Microbiology Techniques

During the practical session we will demonstrate some basic bacteriology techniques and explain the importance of aseptic technique, which is essential in Microbiology. These techniques are used to determine, identify and quantify microorganisms that may be present in a clinical sample.

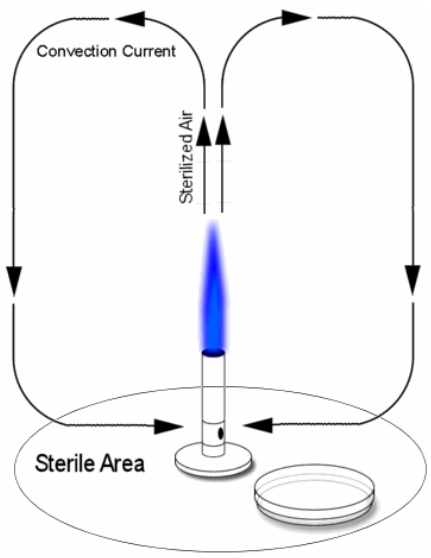
Aseptic Technique

A method of working in which you will be able to inoculate sterile media or transfer bacteria without risk of contamination from other micro-organisms in the environment or from yourself.

The working area must be cleaned before hand with a disinfectant such as 1% Chemgene.

Bunsen burner must be set onto blue flame
All required media and consumables must be placed nearby.

The Bunsen provides an area of sterility close by, this is the preferred working area.

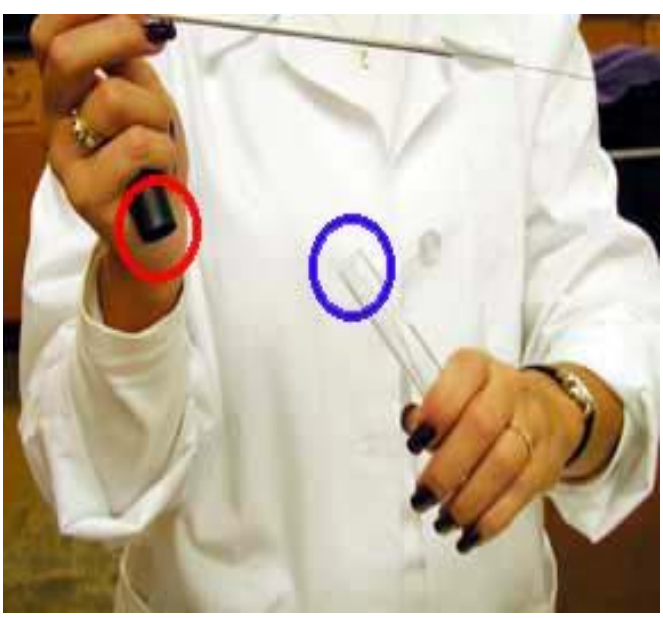


Removing bottle caps

While holding the loop, the cap is unscrewed from the sample bottle using the smallest finger and held there while the loop is dipped into the sample bottle.

The cap is placed back onto the bottle using the same hand that is holding the loop.

If the cap was placed onto the counter, this could cause contamination.



Using a biological safety cabinet:

A biological safety cabinet (BSC) is an engineering control intended to protect laboratory workers, the laboratory environment, and work materials from exposure to infectious or biohazardous aerosols and splashes. Such aerosols and splashes may be generated while manipulating materials containing infectious agents, such as primary cultures, stocks, and diagnostic specimens.

Class 2 safety cabinet

Class 2 containment lab (CL2):

Biological agents of **hazard group 2** may cause infectious diseases in humans. Agents requiring containment level 2 facilities are not usually transmitted by airborne routes. Care must be taken to avoid the generation of aerosols or splashes as these can settle onto bench tops and become an ingestion hazard through contamination of the hands.

Class 3 containment lab (CL3):

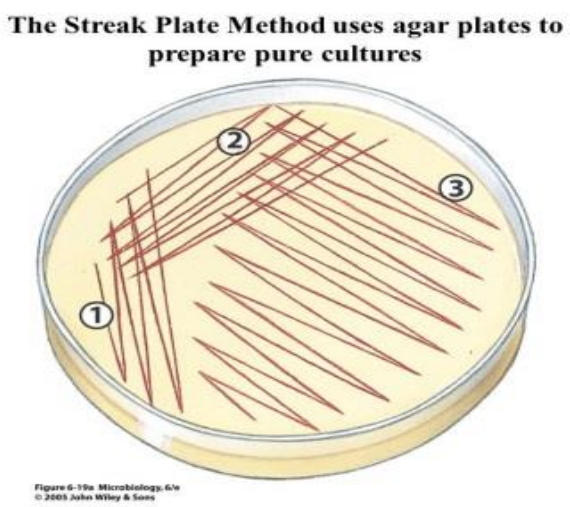
CL3 labs are **sealed, secure rooms** which form part of the containment measures. The labs operate under negative pressure. Prior to entering, a log book is used to record time of entry, worker name and room pressures. The doorway is locked requiring authorisation for entry. Indigenous or exotic microbes that can cause serious or potentially lethal disease through inhalation are handled in this type of facility. Some examples of microbes that would require handling at CL3 are Yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

Isolation of bacteria using the Streak Plate method

In Bacteriology, this is the standard method of determining if bacteria are present in a sample. When the sample is streaked onto a plate this separates out the bacteria into individual colonies for further research, such as Gram Staining to help with identification.

