri dishes closed, turn upside down and mark the <u>bottom</u> (smallest part) with your name, date, sample type and dilution. Turn back around the petri dishes.
- Also mark the test-tubes with the dilutions to make (see attachment)

- Take the Erlenmeyer flask from the rocking platform and leave to stand for a few minutes

Dilution series

- Pull and turn the yellow gas valve on the column above your desk and, with a stretched arm, carefully light the Bunsen burner. Turn the collar (see figure 1) to create a blue, roaring flame.
- To keep sterile materials and liquids free from contamination, the following steps are carried out around the Bunsen burner
- Make sure the micropipette is set at 500 µl
- Open the box with pipette tips, put one of the tips on the micropipette and close the box again
- Open the Erlenmeyer flask, take out 500 µl of your sample extraction and close the flask again.

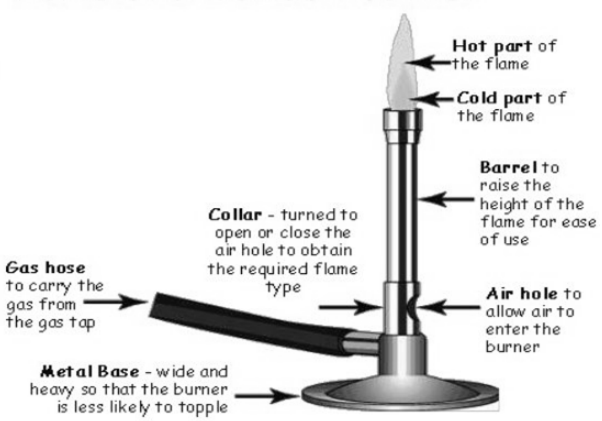


Figure 1: parts of a Bunsen burner

- Get the first test tube ($x10^{-1}$), take off the cap and move the opening of the tube though the flame about three times. Pipette the 500 µl of your sample in this test tube, move the opening through the flame again and close.
- Get a new pipette tip, open the first test tube again, move through the flame and take out 500µl of diluted sample. Get the second test tube and repeat previous steps until you'll reach the final concentration of $x10^{-5}$ (see attachment)

Dispensing sample dilutions and pouring agar

- Still working close to the Bunsen burner: take 1000 µl of the sample extraction from the Erlenmeyer flask in the same sterile way as described above.
- Open up the first petri dish ($x10^{-0}$) a little bit and quickly add the extraction to the bottom of the dish. Close the lid.
- Repeat the steps above for each dilution and corresponding petri dishes.
- Take an Erlenmeyer flask with Plate Count Agar (which is cooled to 56 °C) from the water bath, and make sure to mix the agar before pouring
- Pour around about 25 ml (enough to cover the surface of the bottom of the petri dish, there is no need to fill it) of sterile Plate Count Agar in the petri dish. Don't forget to move the opening of the flask through the flame before pouring
- Carefully mix (watch out for agar against the lid!).
- Let the agar plates solidify.

Incubate at 37°C for 48 hours

4. Assessing the plates

What plates are counted?

After incubation the number of colonies per plate is counted.
The 'ideal' plate contains between 10 and 200 colonies. Statistically fewer than 10 is less favorable. In over 200 colonies it is very likely that one colony was formed from more than one bacteria cell ('overcrowding').
If an ideal plate is not present then we'll have to use the means available. In that case count the plates with just a few colonies! It gets more difficult if we only have plates with many colonies available. If there are a lot then just a quarter of the plate may be counted. Should the plate be packed (over a thousand colonies) then per plate just count twice the number of colonies on 1 cm^2. The average of this on a standard petri dish (diameter 9 cm) is multiplied by factor 100 and the dilution factor and the outcome of this is the germ count. Always mention the fact that it concerns an overgrown plate. Still this outcome is better than the mention "cannot be counted"!

Quantitative assessment

In the quantitative assessment you can count the colonies and calculate the germ count. Germ counts are expressed in numbers per gram (solid samples) or numbers per ml (liquid samples)

Colony Forming Units (CFUs)

The principle of the germ count method is that one microorganism forms one colony. This is a theoretical ideal. However, many observations may be made. For instance: the cells form clusters or strands, in that case a colony is formed from multiple cells. Do all the wanted microorganisms actually grow in these culture conditions? To prevent this discussion we don't speak of the number of living microorganisms, but instead of the number of colony forming units.

Calculating CFUs

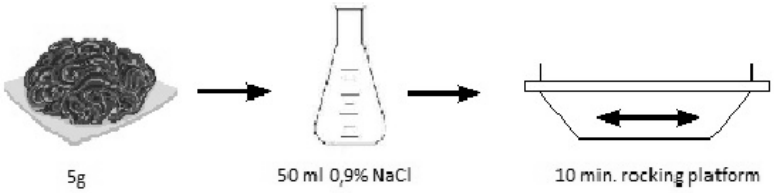
By counting the number of colonies in a dilution of the sample you know the colony count of that dilution. Multiply this number with the dilution made and you'll get the colony count for the original sample.
For example: in a $x10^{-3}$ dilution, 40 colonies have been counted. This means you'll have to multiply the counted colonies with the dilution factor (in this case 1000 times). That makes 40.000 CFUs

You can write down you results in the table below

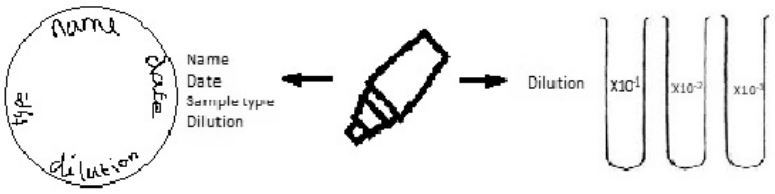
Plate / dilution	CFUs in dilution	CFUs in original sample
$x10^{-0}$		
$x10^{-1}$		
$x10^{-2}$		
$x10^{-3}$		
$x10^{-4}$		
$x10^{-5}$		

Attachment: determination of aerobic colony count in figures

Preparing sample extraction



meanwhile:



Dilution series, dispensing sample and pouring agar