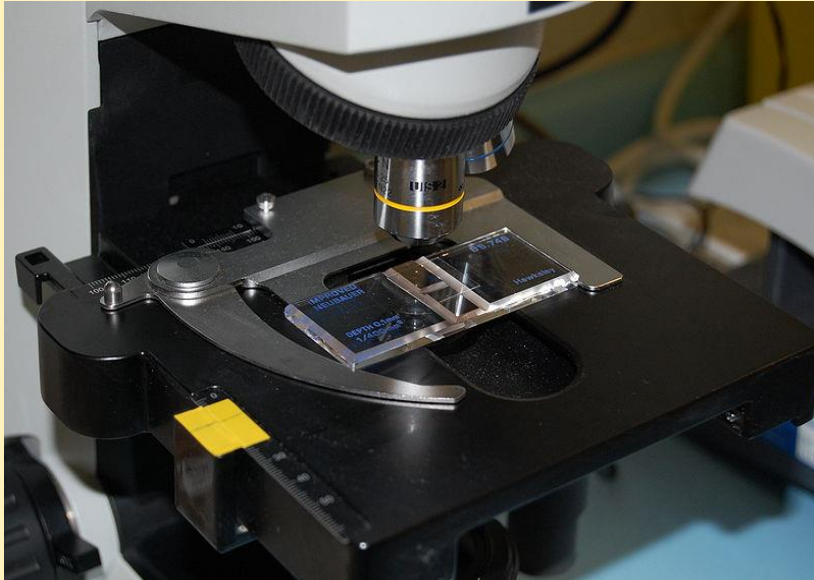


Practical of Urinary Tract Infection



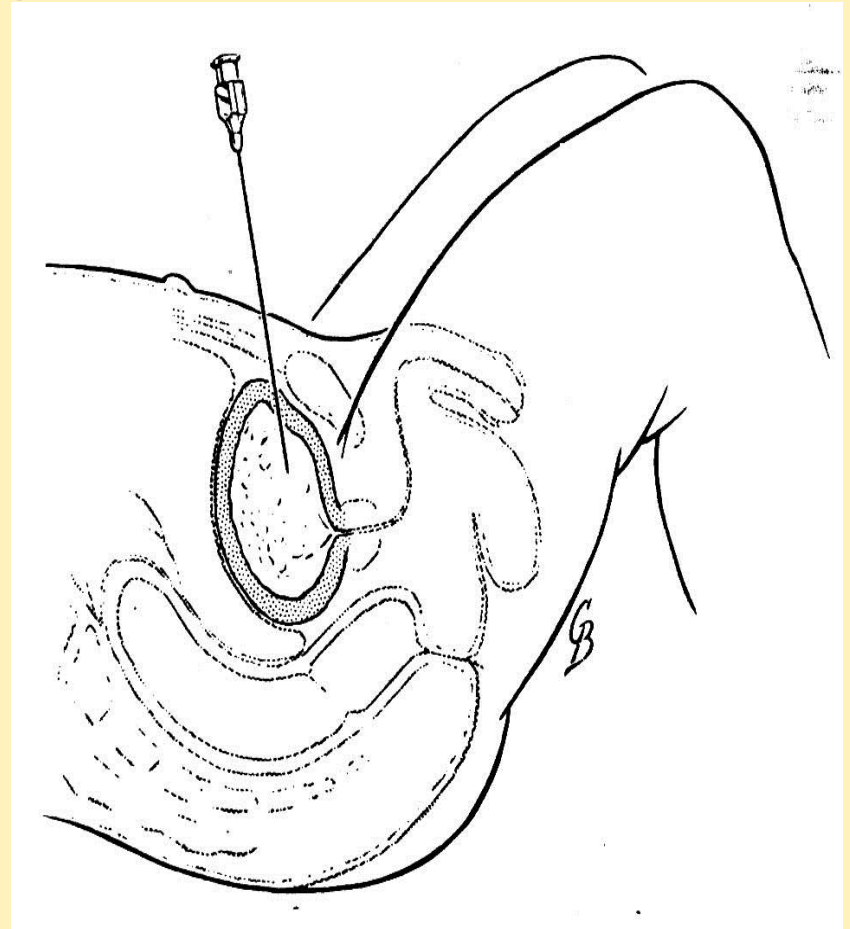
Department of Microbiology

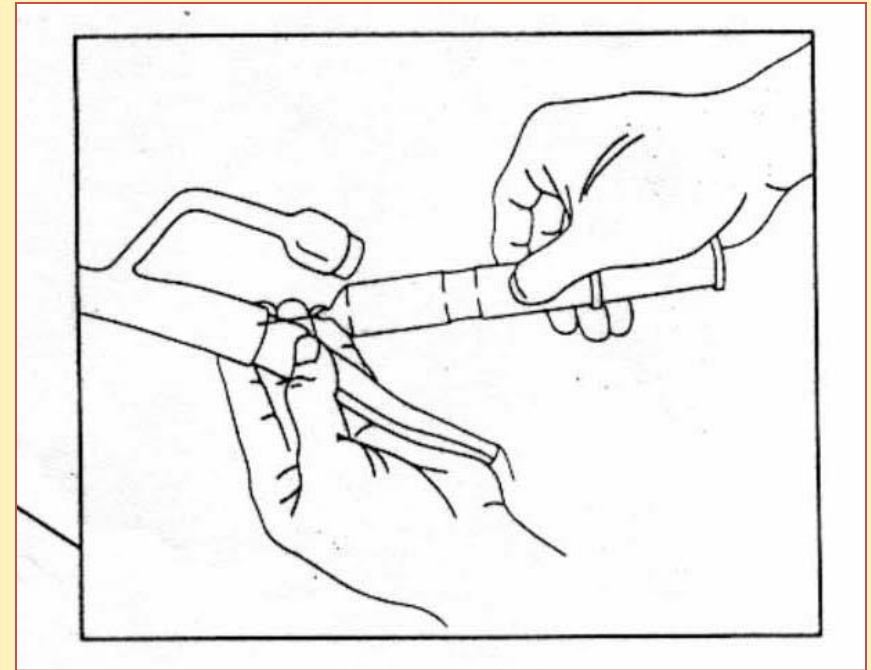
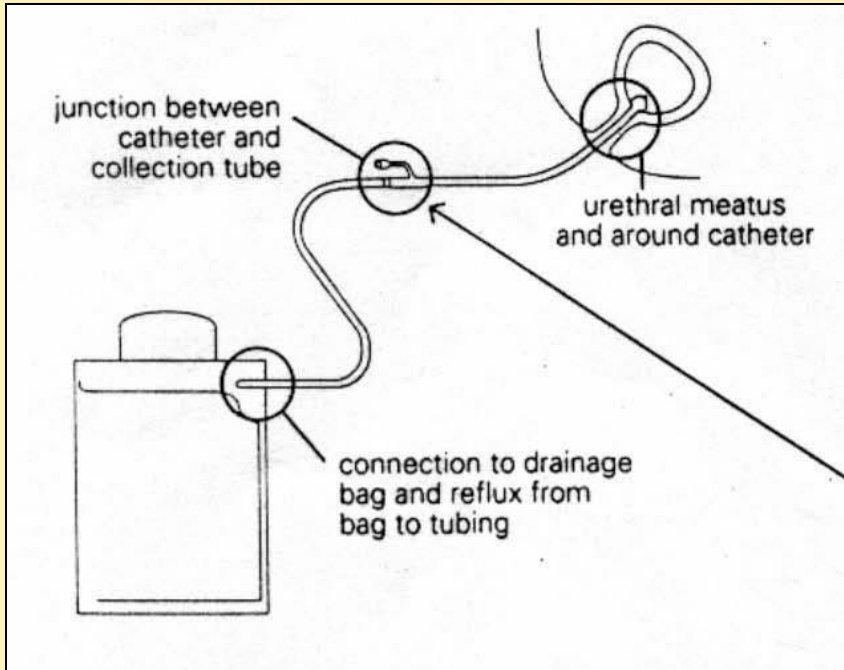
Important aspects of Microbiologic Examination of UTI:

