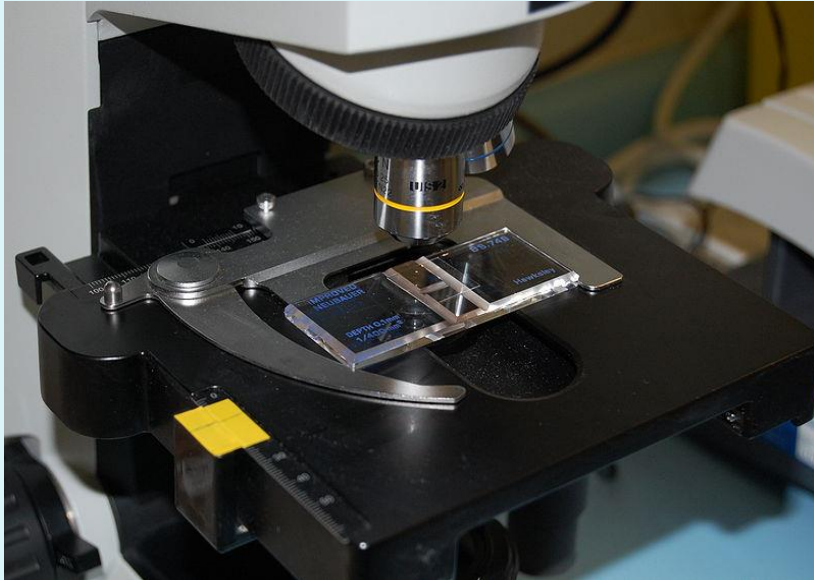










Practical of Urinary Tract Infection



Department of Microbiology



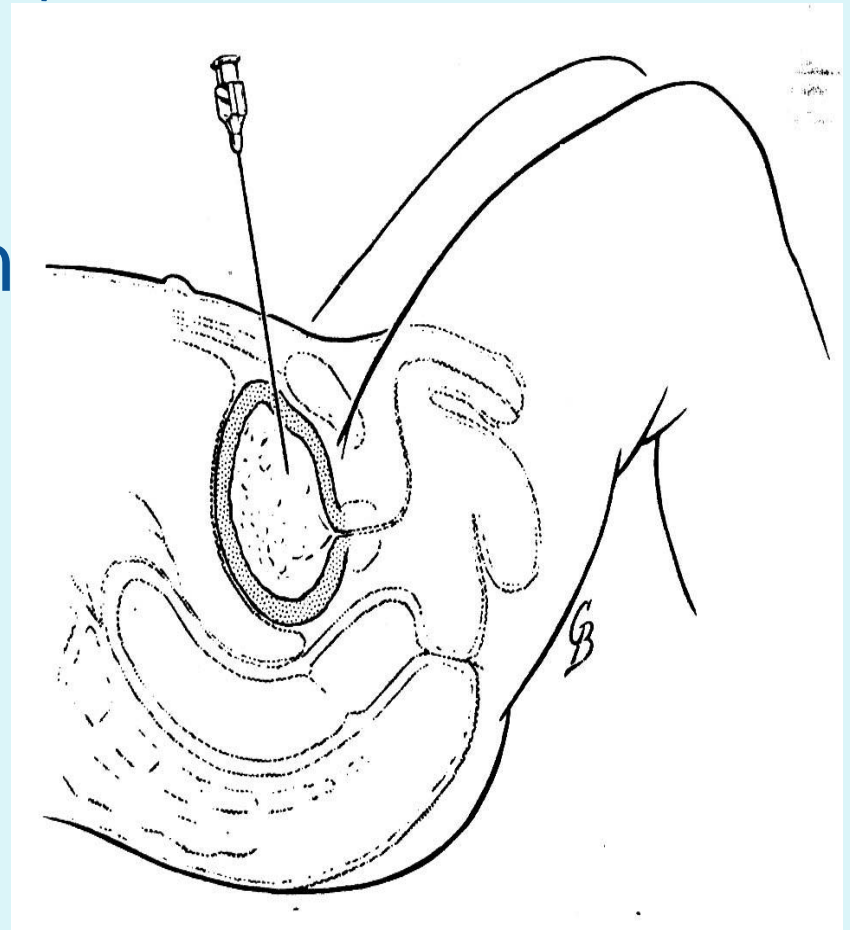
| Organism | Gram Stain | Culture (colony) | Oxidase | | | |
|-----------------|---|---|---------------|-----------|------------|--------------|
| E.coli |  | Lactose fermentative (pink) | - Indole + | | | |
| Klebsiella | | Lactose fermentative (mucoid) | | | | |
| Proteus |  | Lactose non-fermentative (colorless) | - Urea + | | | |
| Pseudomonas |  | Lactose non-fermentative (greenish) | + | | | |
| S.saprophyticus |  | white  | catalase | coagulase | novobiocin | Bile Esculin |
| | | | + | - | R | N/A |
| Enterococcus |  | gray   | - | N/A | N/A | + |

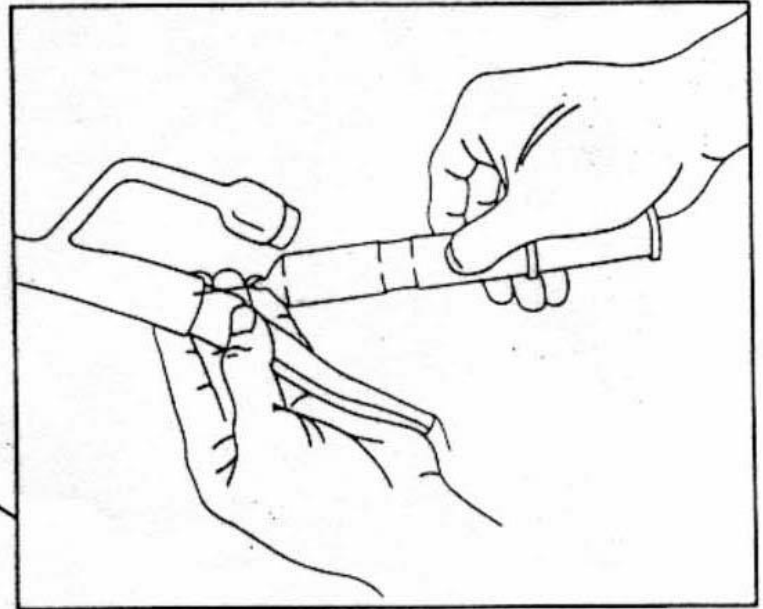
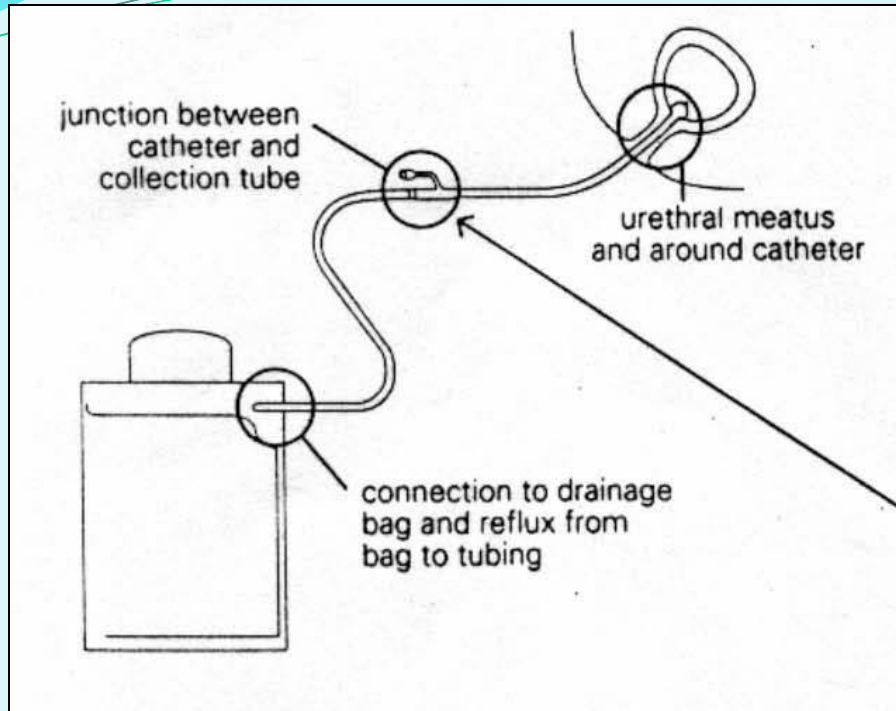
Important aspects of Microbiologic Examination of UTI:

- Urine collection
- Urine analysis
- Interpretation of microbiology laboratory result

Type of Specimens

- Midstream urine (MSU)
- Clean catch
- Adhesive bag
- Suprapubic Aspiration
- Catheter sample



