Enumeration is often employed to determine levels of indicator organisms - those groups of bacteria that may represent the potential presence of, for example, enteric pathogens such as Salmonella. Coliforms, Enterobacteriaceae and E. coli are all classic indicator bacteria groups. To analyse a foodstuff for these organisms, first we must obtain a representative sample - this is usually at the discretion of food manufacturer and **advice should be sought on how best to take samples**. Once at the laboratory, a portion is weighed out (usually 10g) and diluted with a sterile diluent, which should be neither harmful to bacteria nor encourage rapid growth in numbers before the test procedure is completed. **The food and diluent is then macerated** to release micro-organisms into the diluent for further diluting as appropriate.

After dilutions have been prepared, a portion of each dilution is ready to be plated into Petri dishes with agar. Plating commonly takes three formats: spread, pour and spiral. Sensitivities vary between differing plating techniques therefore it is important to ascertain from your **food testing laboratory** what is the minimum detection level for each **enumeration method** and determine if this is suitable for any test specifications you may have.

Agar is a gel to which all manner of nutrients and selective factors can be introduced. Selective factors may be in the form of antibiotics, to which the target organism is not sensitive, whereas other competing organisms are, or as in the case of bile salts when isolating Enterobacteriaceae, a compound found in the gut which favours the growth of the target organism over others. When ready, the prepared plates are transferred to an incubator where temperature can be precisely maintained. **In food microbiology, specific temperatures are employed** to recover the target organism or suppress the growth of non-target organisms. Length of incubation is dependent on growth rate so usually the higher the incubation temperature, the shorter the incubation period. For example 37°C plus temperatures usually require just 24 hours to provide sufficient bacterial growth for examination, whereas mould and yeasts usually require a five day incubation period to produce sufficient visible growth.

On completion of incubation, colonies are counted and after some simple calculations the result is expressed as the number of colonies per gram or millilitre is reported. The basis of **traditional microbiology enumeration** is to assume that in the dilutions plated, any microorganisms were present as individual cells, each of which grew in or on the agar. During the incubation process, these individuals divide to many millions but are unable to spread through the solid agar and so the mass of individual cells forms a single visible colony. However, bacteria are quite often present as small clumps of cells and even after thorough mixing and each clump will still only form one visible colony hence when reporting organism numbers, results are always expressed as “cfu” or colony forming units.

Enumeration methods are suitable for determining levels of spoilage organisms whether this be a total count or specific spoilage bacteria like Pseudomonas and as such, **shelf life determination**

heavily utilises enumeration techniques for relevant counting levels of bacteria and moulds and yeast in foods over the course of storage.