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## Sample Preparation Introduction



Are you preparing samples  
for use in microbiological  
testing?

In the science of microbiology, the methods of sampling are probably the most neglected area. For most methods, sampling is most often described as follows:

Weigh 10g of sample into a sterile container or homogeniser bag and add 90ml of diluent. Homogenise.

Although amounts of sample and diluent may vary and blending/stomaching times may be set, there are usually no fixed rules about the exact nature of the sample taken.

In microbiological testing, emphasis is always on obtaining the 'end result', that is the plate-count obtained after sampling, stomaching and incubation.

Discrepancies in the taking of samples often only come to light when a new method is being validated, for example an impedance method, where the end plate-count result has a great effect on the calibration of an instrument.

Differences in how the operator obtained the sample, how the sample is processed, how long it was left to stand before plating and what sample of the homogenate goes into/onto the plate – all will affect the final result.

## Taking the Sample



If samples were being taken from a slab of meat and from minced meat, then the sampling techniques should be different, especially when looking for specific pathogens that may be present in low numbers.

For the whole piece of meat, it is appropriate to take samples on or near the surface, as this is where the highest level of contamination is likely to occur. For entire carcasses, it is a better idea to swab defined areas of the surface to achieve a microbial count. For the minced product, it is advisable to take a cross-section of the sample, as the microbial levels may be higher in the centre of the mass than on the surface.



As the popularity of ready-made meals grows, so does the challenge of taking a representative sample. It is always down to the operator's judgement as to what is taken as the sample, incorporating all elements of the meal. It is almost certain that no two samples taken incorporate exactly the same mix of ingredients.

## Adding the Diluent

If the diluent is ready-made into fixed aliquots, the dilution factor of the sample may vary. For most foodstuffs the difference between 9.5g and 10.5g may make little difference to the count. However, if low numbers are involved, a significant difference can be seen. With the advent of the gravimetric dilutor, dilution errors caused by inaccurate weighing of samples can be eliminated. The dilutor automatically adds the correct quantity of diluent to achieve the dilution required, depending upon the initial sample weight.

## Pipetting the sample

Taking the sample from the original homogenate is an area where large errors can potentially be introduced. Whilst one operator may immediately pipette the sample once blended into the Petri dish (including all debris, etc.), another may leave the homogenate to stand before processing the sample. The count is unlikely to be comparable in these cases although the original sample is the same. The levels of free bacteria in the supernatant may differ from those in the complete mixture. With all microbiological sampling issues, consistency is the key to getting a reproducible and understandable result. It may be better in these cases to always leave the sample to stand for a defined period and sample from the supernatant, than risk sampling problems and a blocked pipette when using a homogenate with a high particulate content. An excellent way of separating debris from the supernatant is by using filter bags for the initial homogenisation as they automatically separate the two as the homogenisation takes place, leaving the operator to simply draw off the supernatant. Consistency in sampling is especially important when evaluating a new method – the ‘same sample’ is only the same if it has been treated identically.



In order that a representative reproducible microbial result is achieved, the methods of sampling must be considered and standardised for any given sample.

As microbiologists we are asked to check the dates of manufacture and quality control of the media we use, the temperature of the incubators and refrigerators and the calibration of our balances and pipettes. If we can progress to the standardisation of sampling we are moving our discipline one more step from what other departments sometimes consider an art form, to a measurable science.

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