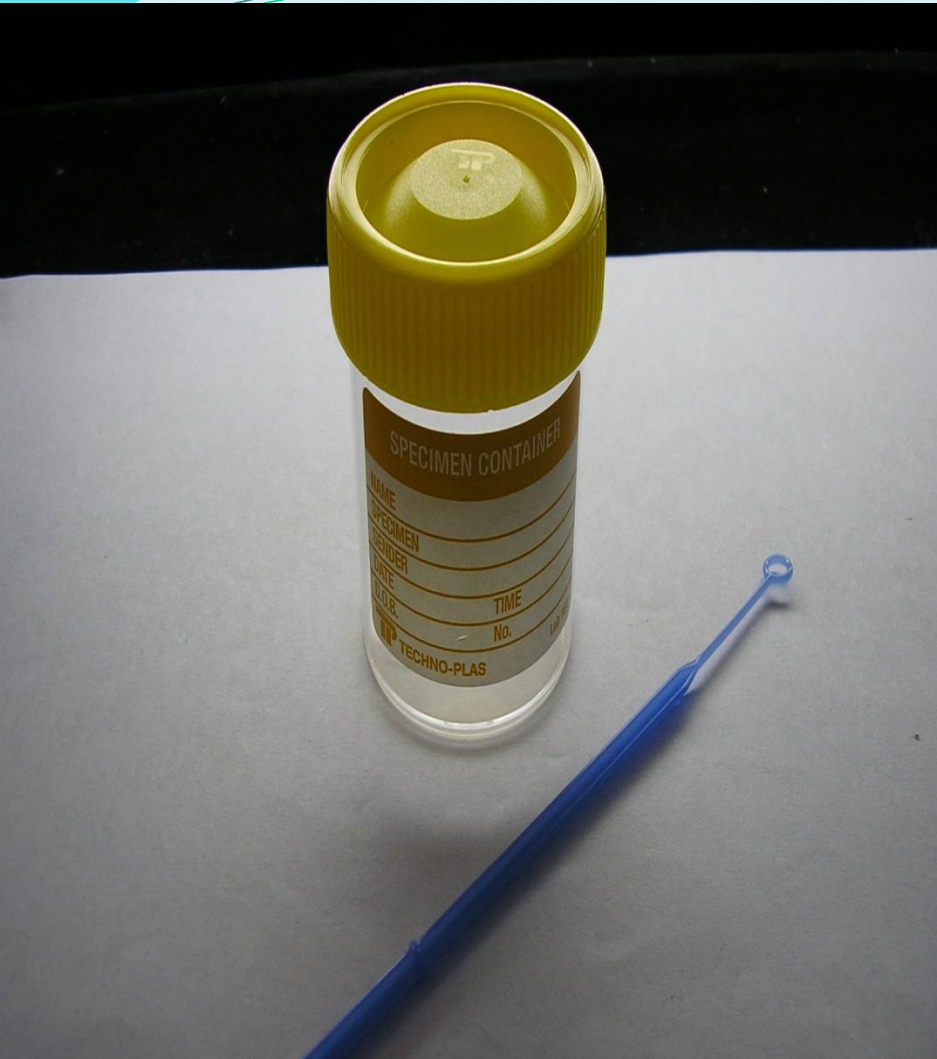


The urinary catheter

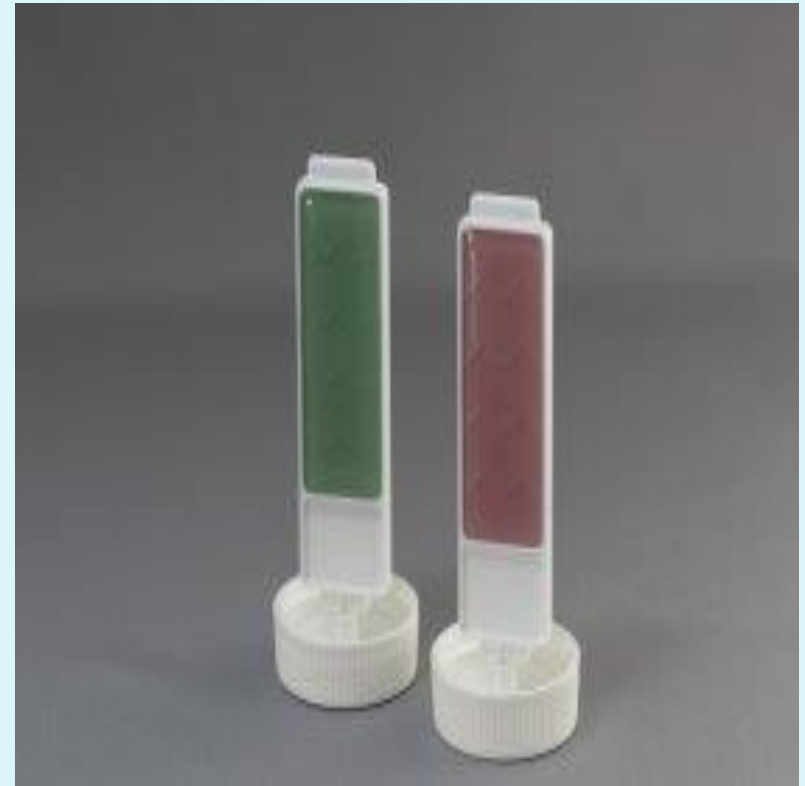
Urine specimens for laboratory investigations can be collected from catheterized patients as shown (left). The second port is for putting fluids into the bladder (right).

Urine from the drainage bag should not be tested because it may have been standing for several hours.

TRANSPORT MEDIA



**Sterile
Urine container**

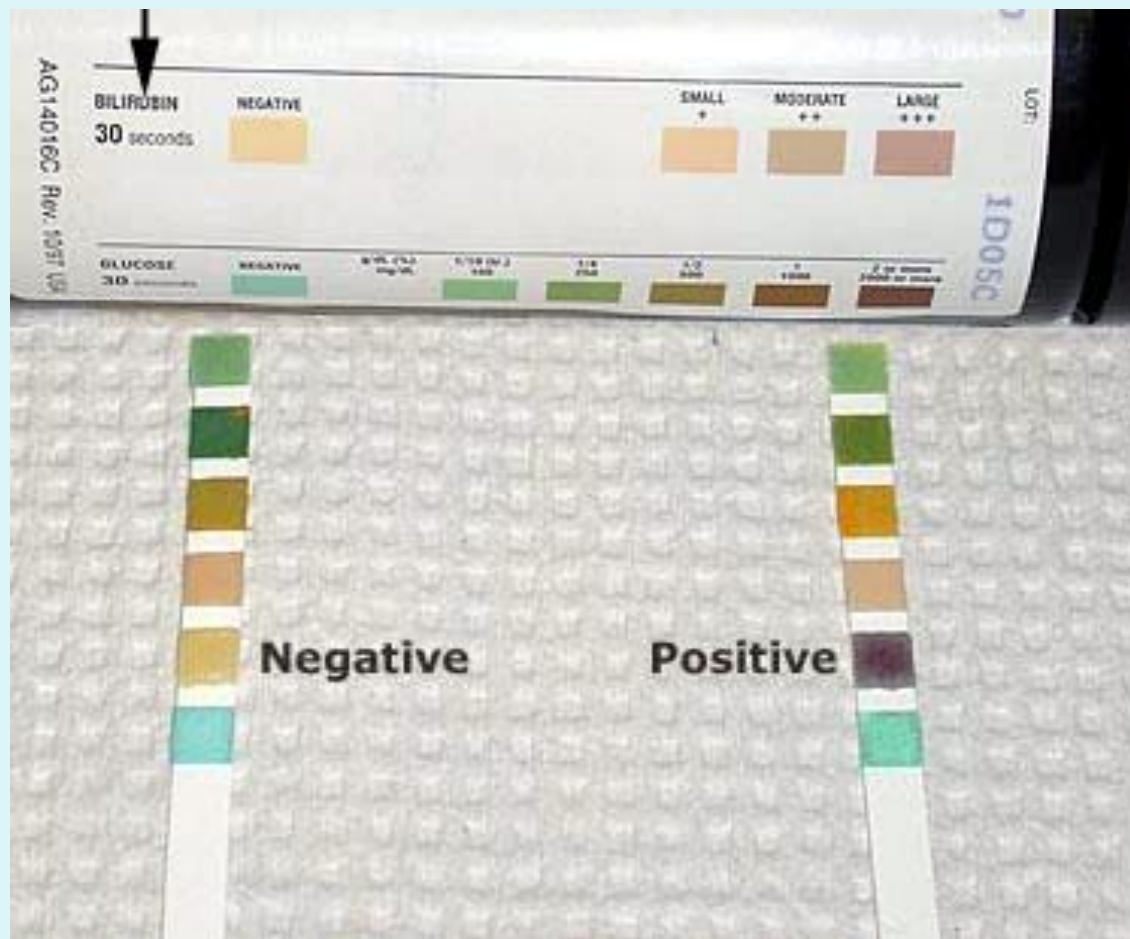


dipslides

One side is CLED media, the other can be MacConkey (MAC) agar or blood agar.