Exercise 1

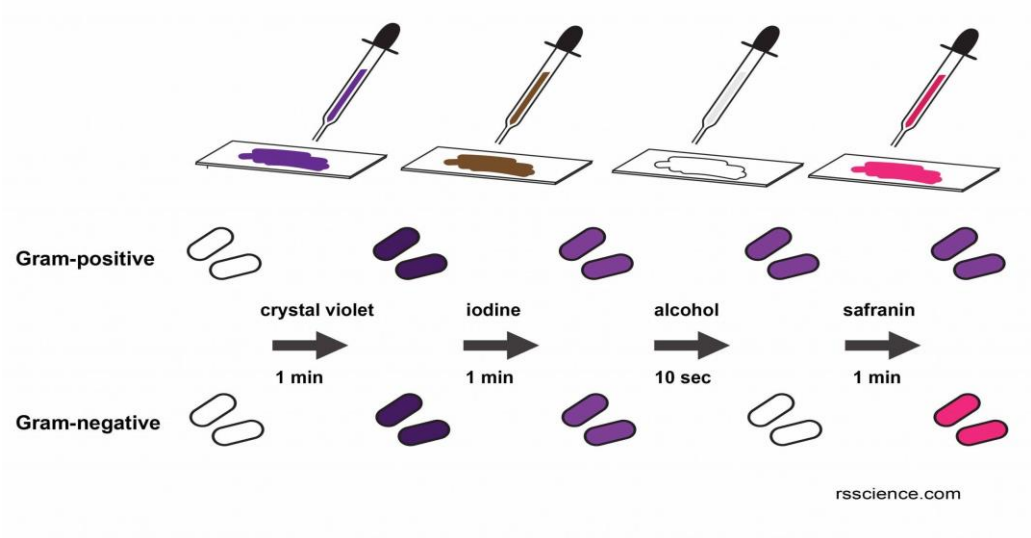
Practice preparing a streak plate using the coloured solution.



The agar plate is incubated at 37C for 24 – 48 hrs. Individual colonies can be selected (picked off with a sterile loop) for Gram stain / further identification / susceptibility testing.

What is Gram Staining and why is it so important?

Gram staining is an important diagnostic test used in Bacteriology.
It determines if bacteria are “Gram positive” or “Gram negative”
The bacteria will stain differently according to their cell wall structure.



Gram Stain Principle

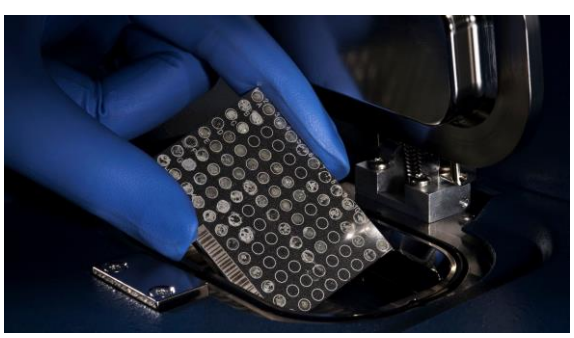
The Gram stain divides bacteria into two classes, gram-positive and gram-negative. The thick peptidoglycan layer of Gram-positive organisms allows these organisms to retain the crystal violet-iodine complex and stain purple. Gram-negative bacterial cell walls consist of a thinner layer of peptidoglycan and lose the crystal violet-iodine complex during the alcohol rinse. Gram-negative bacteria stain with safranin and appear red.

Most clinically important bacteria can be detected using the Gram stain. Exceptions are organisms that exist almost exclusively within host cells (e.g. chlamydia), those that lack a cell wall (e.g. mycoplasma and ureaplasma), and those too small to be resolved by light microscopy (e.g. spirochetes). Factors that may alter the true Gram reactivity of a bacteria include loss of cell wall integrity because of antibiotic treatment, age of the bacteria, action of autolytic enzymes, and as mentioned in Step 3 of Gram Stain Procedure, overheating the slide during heat fixation.