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

Type of Specimens

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



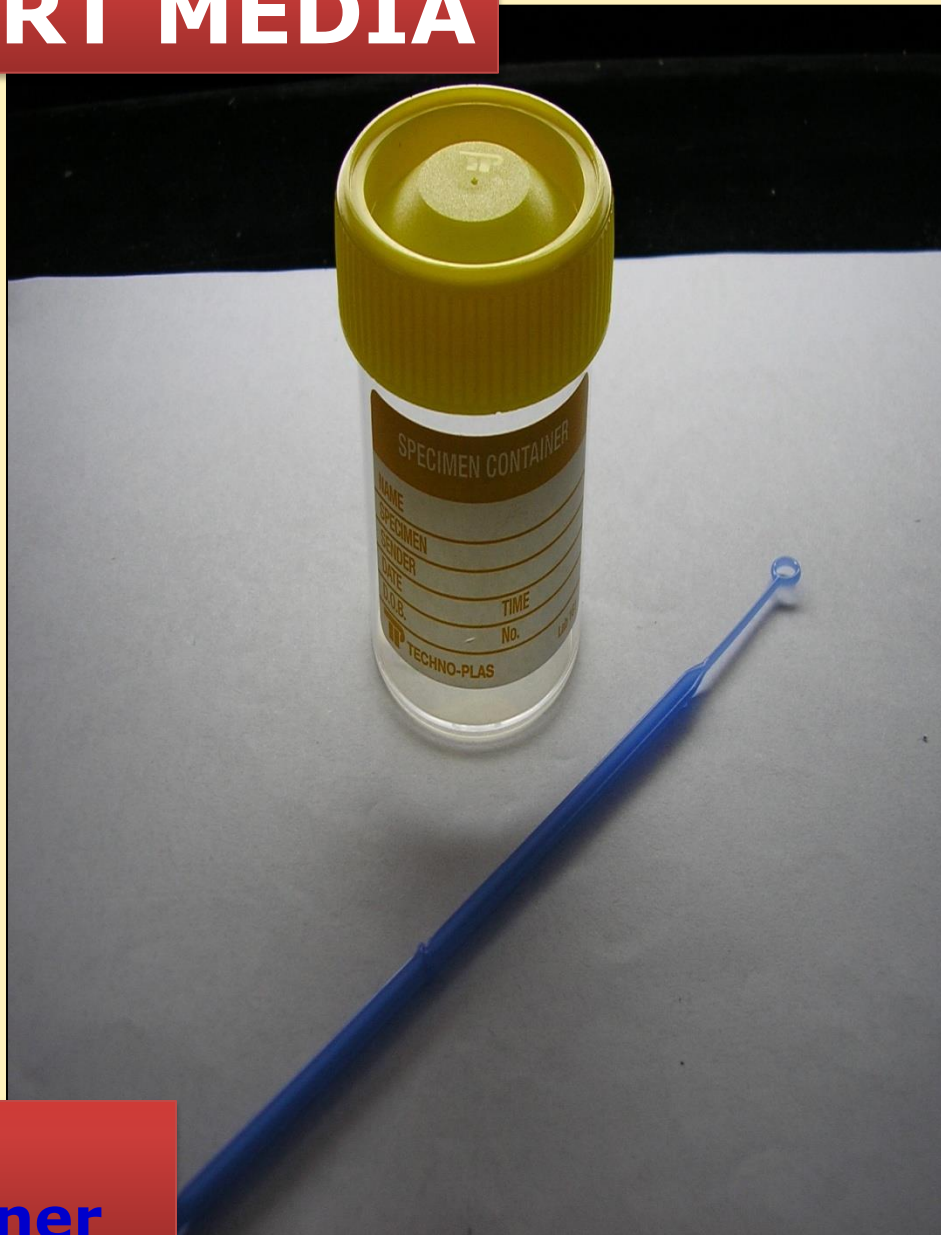


The urinary catheter

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

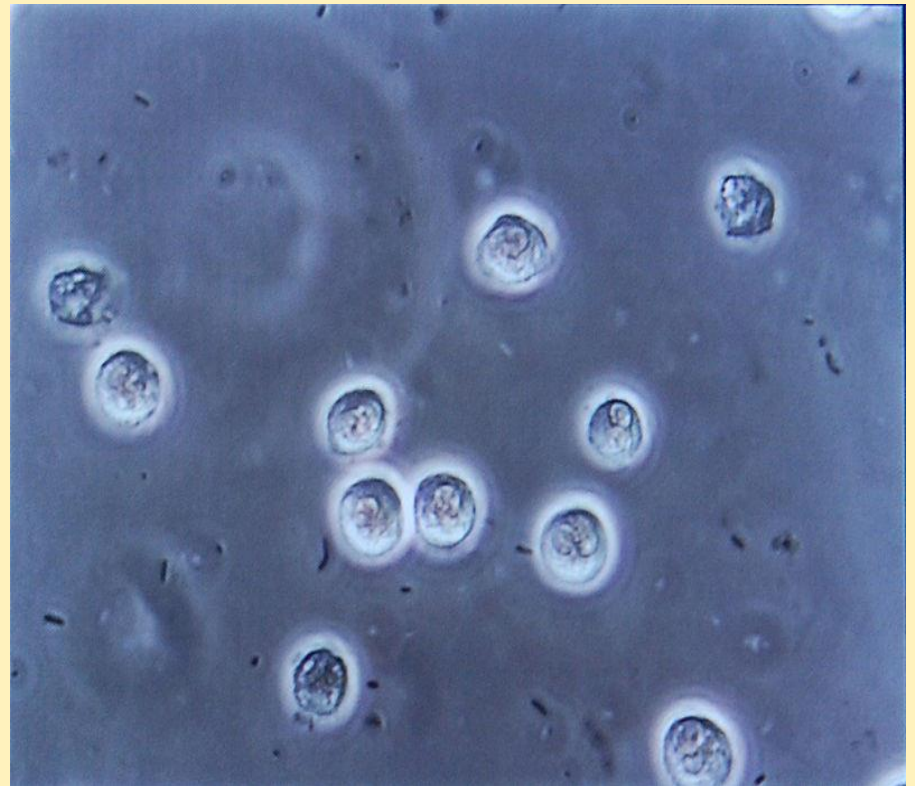
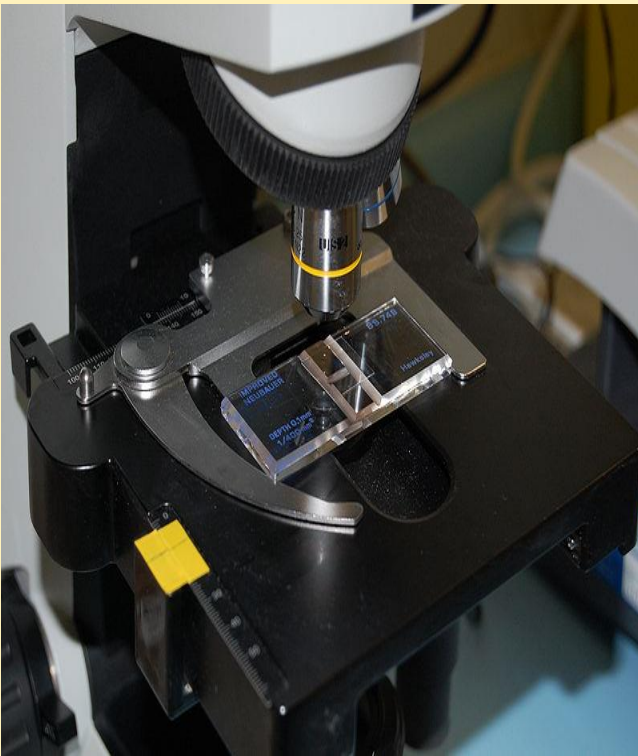