Urine analysis;

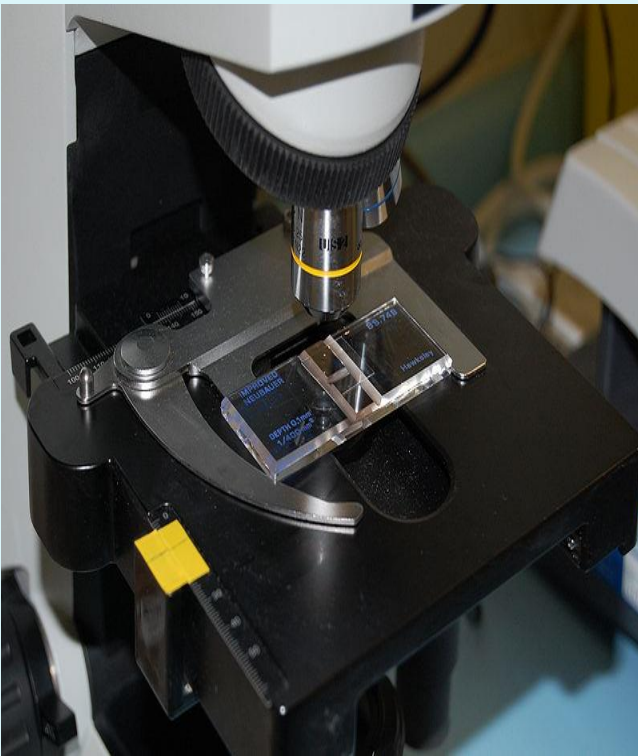
1- Dip stick (leukocyte esterase ,nitrate test)



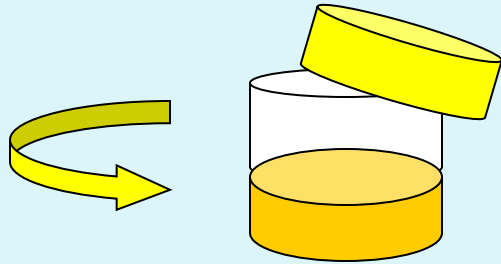
Urine analysis;

1- Dip stick (leukocyte esterase ,nitrate test)

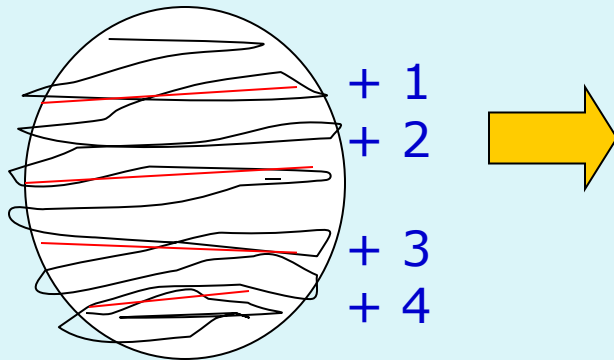
2-microscopic ex; cell-counting chamber



Laboratory examination of urine



Quantitative (Colony counts)



a urine sample is streaked on surface of
Blood Agar plate and CLED agar / Mc
Conkey agar with a special loop
calibrated to deliver a known volume.

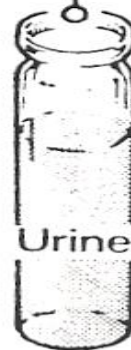
Over night incubation

Isolation of colonies,
Biochemical tests,
Drug susceptibility test,

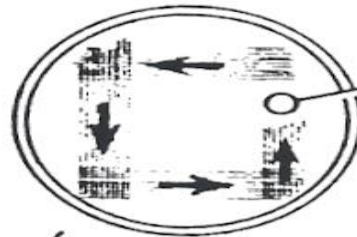
Over night incubation

RESULT

(3 mm internal diameter)



Inoculate plate
without flaming
loop between
strokes

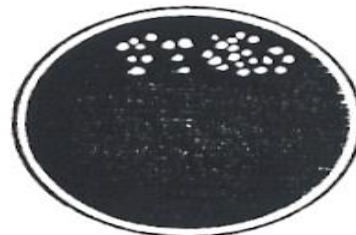


Loop charged with
approximately 1/300 ml
of urine

Possible results



<30 colonies
<10⁴ organisms/ml
Not significant



30 colonies
10⁴ organisms/ml
Doubtful significance



>300 colonies
>10⁵ organisms/ml
Significant

Diagram illustrating the semi-quantitative culture of urine specimens.

| GRAM NEGATIVE | GRAM POSITIVE |
|---|--|
| <i>Escherichia coli</i> | <i>Enterococcus</i> |
| <i>Klebsiella</i> | <i>Staphylococcus saprophyticus</i> |
| <i>Proteus</i> | <i>Streptococcus agalactiae</i> (group B) |
| Other <i>Enterobacteriaceae</i> (<i>Enterobacter</i> , <i>Citrobacter</i>) | <i>Staphylococcus aureus</i> ¹ (Associated with staphylococemia) ⁽ |
| <i>Pseudomonas aeruginosa</i> | |

- Other organisms ;
 - *Candida albicans*
 - *Schistosoma haematobium*
 - *Tricomonas vaginalis*

Causes of UTI's

Outpatients
(%)

Inpatients
(%)

| | | |
|---|-------|-------|
| <i>Escherichia coli</i> | 53-72 | 18-57 |
| Coagulase negative <i>Staphylococcus</i> | 2-8 | 2-13 |
| <i>Klebsiella</i> | 6-12 | 6-15 |
| <i>Proteus</i> | 4-6 | 4-8 |
| <i>Morganella</i> | 3-4 | 5-6 |
| <i>Enterococcus</i> | 2-12 | 7-16 |
| <i>Staphylococcus aureus</i> | 2 | 2-4 |
| <i>Staphylococcus saprophyticus</i> | 0-2 | 0.4 |
| <i>Pseudomonas</i> | 0-4 | 1-11 |
| <i>Candida</i> | 3-8 | 2-26 |

culture media

blood agar



an enriched medium

MacConkey agar



a differential medium

CLED agar



Selective medium

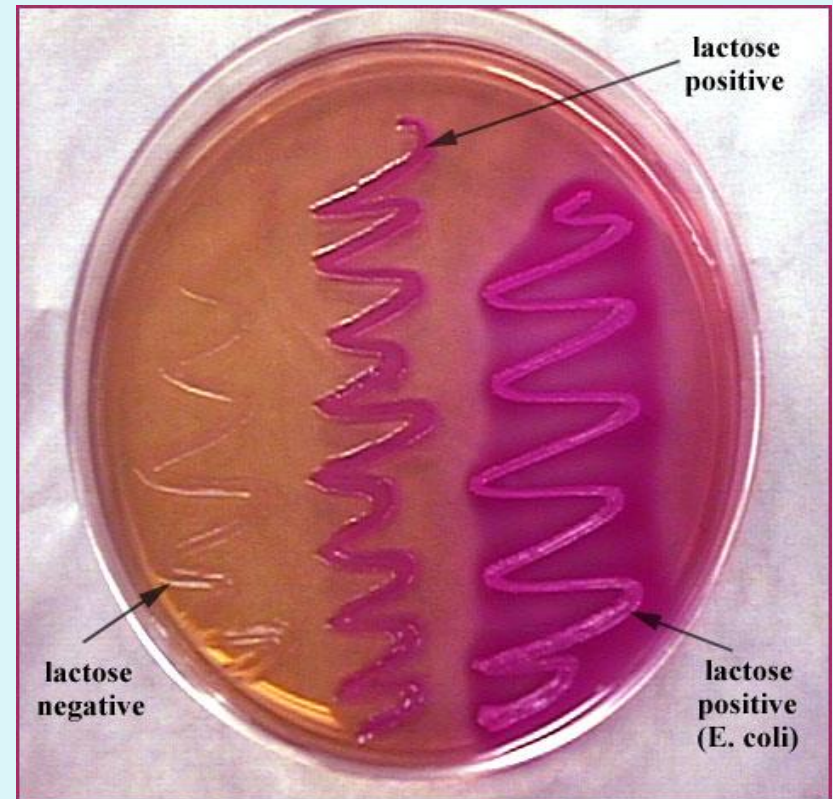
Blood agar

An enriched medium, especially for culturing fastidious microorganism and observed the hemolytic reaction