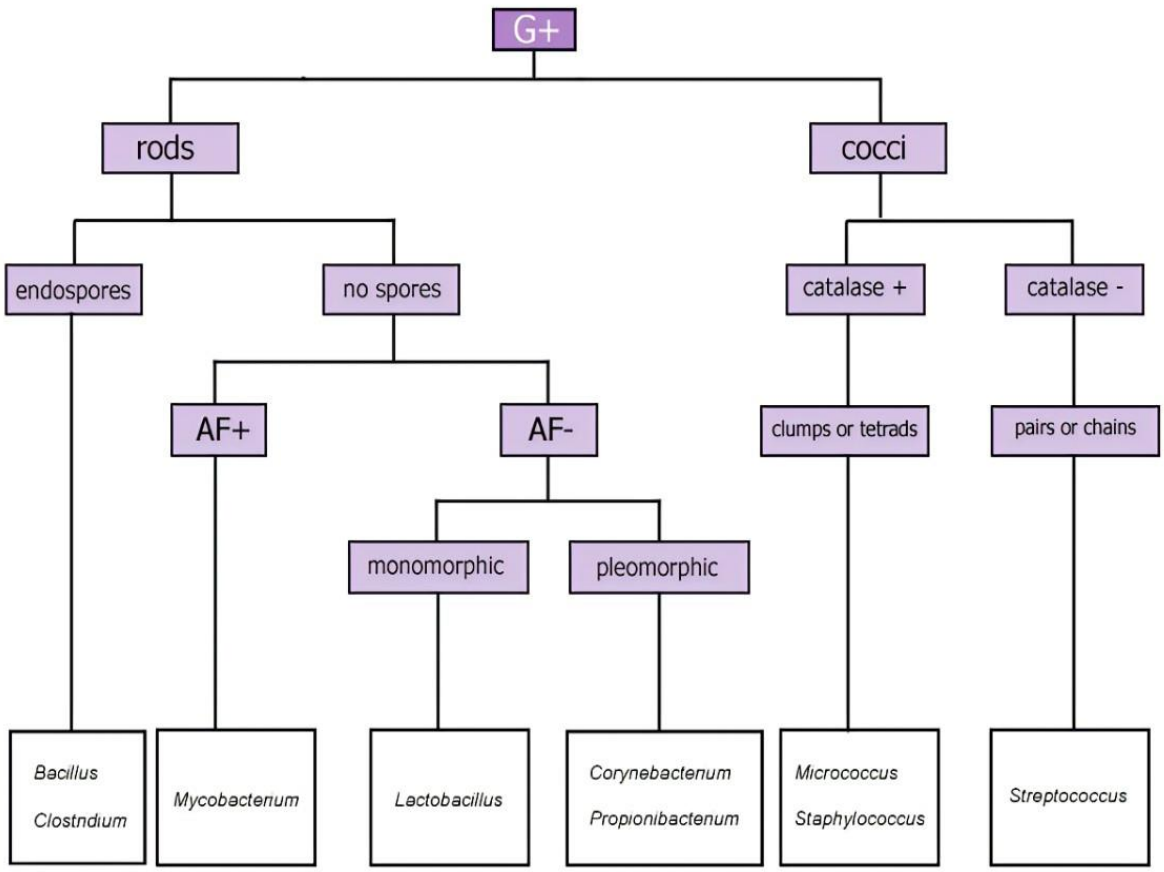
Other methods of identification:

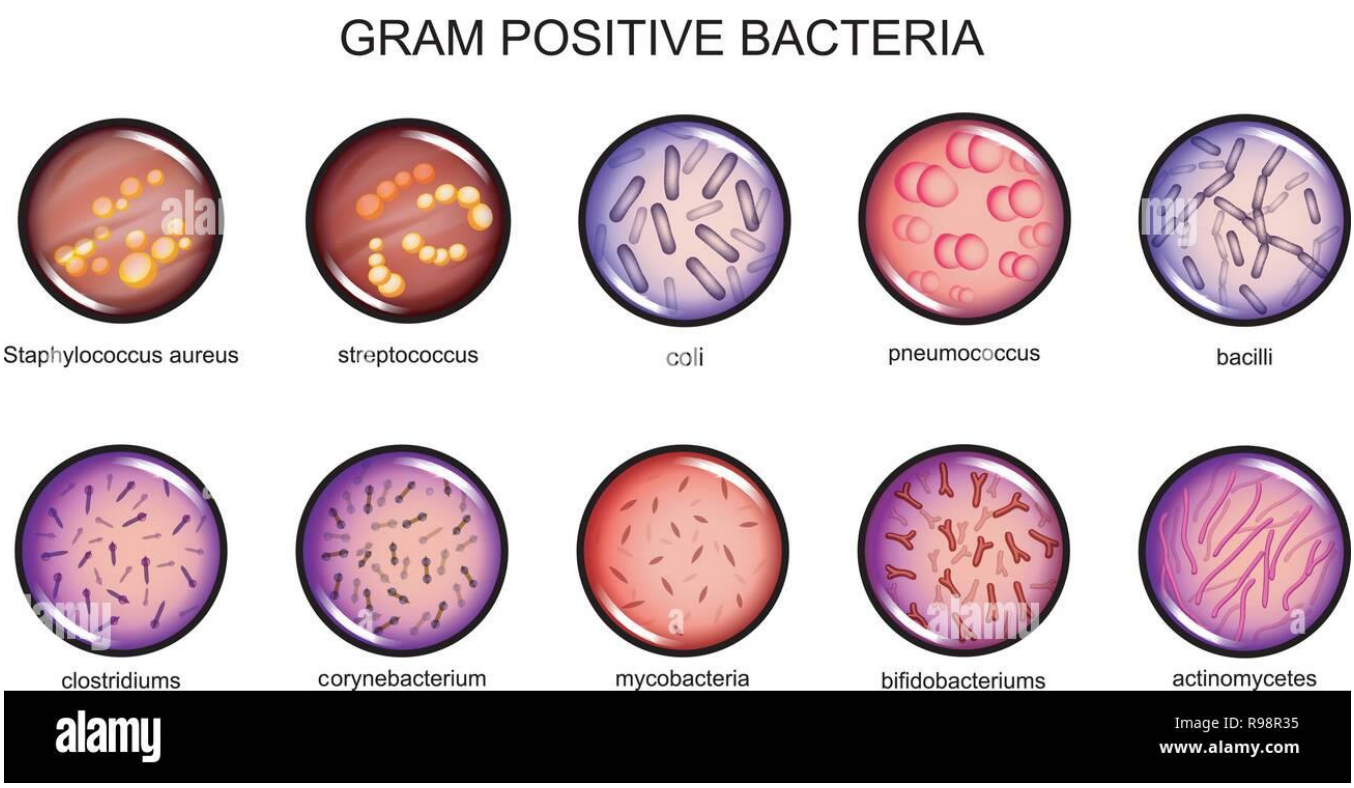
* PCR: quantitative and qualitative **(covered in other session)**

