APPENDIX IV - TESTS

1. Catalase
   - bacteria that contain cytochrome enzymes are catalase positive and those that don't are catalase negative
   - *Staphylococcus* +
   - *Streptococcus* and *Enterococcus* -
   - flood bacteria with 3% hydrogen peroxide and observe for bubbles

     catalase

   a) H_2O_2 (3%) $\rightarrow$ Catalase peroxide + H_2O
   b) Catalase peroxide + H_2O_2 $\rightarrow$ Catalase H_2O + O_2

2. Coagulase
   - the ability to clot plasma
   - two different coagulase tests can be performed, a tube test for free coagulase and a slide test for bound coagulase, or clumping factor
   - *Staphylococcus aureus* +
   - Coagulase negative *Staphylococcus* -

3. Bile esculin
   - to detect beta glucoside which breaks down esculin to form a black precipitate due to the presence of ferric ions
   - *Enterococcus faecalis* +
   - Beta-haemolytic group B streptococcus -

   Esculin (β glucoside) $\rightarrow$ Esculetin + ferric ions (ferric citrate in medium)
   $\downarrow$
   black precipitate

4. MUG
   - this test is mainly done on the urine bench
   - to detect beta-glucuronidase
   - colourless substrate is broken down to produce a yellow compound
   - Kovac's reagent is added to detect indole production
   - *Escherichia coli* is MUG + and indole +

tryptophanase (produced by bacteria)
Trytophan → ammonia + pyruvic acid + indole

↓

reacts with p-dimethylamino benzaldehyde (Kovac's reagent)

↓

quinoidal red violet

[Indole spot test uses Erlich's reagent 1% dimethylaminocinnamalaldehyde]

↓

blue colour

5. Oxidase
   - to test for the production of oxidase
   - spot inoculate organism on to a filter paper soaked with 1% tetramethylphenylene diamine dihydrochloride - positive is purple, negative is yellow
   - *Pseudomonas aeruginosa* +
   - *Escherichia coli* -

Oxidizing reaction
Reagent 1% Dimethyl or Tetramethyl para-phenylenediamine
↓ on colonies
↓
Indopheneloxidase (produced by bacteria)
↓
↓
Indophenol = black colonies (with dimethyl)
= magneta colonies (with tetramethyl)

The following tests are done on the stool bench to screen for *Salmonella*, *Shigella* and *Yersinia.*

6. Urease
   - Detects urease production
   - Peptones in media utilized producing an alkalinity. Phenol red indicator

Urea →→ammonia

7. TSI
   - Triple sugar iron agar. 0.1% glucose, 1% sucrose and lactose.
   - If the glucose is fermented, only a small amount of acid will be produced, which will be neutralized by alkali from peptone metabolism along surface of slant.
   - Oxidation of peptone cannot take place in the anaerobic conditions in the depth of the medium.
   - Therefore when glucose only is fermented, the butt of the medium becomes yellow and the slant remains red.
   - If lactose or sucrose is fermented, the amount of acid produced is large enough to offset alkali production and a yellow slant is produced.
   - Production of hydrogen sulphide is shown by formation of iron sulphide from the ferrous sulphate.

8. ONPG - To detect enzyme β-D-galactosidase in lactose fermenting organisms. O-nitrophenol-B-D-galactopyranoside→→ O-nitrophenol (yellow)

9. PPA - To detect Phenylalanine deaminase production. Phenylalanine→→Phenylpyruvic acid + $FeCl_3$ (ferric ions) = blue green