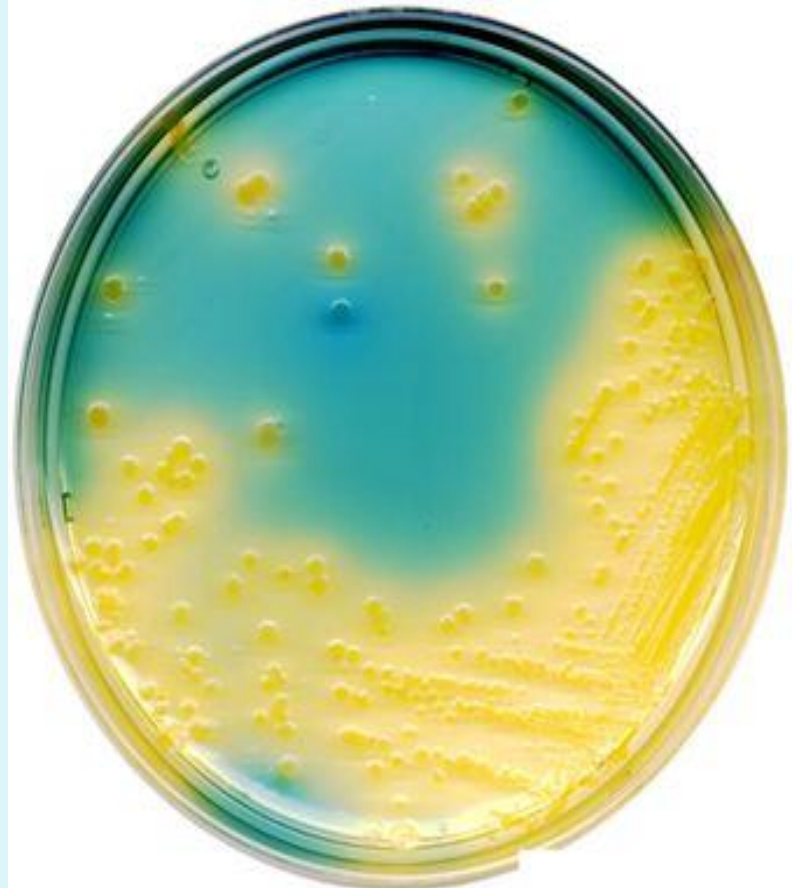


MacConkey's agar showing both lactose and non-lactose fermenting colonies. **Lactose** fermenting colonies are **pink** whereas **non-lactose** fermenting ones are **colourless** or appear same as the medium.]



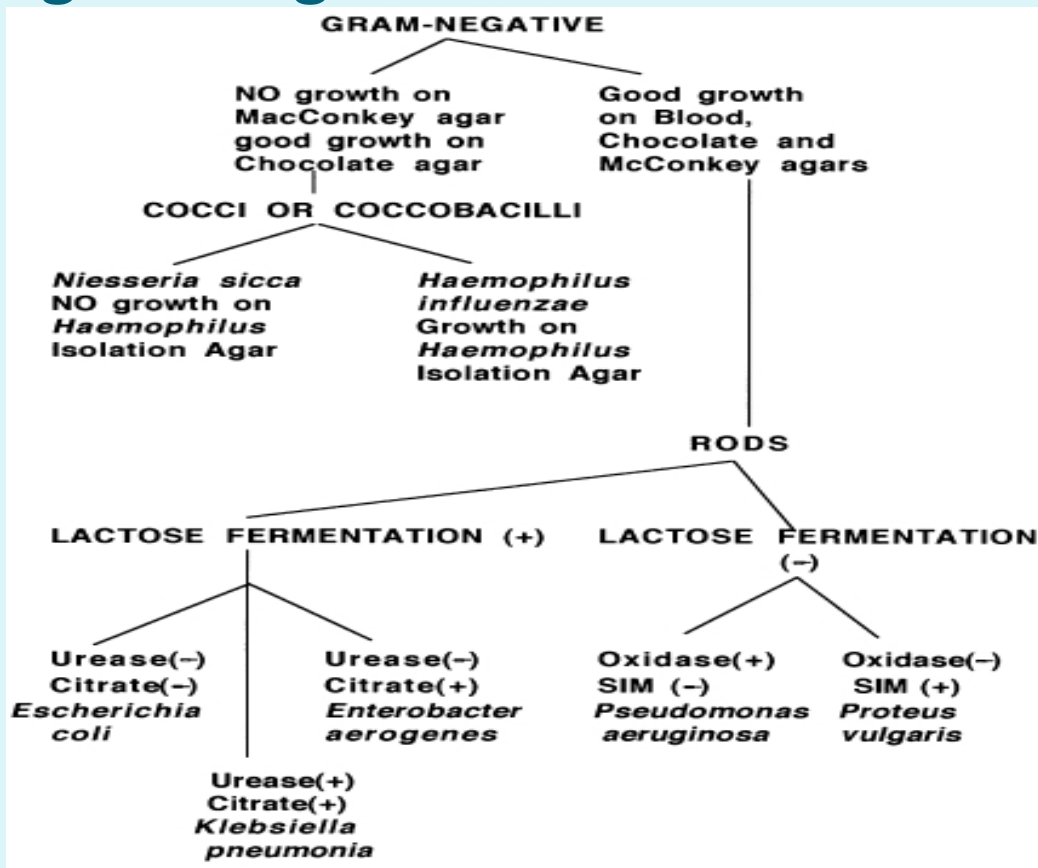
CLED agar

Selective culture medium for detection and isolation Of *Escherichia coli* and *coliform* bacteria in urine

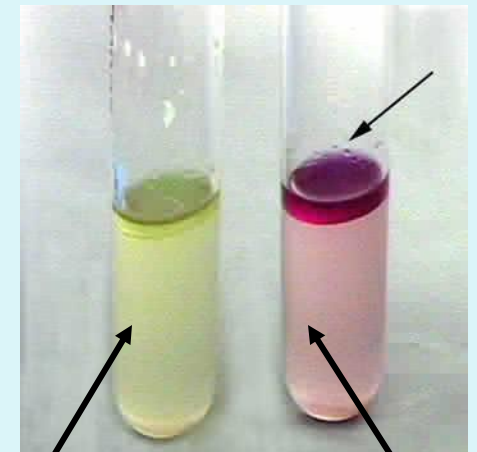
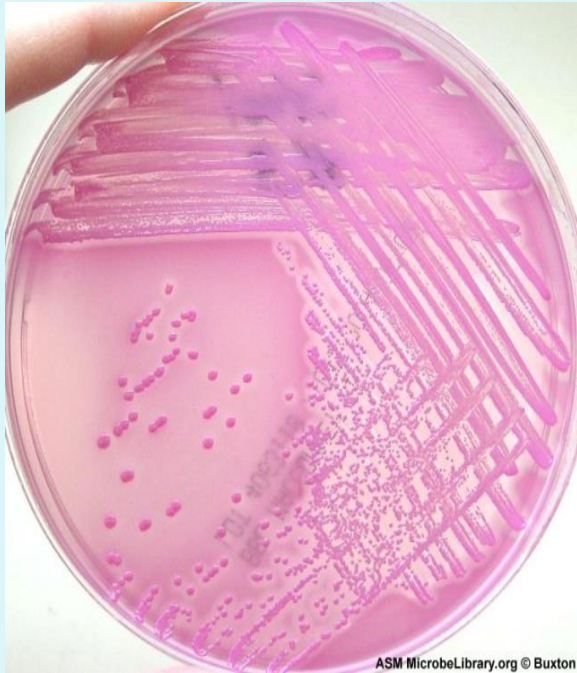