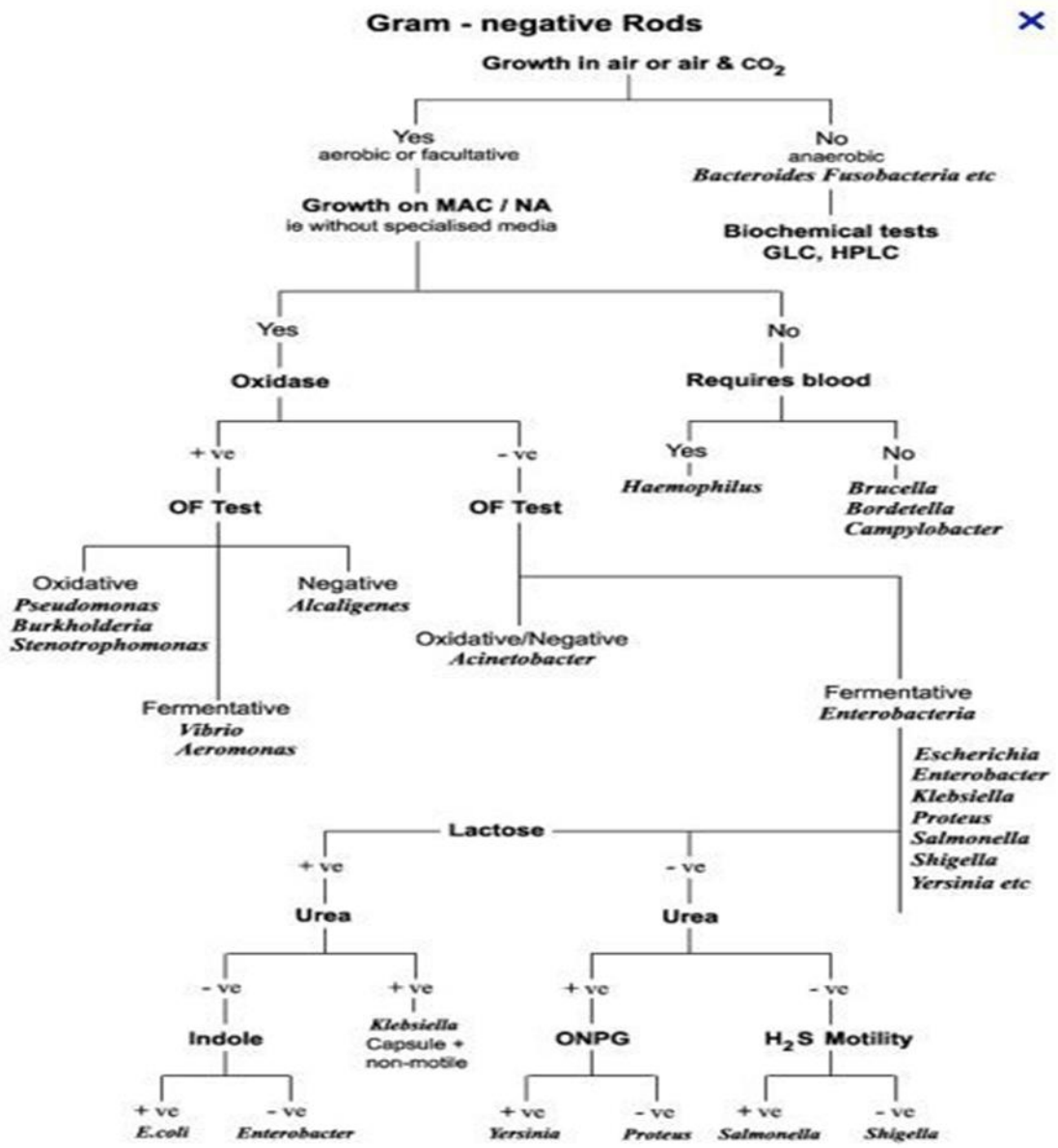
* MALDI-TOF: Stands for Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight. A spectrophotometer used in microbiology as a rapid, accurate and cost effective method for identifying microorganisms (bacteria, fungi and viruses).