**Sterile
Urine container**

Urine analysis;

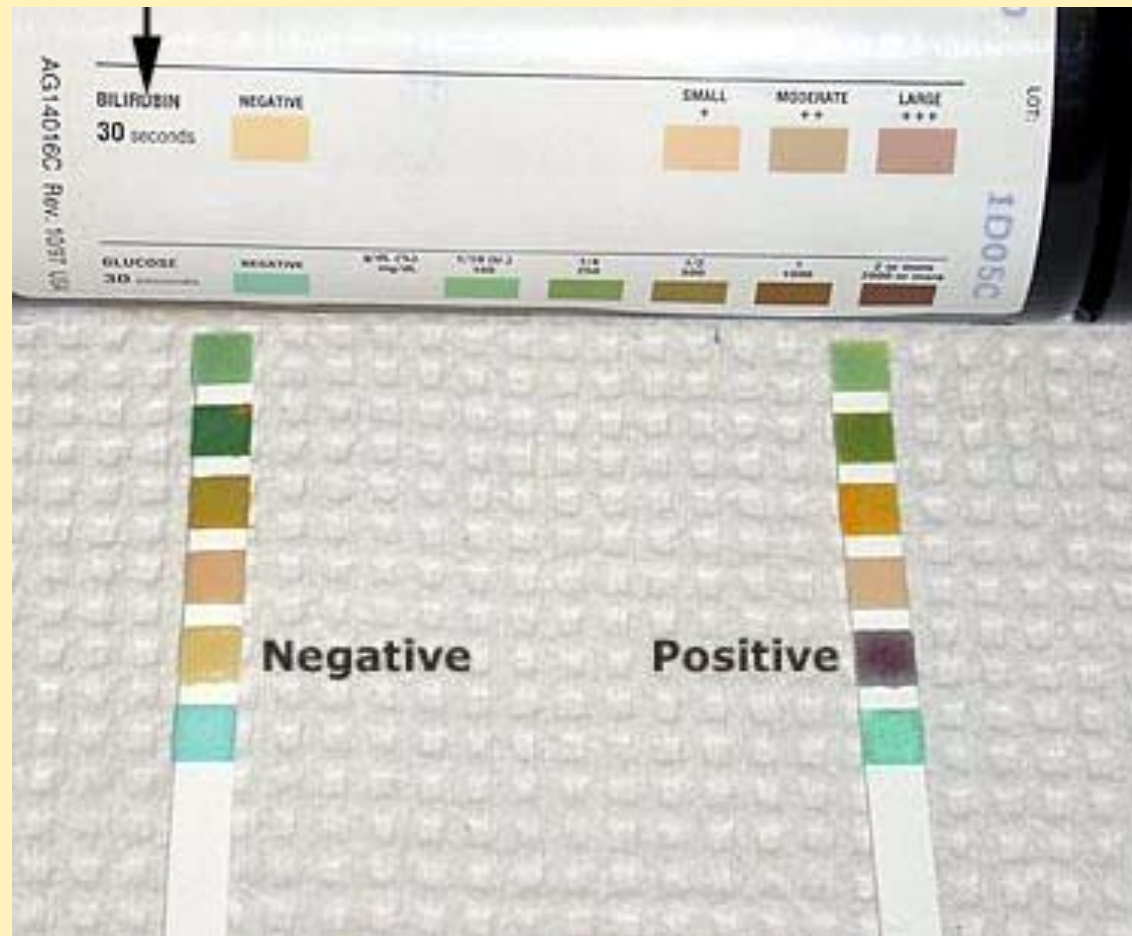
1- Dip stick (leukocyte esterase ,nitrate test)

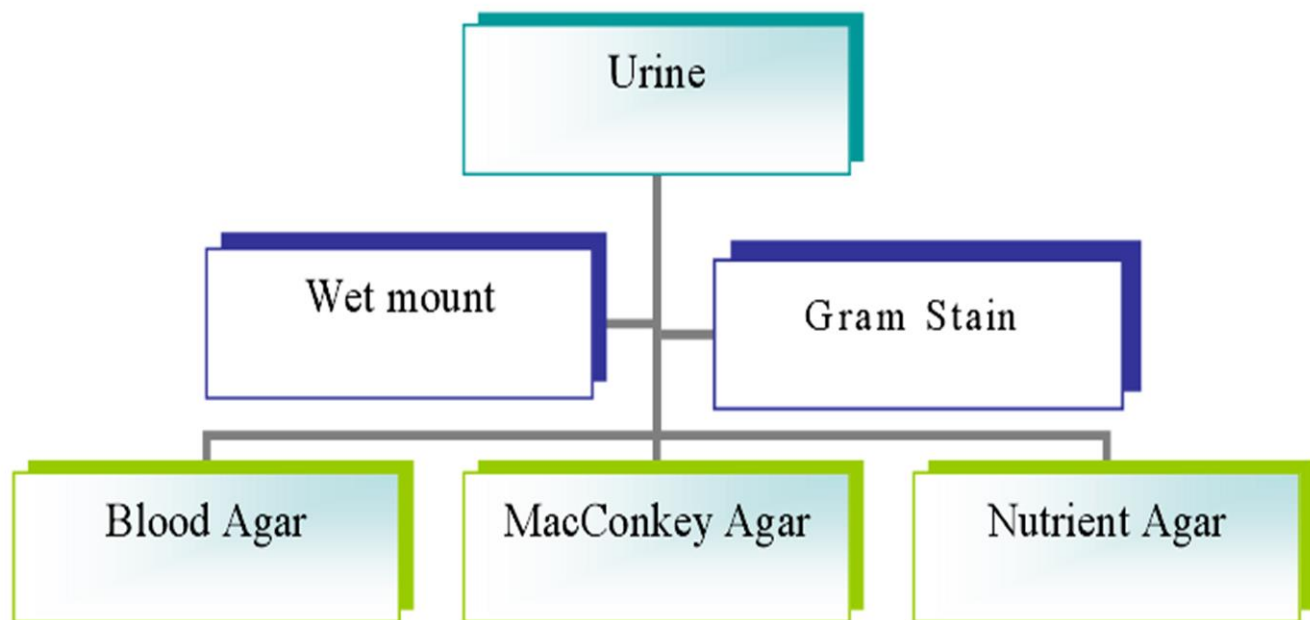
2-microscopic ex; cell-counting chamber



Urine analysis;