gram negative bacilli

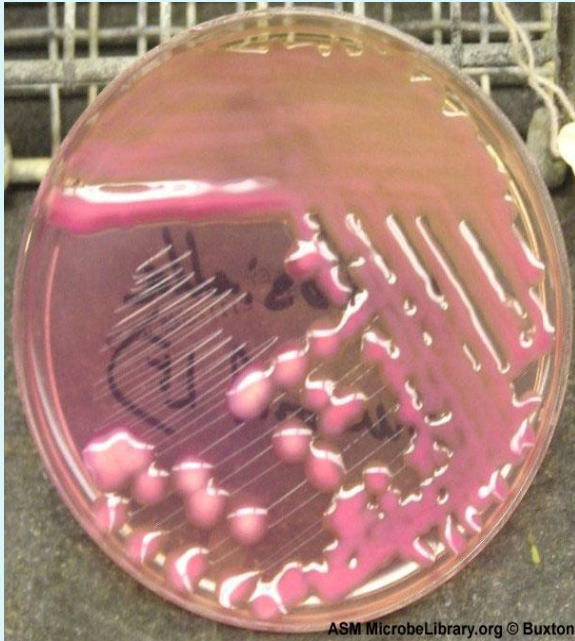


E coli

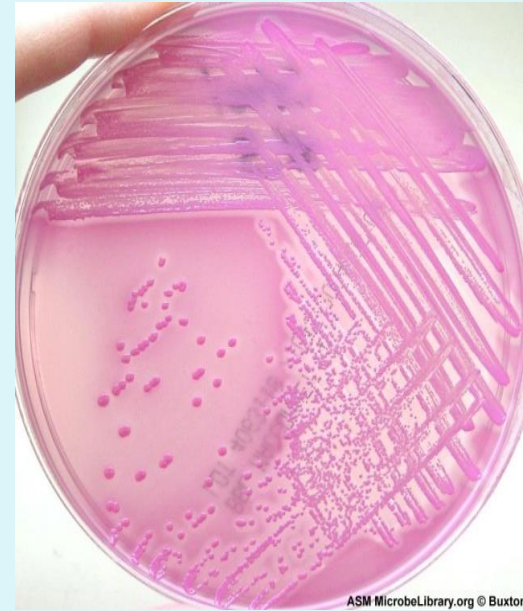


Indole Reactions
Negative **Positive**

Klebsiella pneumoniae

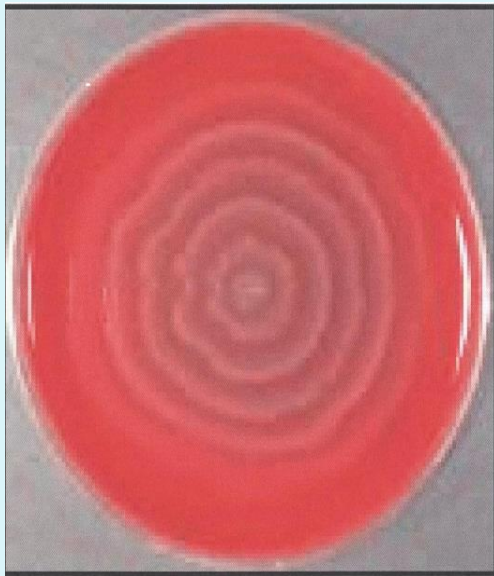


E coli



Klebsiella

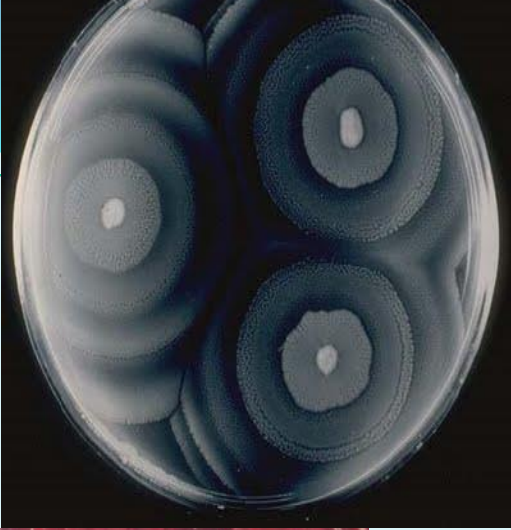
Proteus growth : Swarming



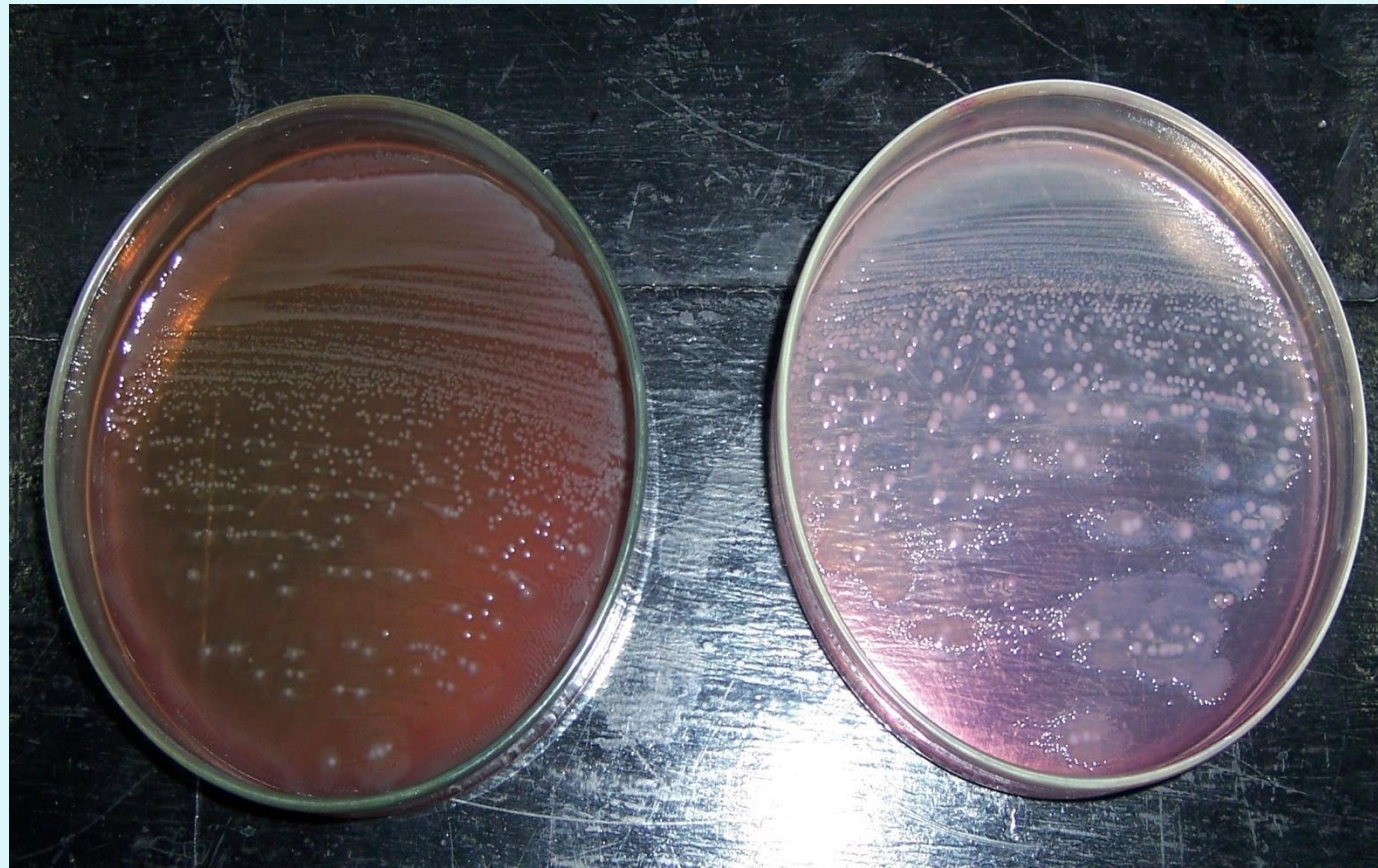
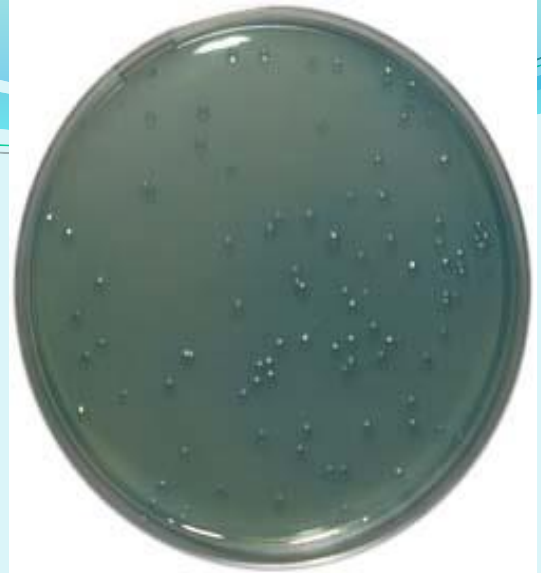
CLED [(Cystine-Lactose-Electrolyte-Deficient) - inhibits the proteus swarm