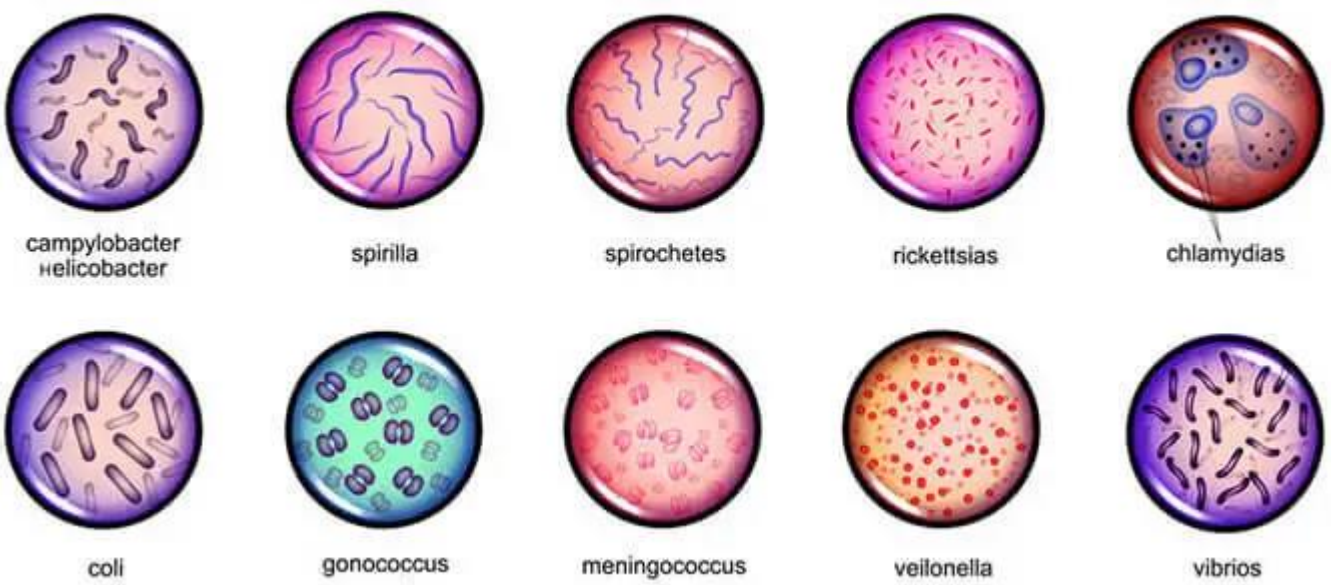
Exercise 2: Examine 2 slides of stained bacteria to determine which one is Gram negative and positive.