1- Dip stick (leukocyte esterase ,nitrate test)





culture media

blood agar



an enriched medium

MacConkey agar



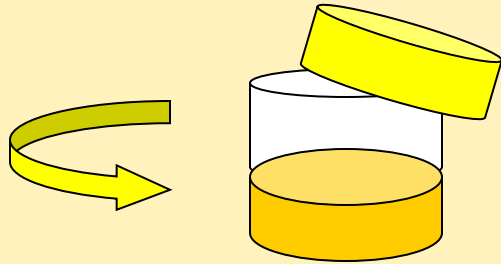
a differential medium

CLED agar

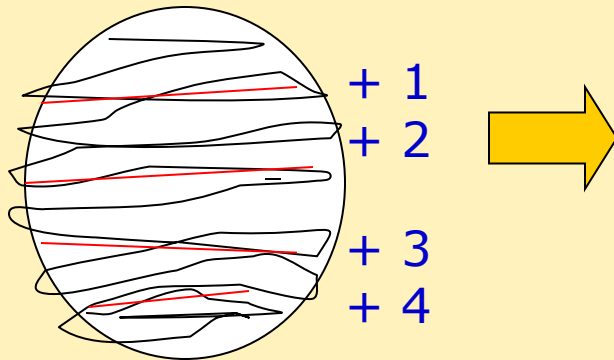


Selective medium

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,

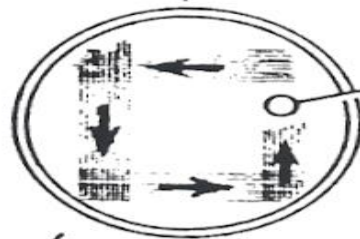
Over night incubation

RESULT

(3 mm internal diameter)

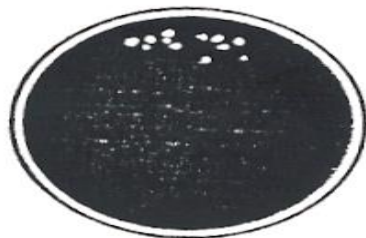


Inoculate plate
without flaming
loop between
strokes

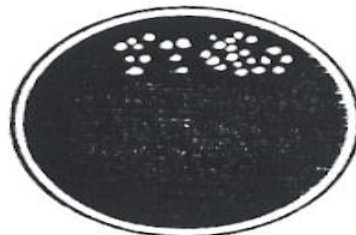


Loop charged with
approximately 1/300 ml
of urine

Possible results



<30 colonies
<10⁴ organisms/ml
Not significant



30 colonies
10⁴ organisms/ml
Doubtful significance



>300 colonies
>10⁵ organisms/ml
Significant

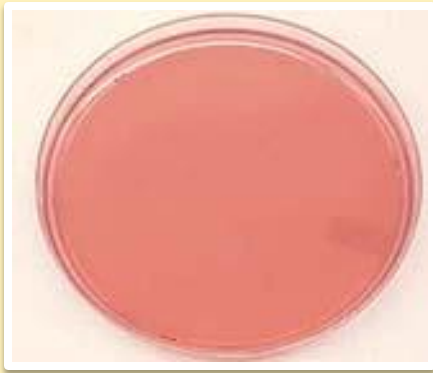
Diagram illustrating the semi-quantitative culture of urine specimens.

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>	

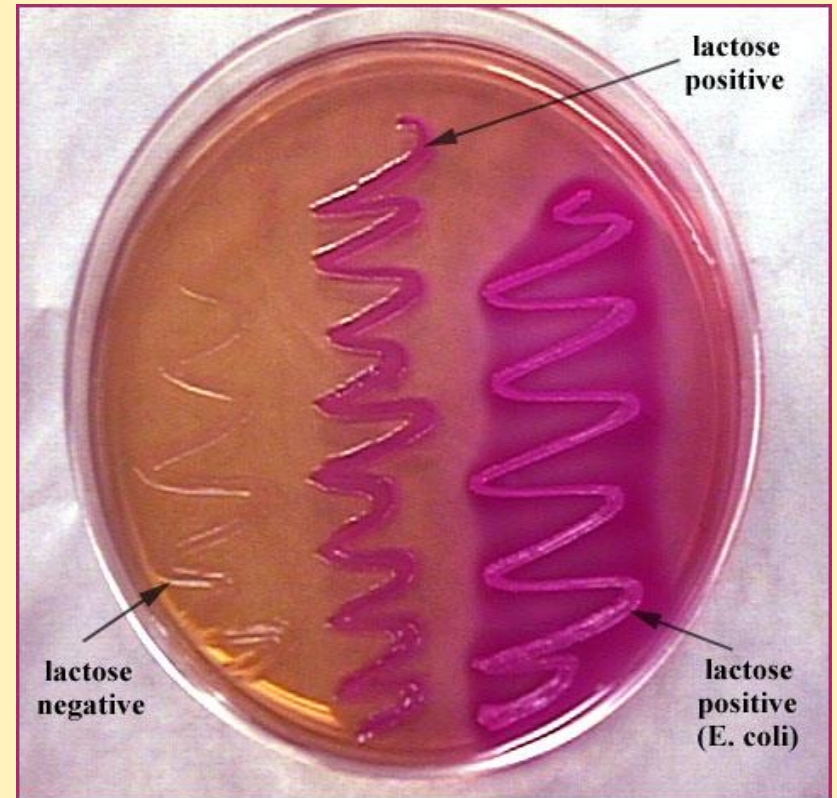
• Other organisms ;