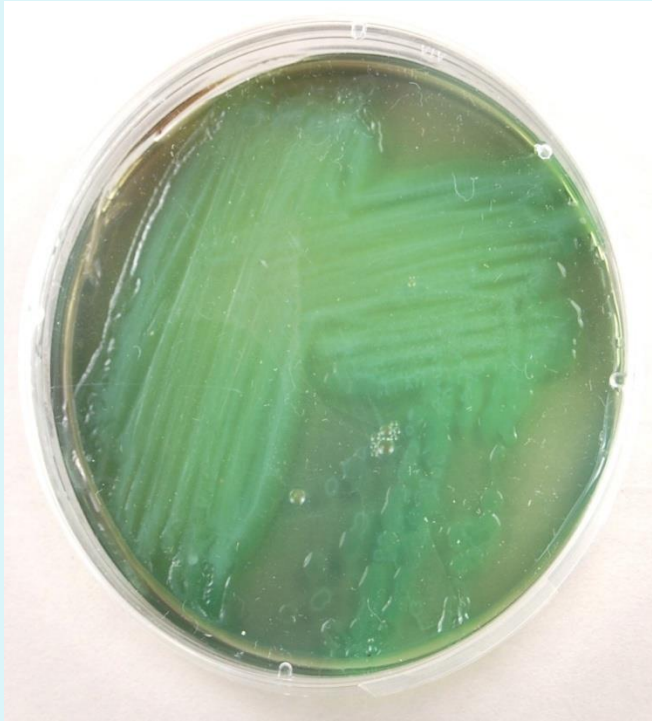
**proteus is Urease positive
Urease splits urea into ammonia; and alkalinizes the urine with production of crystals**



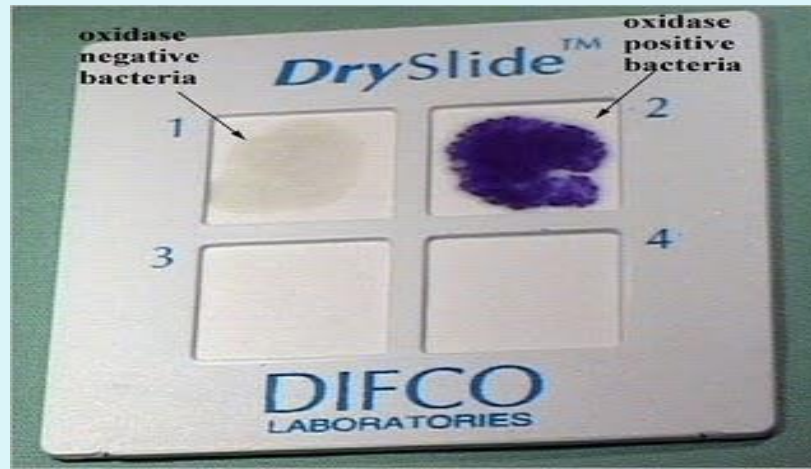
Proteus spp,



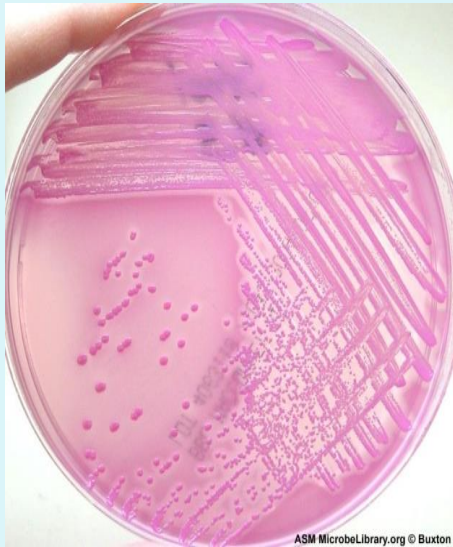
Pseudomonas aeruginosa



ASM MicrobeLibrary.org © Buxton



E coli



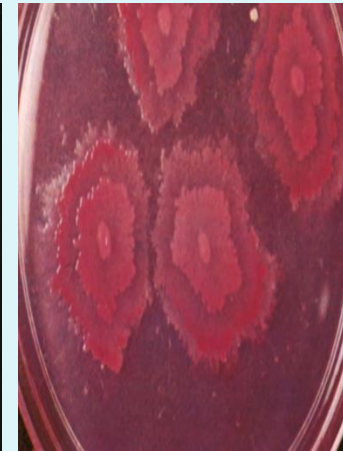
Klebsiella pneumoniae



Pseudomonas aeruginosa



Proteus spp,

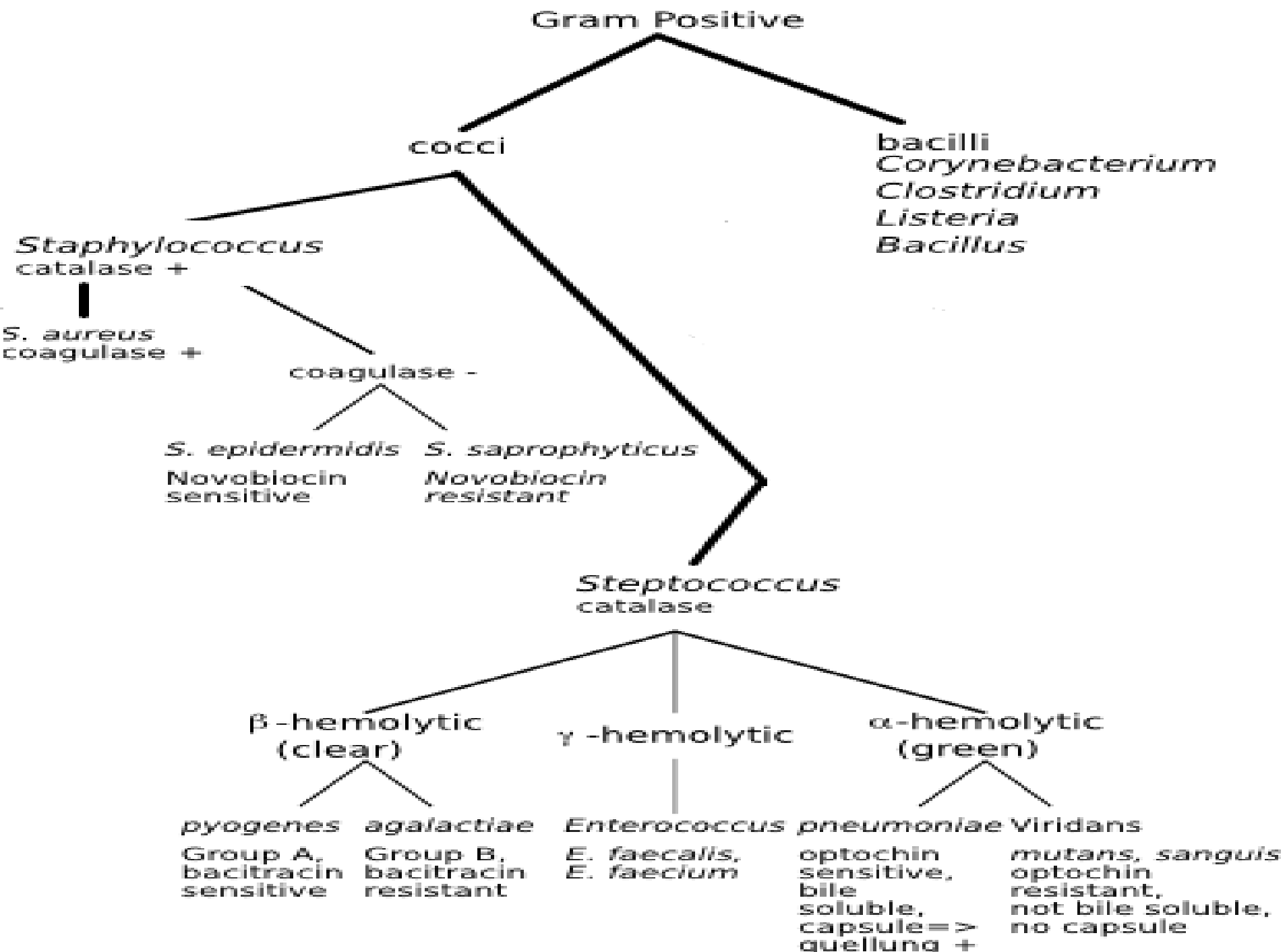




Three API 20E strips :

- Immediately after inoculation
- After 24 hours incubation
- That in (b) after addition of reagents to certain wells.

The organisms here is *Escherichia coli*. Here the first carbohydrate well (glucose) is also used for the nitrate reduction test



Enterococcus species



Biochemical Identification

- Bile Esculin hydrolysis



Both Group D streptococci and enterococci produce a positive (left) bile Esculin hydrolysis test.