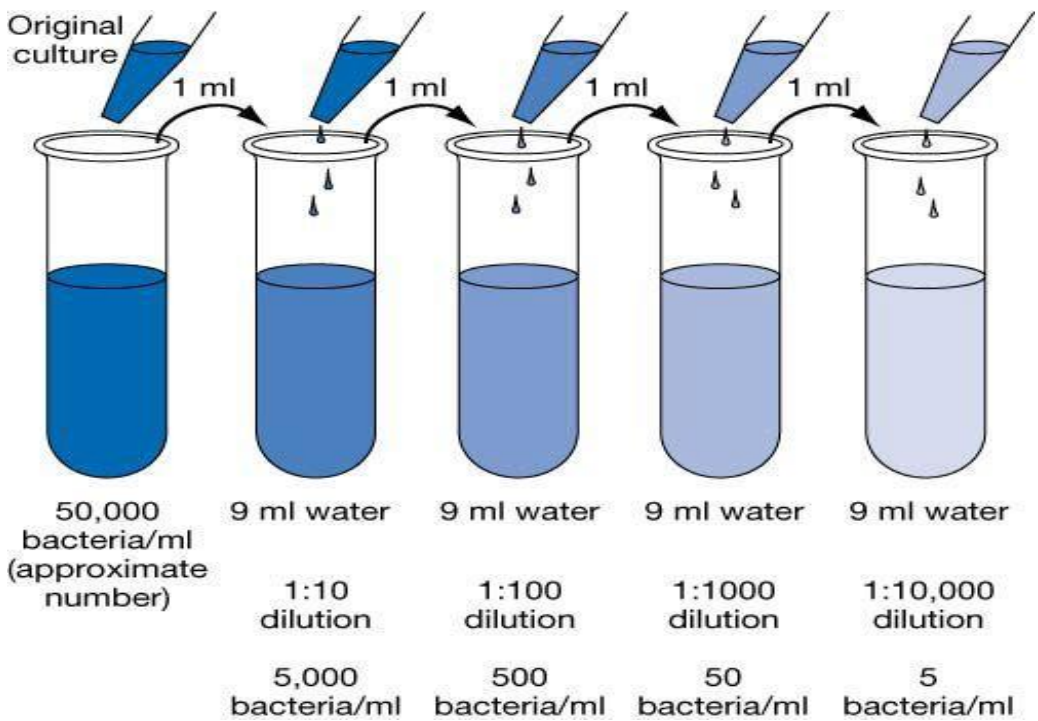
SOME EXAMPLES OF GRAM NEGATIVE BACTERIA

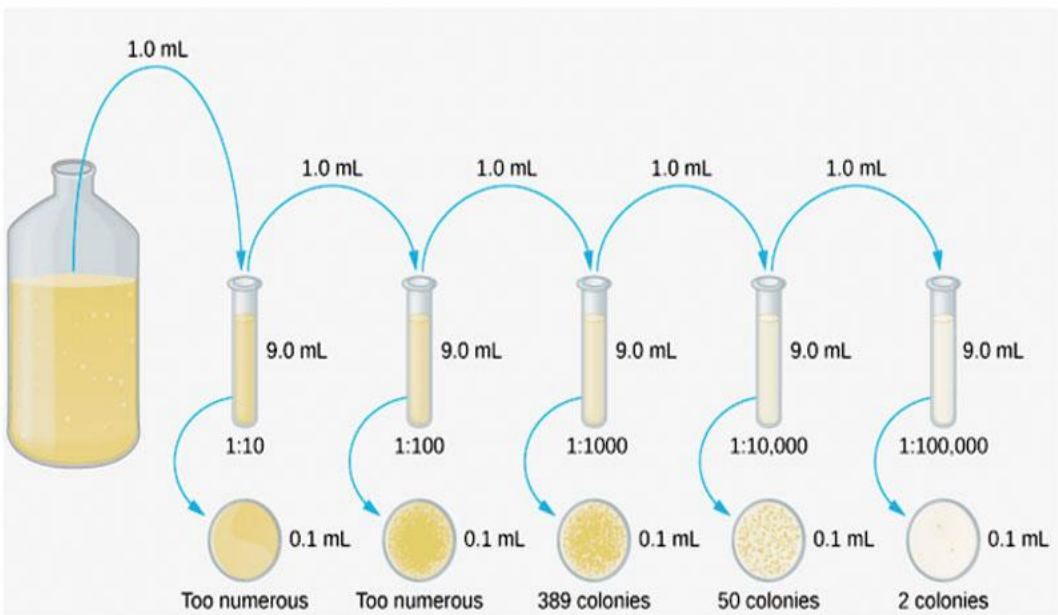


Serial dilution principle:

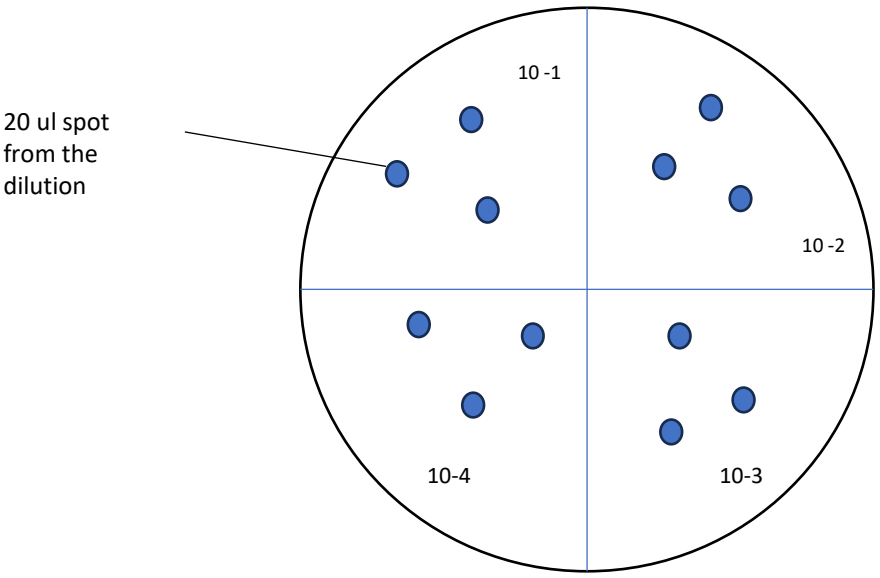
Serial dilutions are prepared to determine the number of bacteria in a sample.

The Miles and Misra technique can be used to count the bacteria and work out the concentration which can be expressed as a log number or a number to the power of 10.
This will be shown in Exercise 3.

