Microbiology Lab – UGS

I. *E. coli*:

Urine for analysis must be placed in a sterile container. In most cases, a "midstream" specimen of urine is collected. The urine is then inoculated on MacConkey agar* or CLED agar, which will allow the growth of *E. coli*. A standard loop with known size is used for inoculation. The number of colonies on the agar is then estimated and diagnosis is made based on the number of colonies according to the following:

Number of colonies	Infection
$>10^5$	Significant
10^3-10^5	Intermediate
$<10^3$	Not significant

*MacConkey agar: a selective and differential medium.

- Selective: allows only Gram-negative bacteria to grow, mainly enteric bacteria.
- Differential: can differentiate between lactose fermenters and non-lactose fermenters:
  → Lactose fermenters: **Pink**, such as: ***E. coli***.
  (Weak fermenters give light pink, such as Klebsiella).
  → Non-lactose fermenters: **Yellow**.

Four tests done on *E. coli*:

1. Krigler test:

Tilted agar, with slant and butt, **normally red**.

Lactose fermenter: **slant** becomes **yellow**.

Glucose or dextrose fermenter: **butt** becomes **yellow**.

***E. coli* is both lactose and glucose fermenter,** so both slant and butt become **yellow**. This is also referred to as acid/acid.

Note: if H_2S is produced, a black line appears (ex: Proteus).

2. Urease test:

Negative	**Yellow**	***E. coli***
Positive	Pink	Proteus

3. Citrate test:

Negative	**Green**	***E. coli***
Positive	Blue	Proteus

4. SIM:
   (bacteria is inserted into media using an inoculation needle, producing a line of bacteria)
   S = H_2S production = black color (ex: Proteus). ***E. coli* is negative.**
   I = indole production: Add Kovac's reagent (brown color) to SIM → if there is indole, a **red ring** appears (ex: *E. coli*). ***E. coli* is positive.**
   M = motility = branching from line of bacteria. ***E. coli* is positive.**

II. *Streptococcus*:

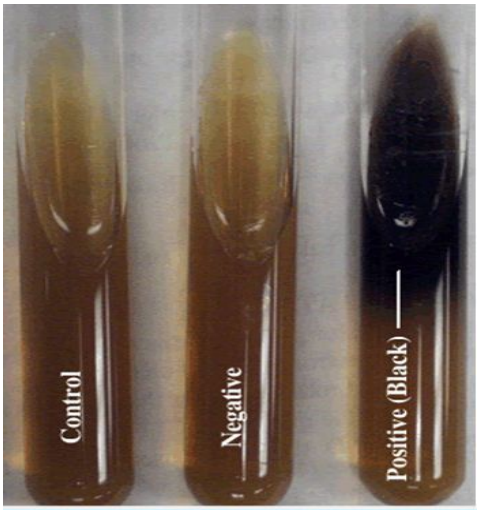
Gram-positive cocci, arranged as a chain.

Inoculation on blood agar (for 24 hours at 37°C) → **hemolysis**:

- Partial: green → α-hemolytic.
- Complete: colorless → β-hemolytic – 2 groups → add Bacitracin disc to differentiate:
  → Inhibition zone = group A.
  → No inhibition = group B.
- No hemolysis: red → ɣ-hemolytic → group D (***Enterococcus***).

<u>Bile Esculin test for *Enterococci*:</u>

Media with very high salt concentration, **selective** for group D *Enterococci* → hydrolysis of esculin will produce ferric citrate (**black** color).



Bile Esculin Test

III. *Neisseria*:

- Gram-negative diplococci (kidney-shaped).
- Can be seen intracellular or extracellular.

IV. *Candida*:

- Yeast under microscope: Gram positive round cells.
- On **Sabouraud dextrose agar**: very large colonies, convex-shaped, **creamy** color, smell of yeast.
- Gram-tube test: serum + *Candida albicans* → incubate for 4 hours → reproduce → sample taken by loop → place on microscopic slide → Gram stain → pseudo-hyphae (elongations). If **pseudo-hyphae** are seen, this confirms the Candida is of *albicans* species. Other species show budding. *Candida albicans* is the most pathogenic.
- To distinguish between 4 species of Candida: **CHROMagar**: (expensive – not commonly used)

Candida albicans	**Green**
Candida tropicalis	**Blue**
Candida glabrata	**Dark pink-violet, glistening**
Candida krusei	**Rough, pale pink**

Good Luck

– Ala'a Farkouh