Staphylococcus spp

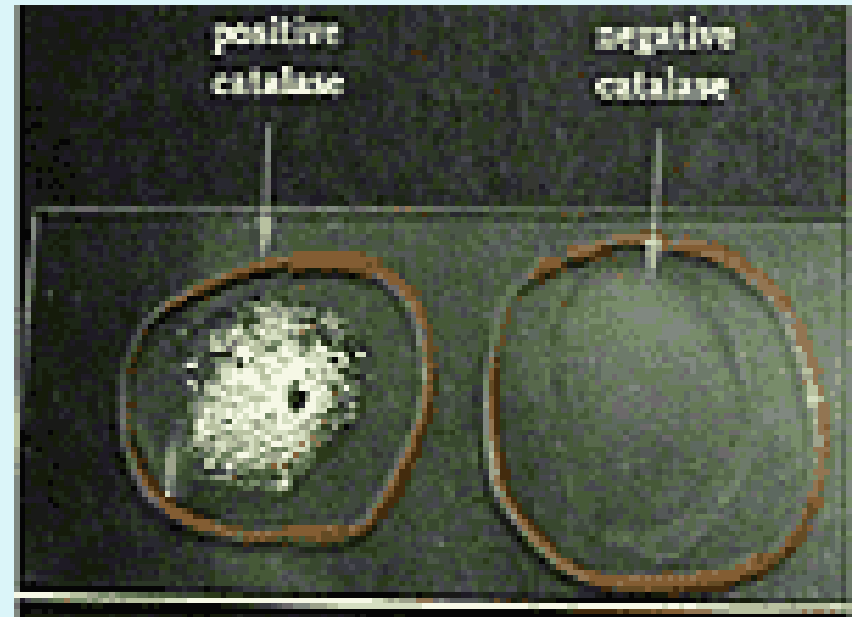
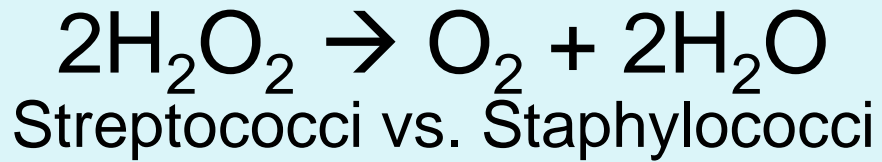


Staphylococcus aureus
Golden colonies (yellowish)

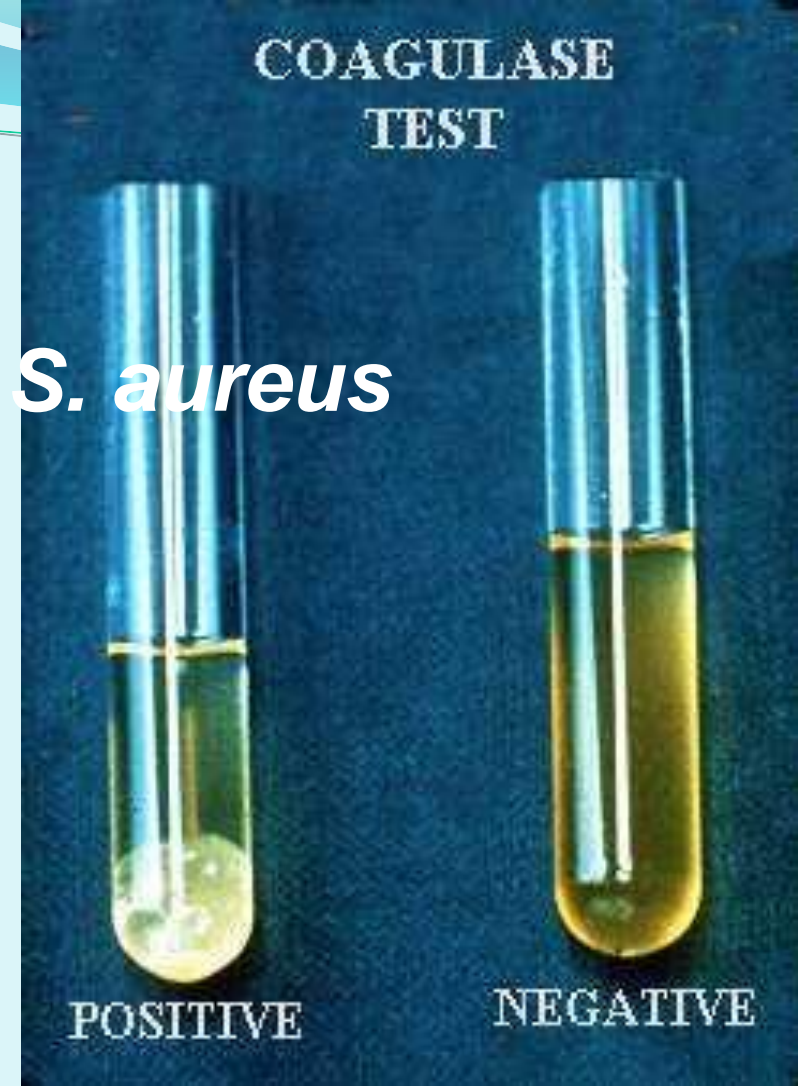
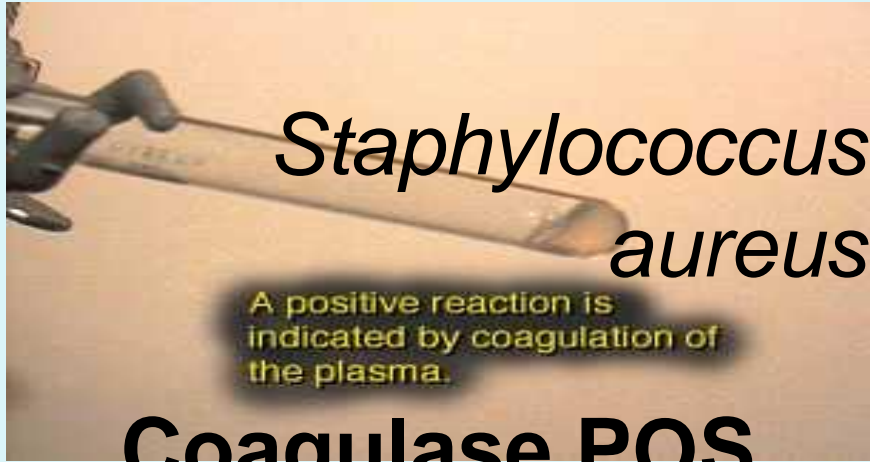
Staphylococcus epidermidis
white colonies

Differential Characteristics

Catalase



Differential Characteristics



NOVOBIOCCIN TEST



***Staphylococcus saprophyticus*
(resistant-Novobiocin)**

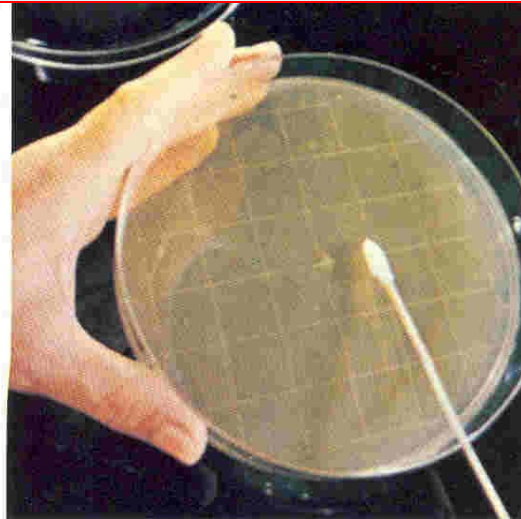
***Staphylococcus epidermidis*
(sensitive-Novobiocin)**



[A]



[B]



[C]