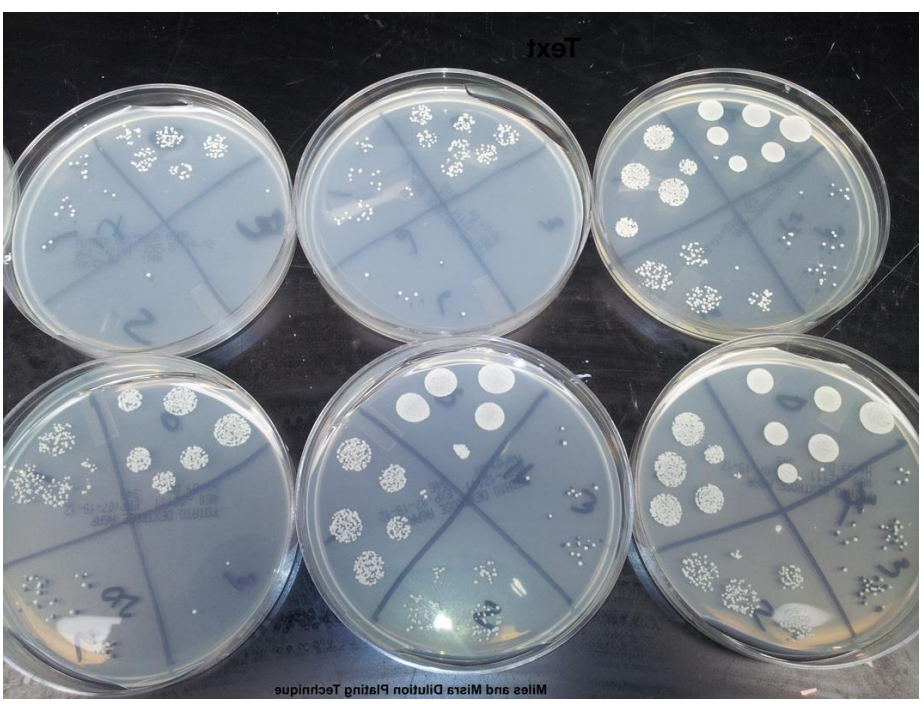




Miles and Misra surface drop technique for counting bacteria:
For this exercise, serial dilutions have already been prepared for you. (see previous sheet)
Pipette 20 ul from each of the dilution tubes labelled 10-1, 10-2, 10-3, 10-4 into each segment 3 times.
See diagram below.



The plates are allowed to dry and incubated at 37C for 24 – 48 hours.

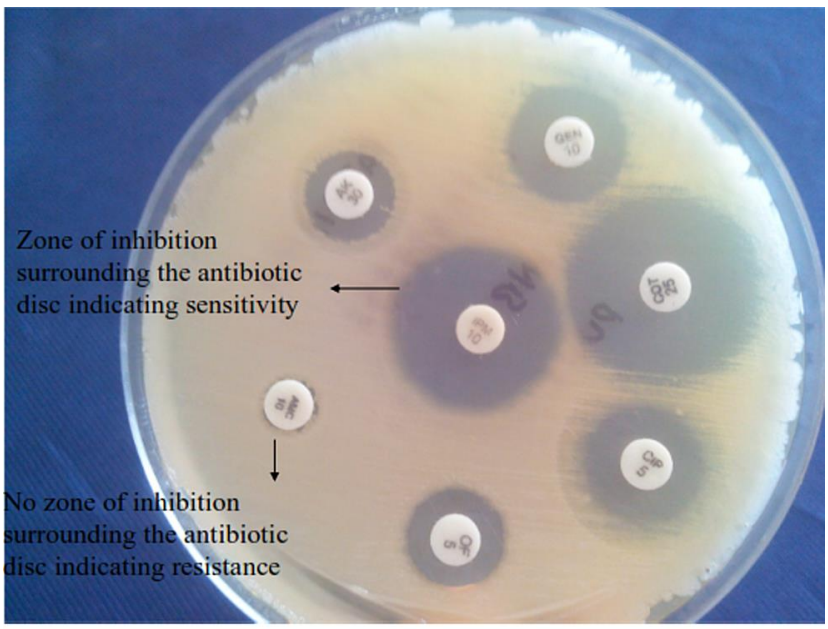


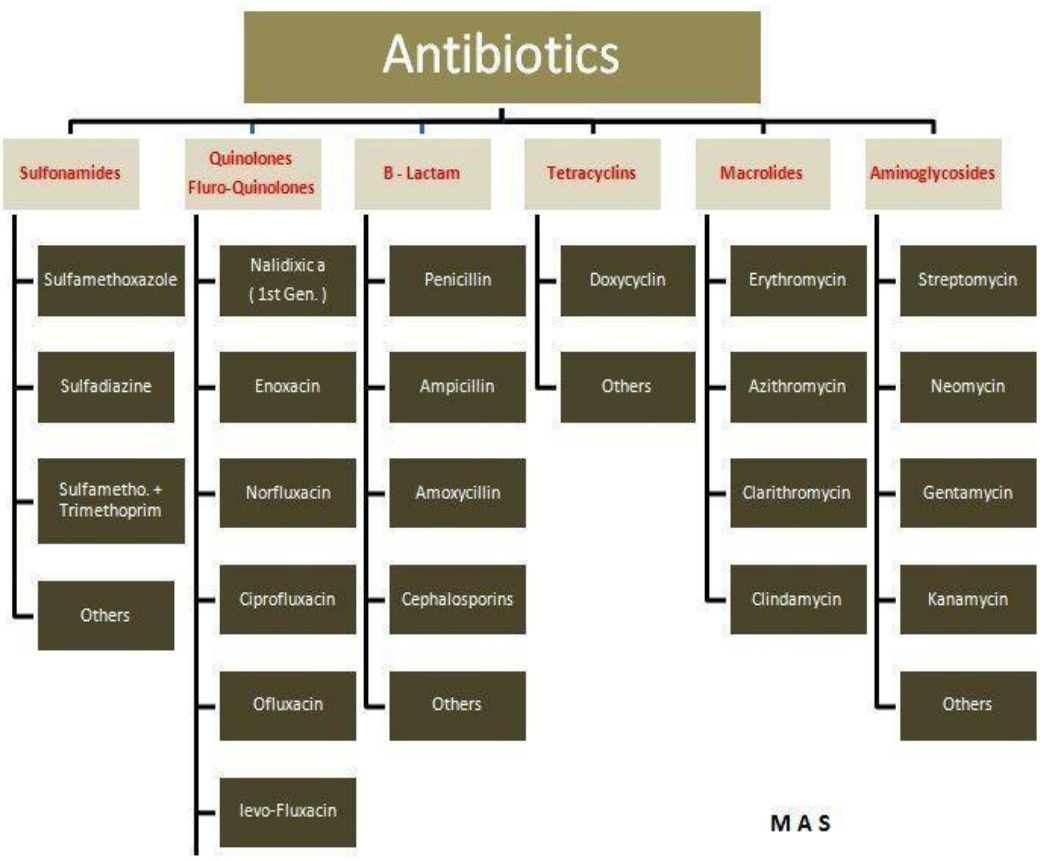
Colonies are counted. 10 colonies or less are classed as Too Few To Count (TFTC)
Colony counts of between 30 and 300 hundred are acceptable.
From the counts obtained on the plates, the concentration of bacteria per ml can be calculated.