- *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



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

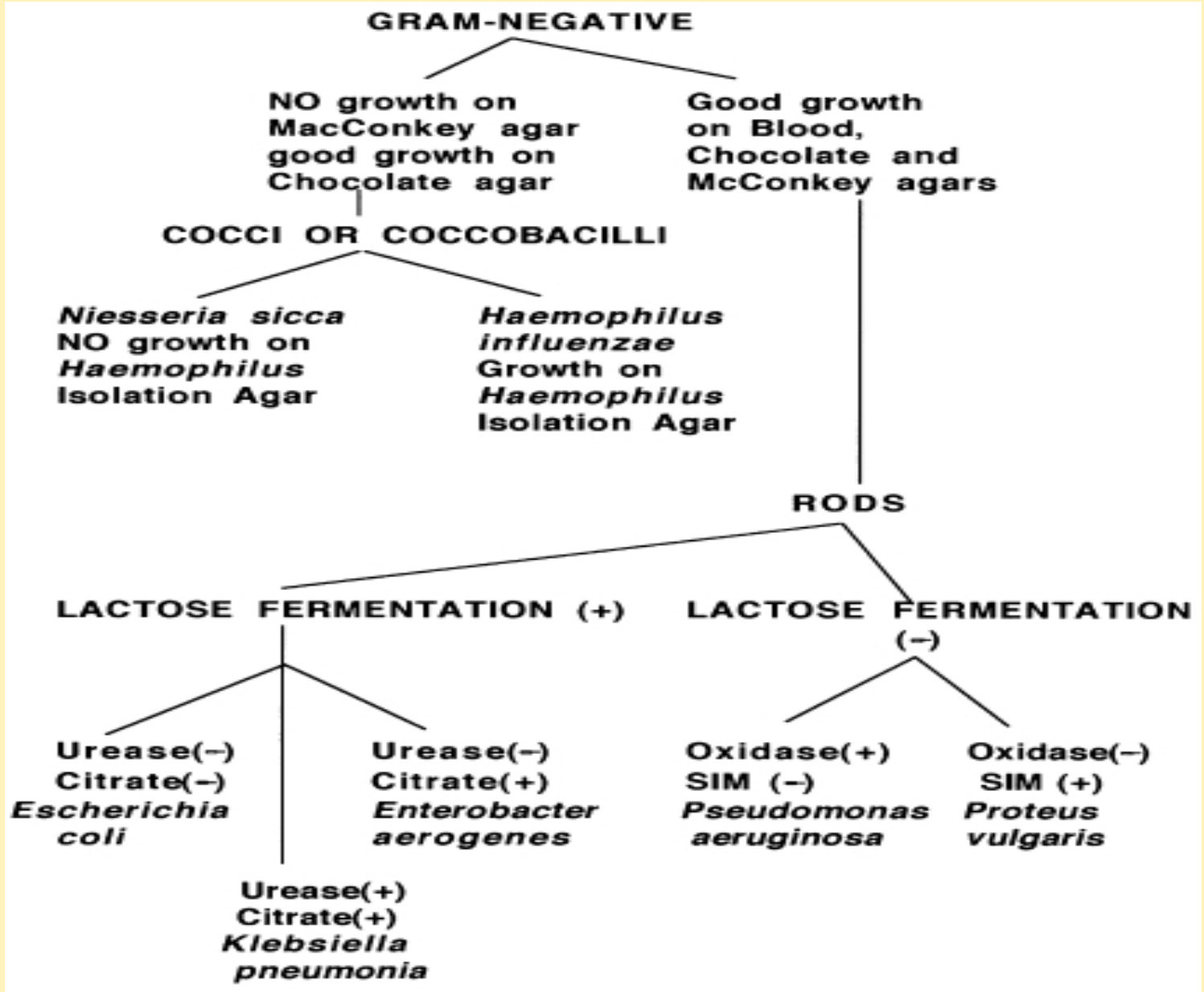


MICROSCOPIC APPEARANCE:

Gram negative bacilli



gram negative bacilli

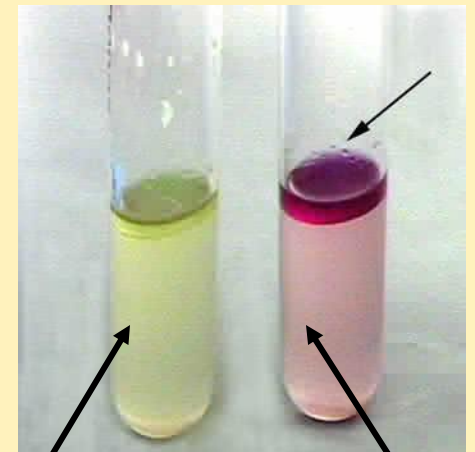
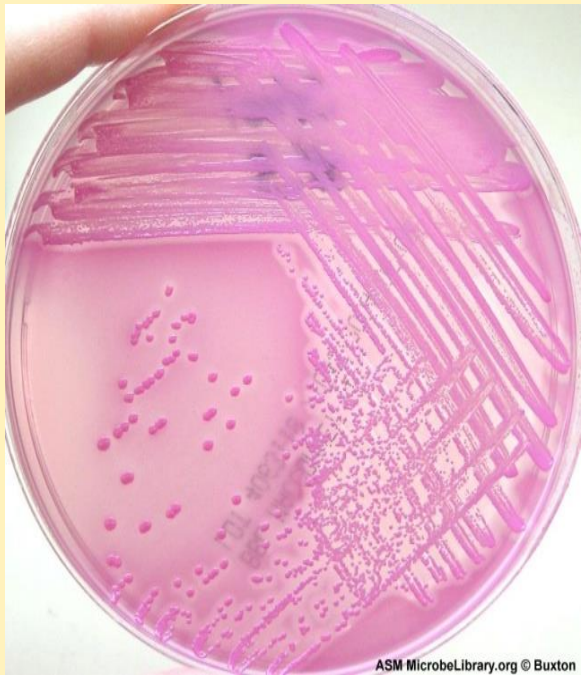


MacConkey's agar plate showing growth of:
Lactose fermenter pink colonies

e.g. *E. coli*



E coli



Indole Reactions
Negative **Positive**

Indol Test

Indol Test



**Pink color ring
Indol positive**



**+Ve
*E. coli***

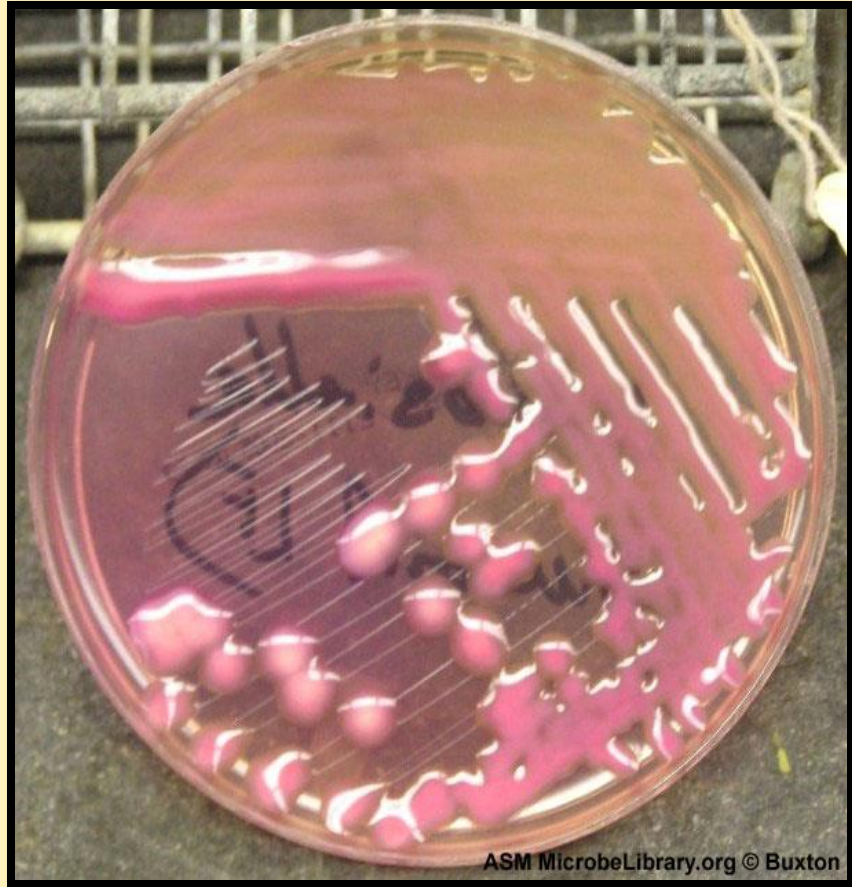
**No change in color
Indol negative**



**-Ve
*Klebsiella
Enterobacter***

MacConkey's agar plate showing growth of:
Lactose fermenter pink colonies

Klebsiella



CLED agar plate showing growth of:
mucooid coloneis

Klebsiella



MacConkey's plate showing growth of:
Non - Lactose fermenter pale colonies

e.g. *Proteus*



Blood culture palate showing :