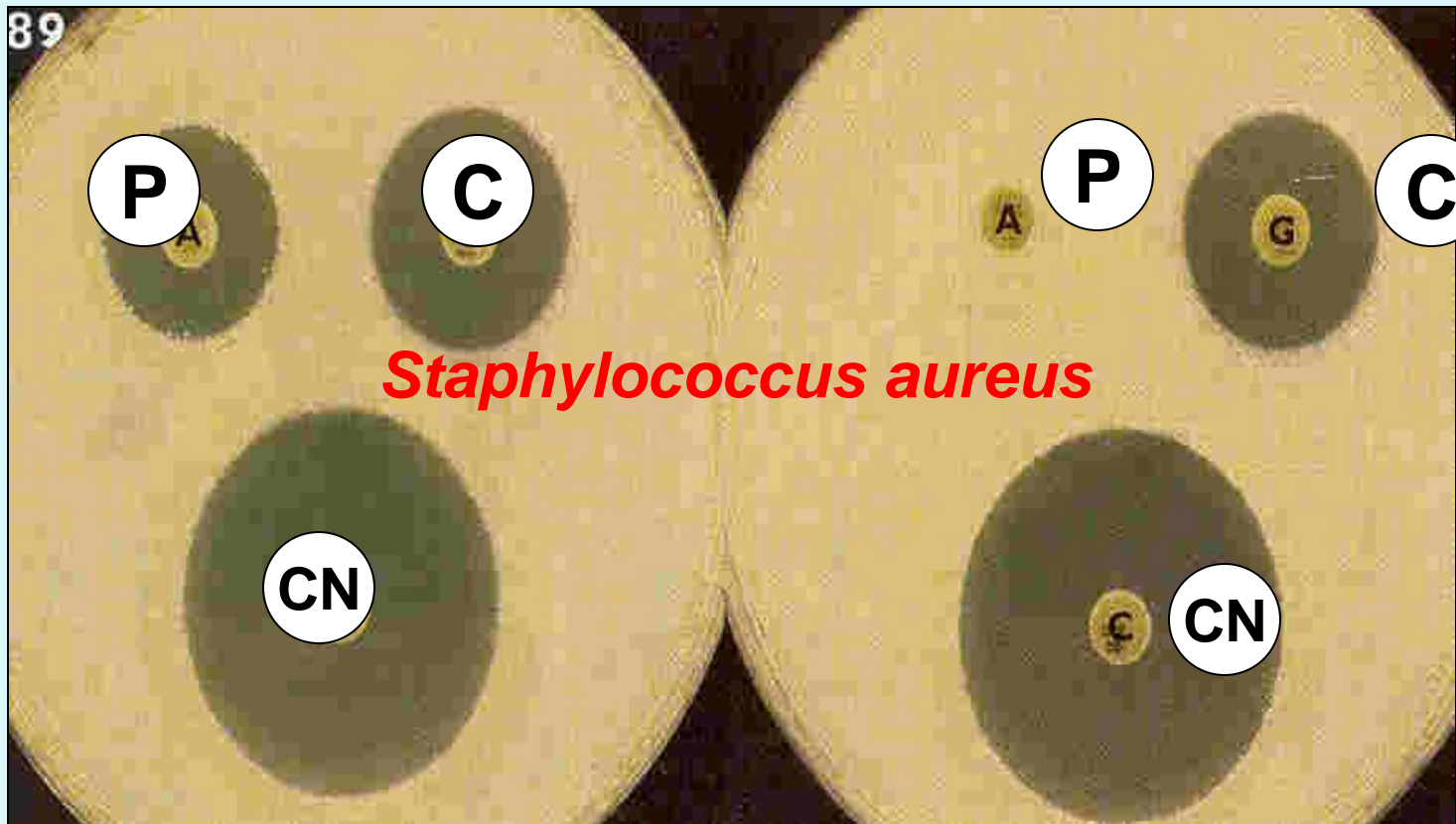
[D]



[E]

Gentamisin (CN) : 12 - 15
Chloramphenicol (C) : 12 - 18
Penicilin (P) : 28 - 29

R (Resistant) ; S (Sensitive)



Antibiotic sensitivity test: Agar diffusion method



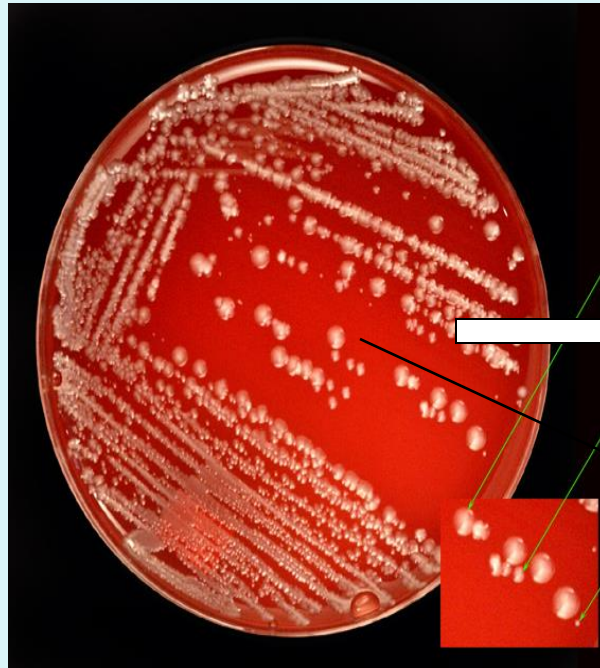
Case 1

The blood agar plate and CLED plate provided were inoculated with a sample of urine from a patient with a suspected urinary tract infection. Examine the plates and photographs provided.

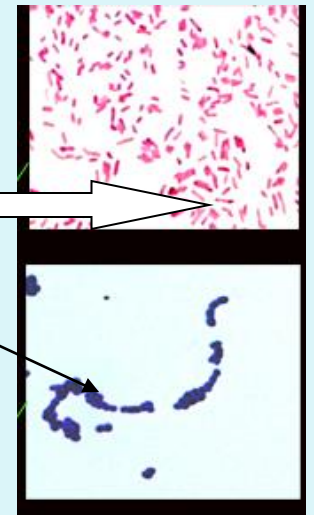
- Identify the colonies on the blood agar plates and photographs. The photographs show the results of the Gram stain of each colony type.
- Large colonies are Gram.....and small colonies are Gram.....



CLED plate



Blood agar



Gram stain

Case 2

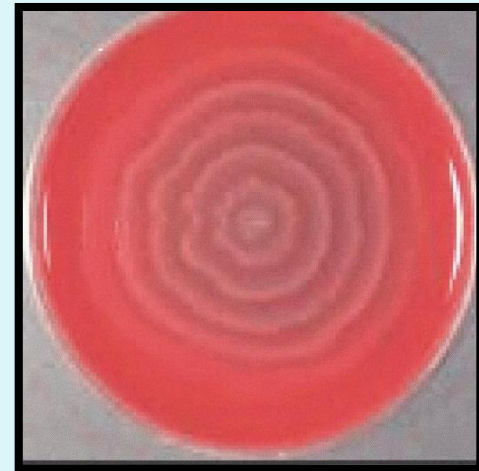
These Blood agar and CLED agar plates were inoculated with MSU from a 45 years old man suspected of having bladder stone and complaining of burning micturation.

Urine examination showed :

Moderate number of WBC and a PH of 8



CLED

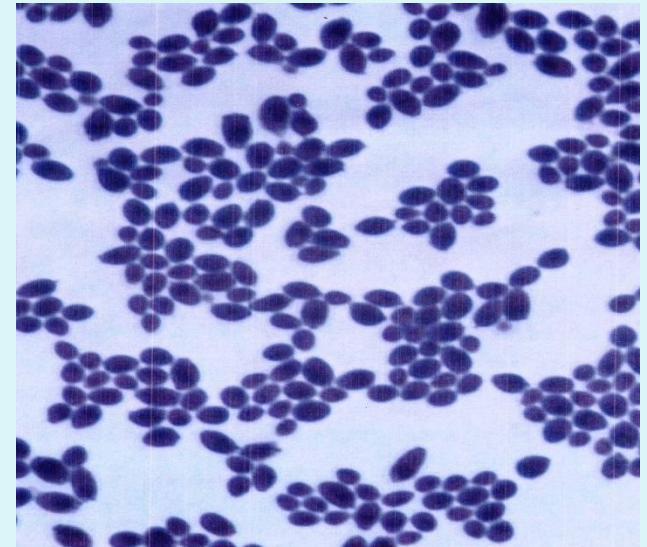
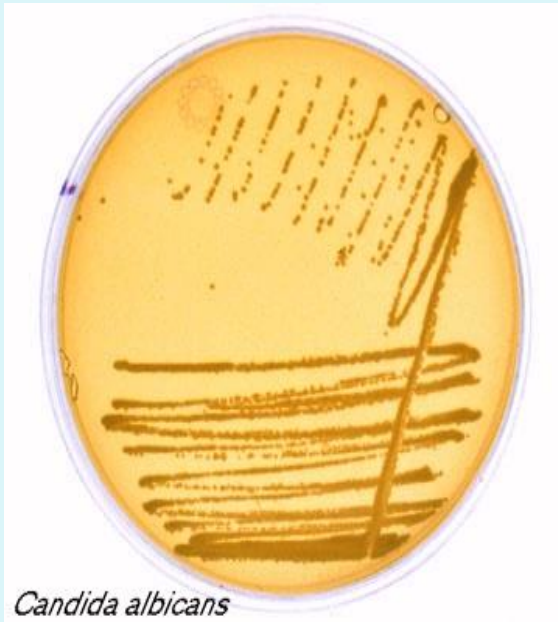


- A) What is the likely this pathogen?**
- B) How would you confirm the identity of this pathogen?**
- C) What is the role of this organism in forming stones?**

Candida albicans

Growth on Sabouraud's
Dextrose Media

Candida albicans on blood
agar;



Schistosoma haematobium



***Schistosoma
haematobium***
(urine; eggs 115-170 x 45-
65 micrometers)
(primates)

Candida albicans: identification tests

- Chlamyospore
- Germ tube test



discussion

Mention one organism from each of the following which may cause urinary tract infection

- A) Bacteria**
- B) Parasites**
- C) Fungi**