Antimicrobial susceptibility testing:

Resistance to antibiotics is a major concern globally
Resistance to E.coli is of particular concern.

Determining the susceptibility of a specific bacteria against a range of antimicrobials by placing antibiotic impregnated discs onto a lawn of bacteria of know concentration.
After 24 hours incubation, the clear zones around the discs are measured and susceptibility, or resistance to an antibiotic can be determined.
Covered in lectures.

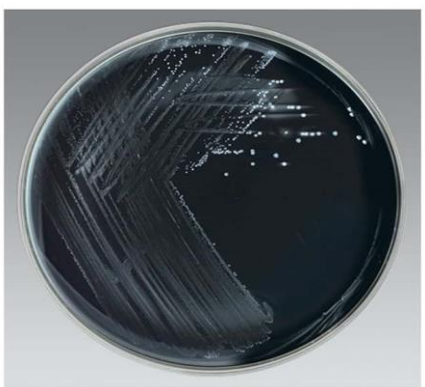




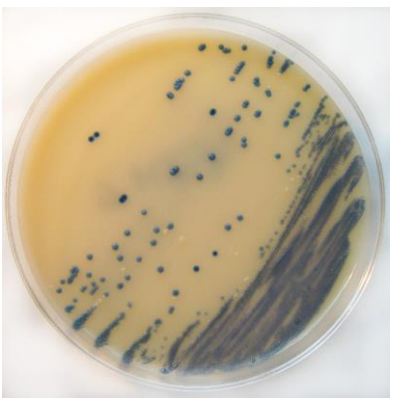
Examples of microorganisms growing on selective agar:

Colonies taken from the original streak plate can be streaked onto selective agar that is specific for a particular type of bacteria.

On a nutrient agar plate the colonies can all look very similar.
On selective agar they have specific characteristics and may also require different incubations conditions.
The bacteria below all grow aerobically at 37'C with the exception of Campylobacter*.

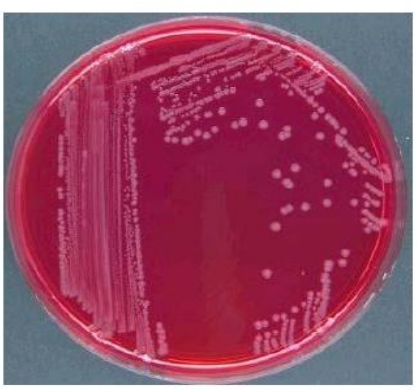


***Campylobacter growing on CCDA charcoal agar. White, milky colonies. Microaerophilic: An aerobic bacterium that requires oxygen, but less than is present in the air, and grows best under modified atmospheric conditions.**



MRSA Staphylococcus aureus growing on MRSA Brilliance media. Denim blue colonies for MRSA. S. aureus colonies are white / pale blue.

Salmonella spp. Growing on XLD agar. Black colonies.
Shigella colonies are pink on XLD.

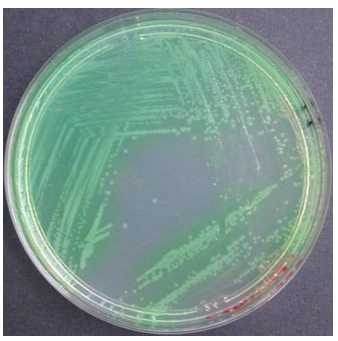


Salmonella spp. Colonies are pink on Brilliant Green agar.

E. Coli colonies are yellow on BGA.

E. Coli colonies on EMB agar have a metallic sheen.

Staphylococcus aureus



Pseudomonas aeruginosa