Swarming growth of *Proteus*



Urease Test

Urease Test



Pink colour
Urease positive



+Ve
Proteus

Yellow colour
Urease negative



-Ve
Salmonella
Shigella

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

Proteus growth : Swarming



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

Nutrient agar plate showing growth

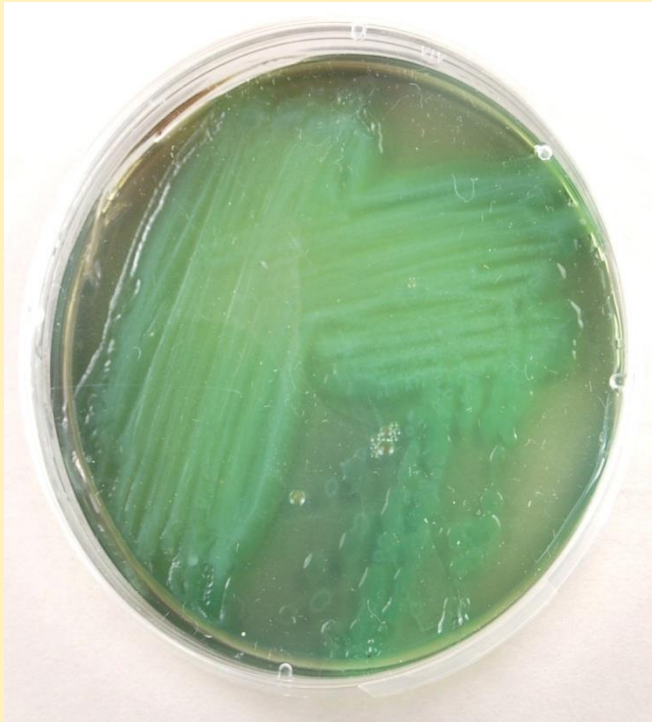
of:

Blue-green colonies

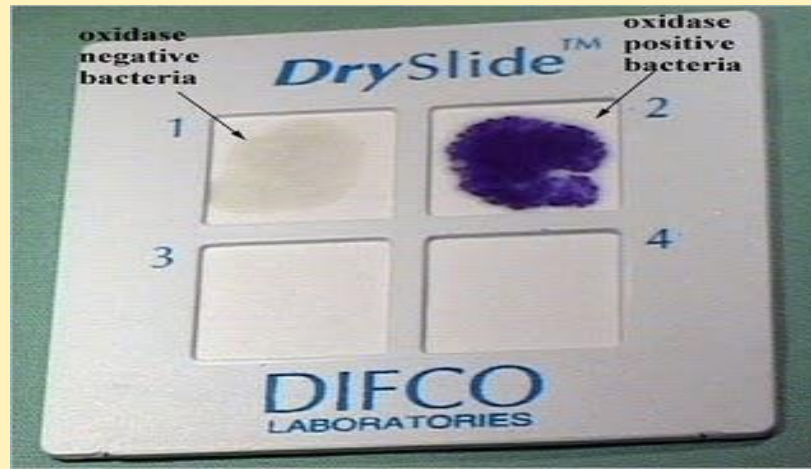
Pseudomonas



Pseudomonas aeruginosa



ASM MicrobeLibrary.org © Buxton



a

b

c



Three API 20E strips :

- a. Immediately after inoculation
- b. After 24 hours incubation
- c. 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



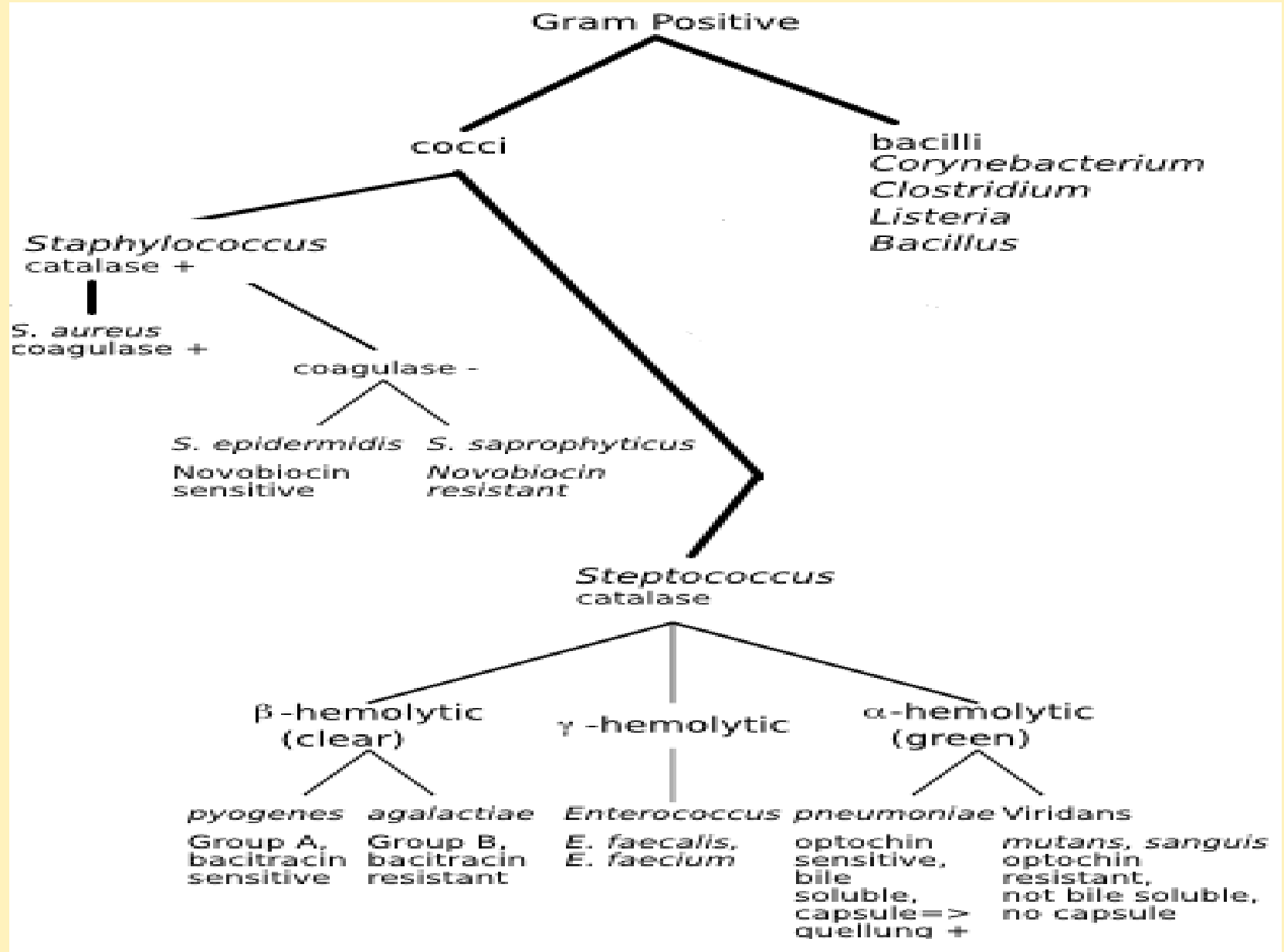
Salmonella



E. coli



Klebsiella pneumoniae



Enterococcus species



Bile Esculin hydrolysis



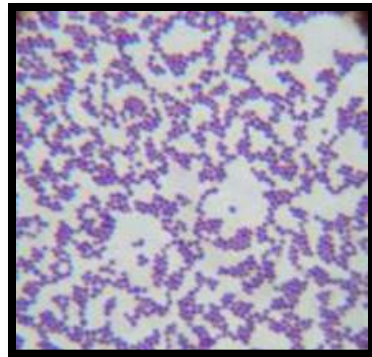
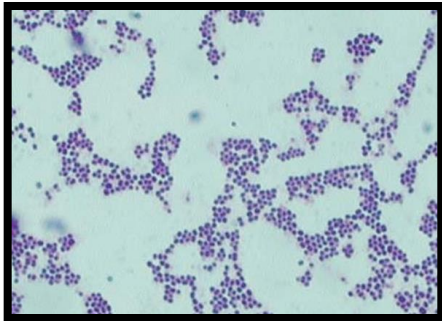
Both Group D streptococci and enterococci produce a positive (left) bile Esculin hydrolysis test.



MICROSCOPIC APPEARANCE

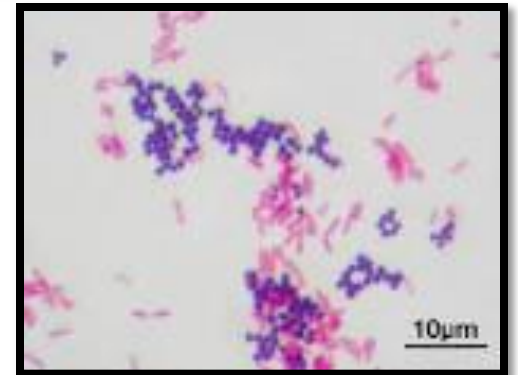
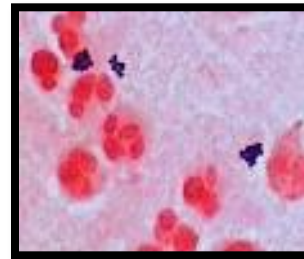
Gram positive cocci in clusters most likely staphylococci

FROM CULTURE



SMEAR FROM SPESIMEN:

Pus cells & Gram positive cocci in clusters



To differentiate between **Staphylococcus aureus** & **Staphylococcus epidermidis** use the following test:

1.COAGULASE TEST:

- **Tube coagulase test**
- **Slide coagulase test**

2. DNAase TEST

3.MANITOL FERMENTATION TEST

1-COAGULASE TEST

Slide coagulase test

Procedure:

Mix bacterial colony with plasma on a

S



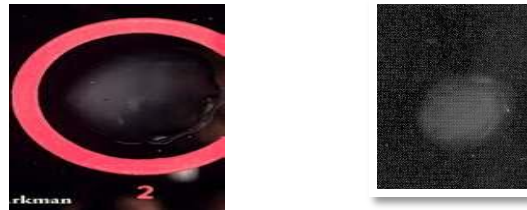
Granules



+Ve

Staphylococcus aureus

smooth

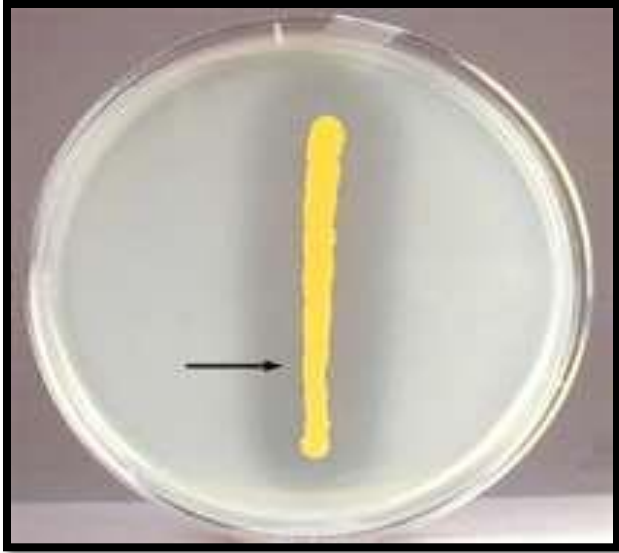


-Ve

Staphylococcus epidermidis

2-DNAase TEST

Clear area around the growth



Staph. aureus

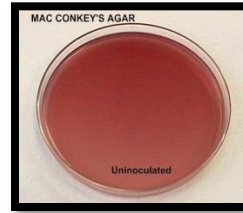
NO clear area around the growth



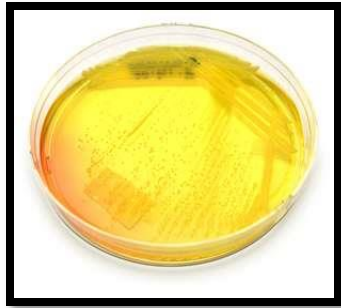
Staph. epidermidis

3-MANETOL FERMINTATION TEST

Uniculated Manitol Salt Agar



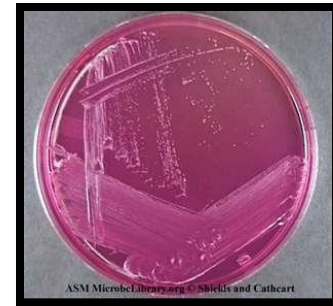
Yellowish colored colonies and medium



+Ve

Staphylococcus aureus

White color colonies with no change in medium



-Ve

Staphylococcus epidermidis

Blood agar plate showing growth of :

Staphylococcus aureus



Colonies are golden yellow in color

Staphylococcus spp



Staphylococcus aureus
Golden colonies (yellowish)

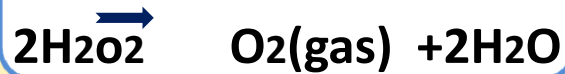
Staphylococcus epidermidis
white colonies

CATALASE TEST

Procedure:

Mix the colony in a drop of hydrogen peroxide (H₂O₂)

Bubbles come out



+Ve

Staphylococcus

No Bubble

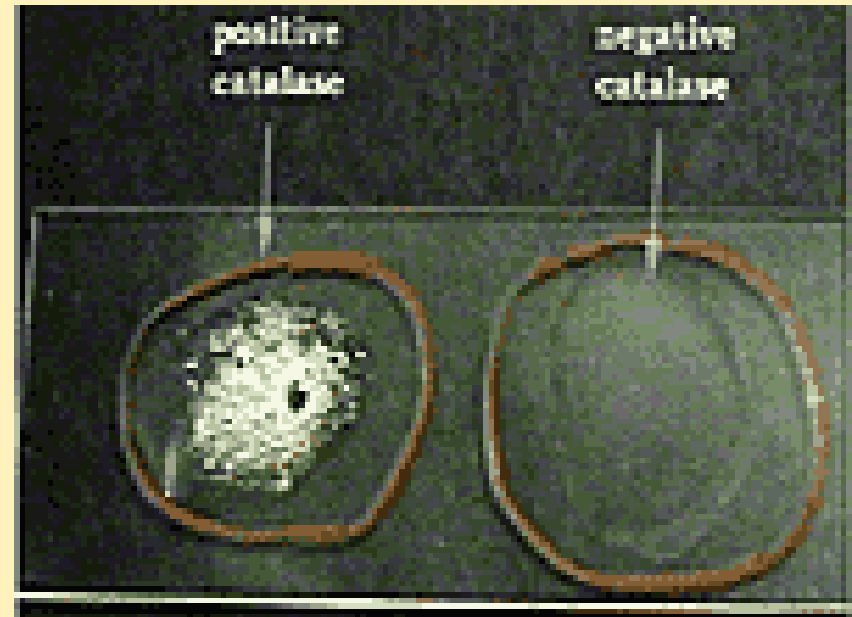
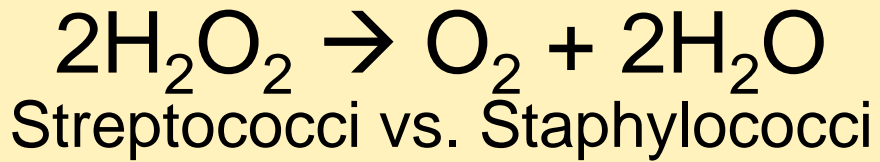


-Ve

Streptococcus

Differential Characteristics

Catalase

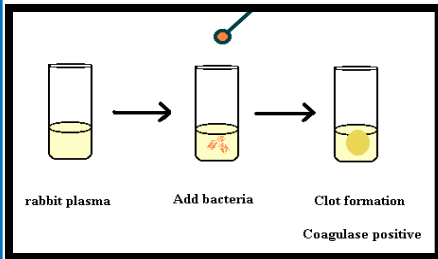


COAGULASE TEST

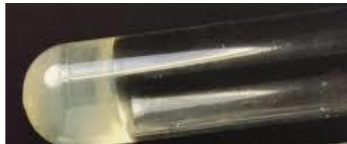
Tube coagulase test

Procedure:

Mix bacterial colony with plasma in tube



Clotting of plasma



+Ve

Staphylococcus aureus

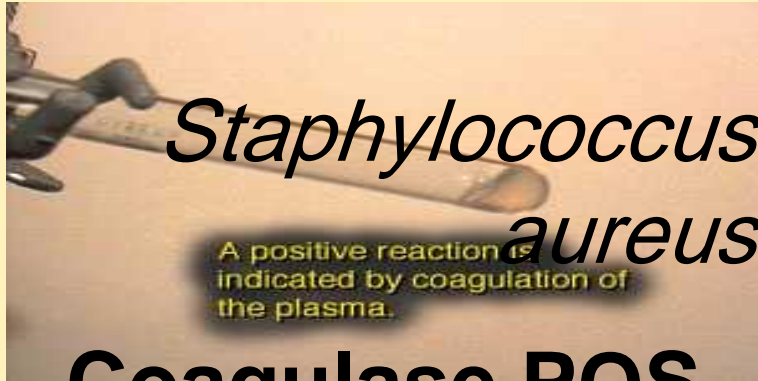
No clotting



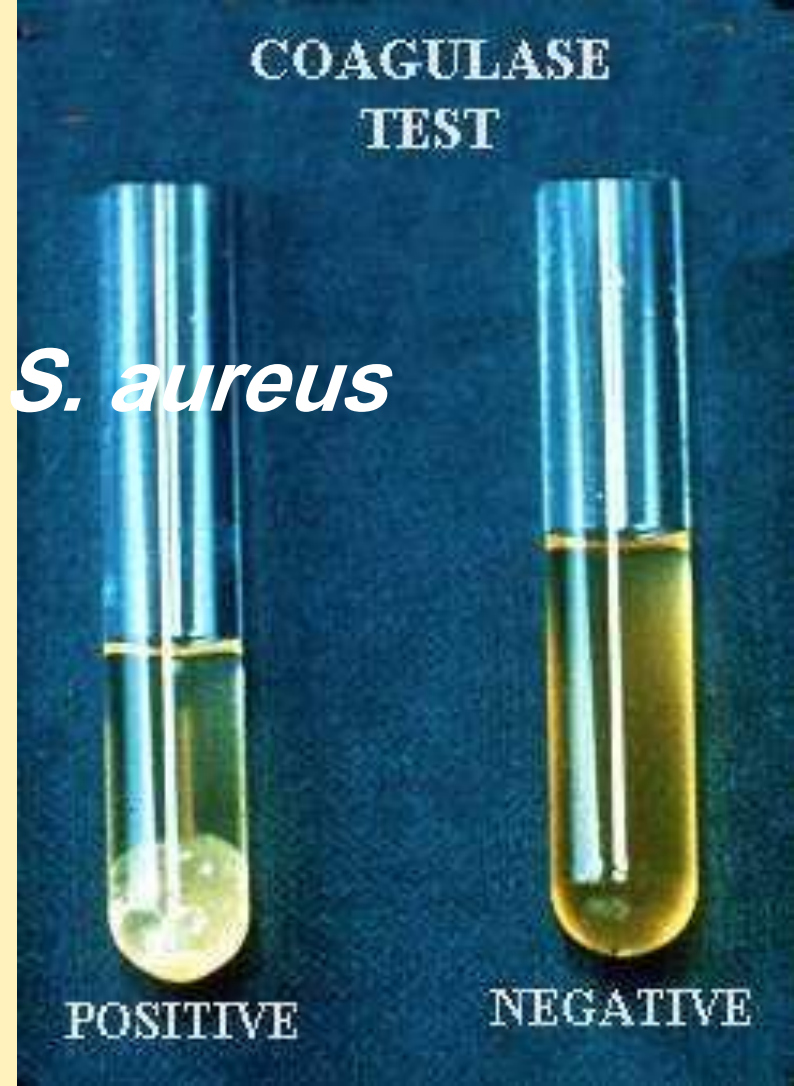
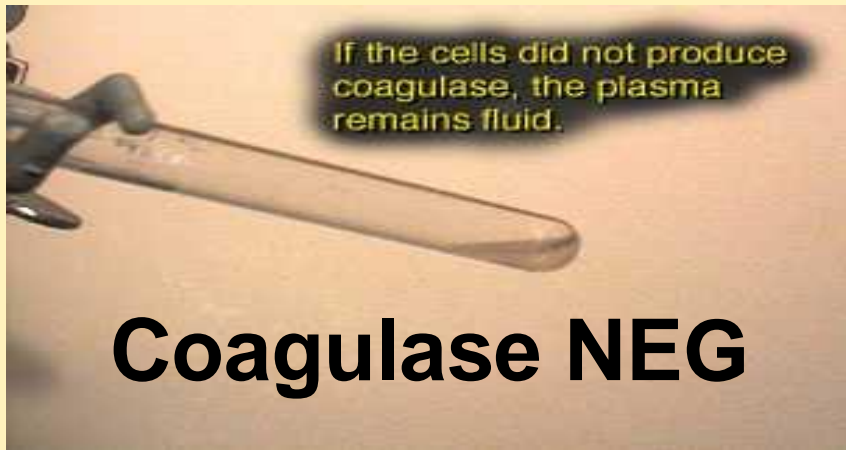
-Ve

Staphylococcus epidermidis

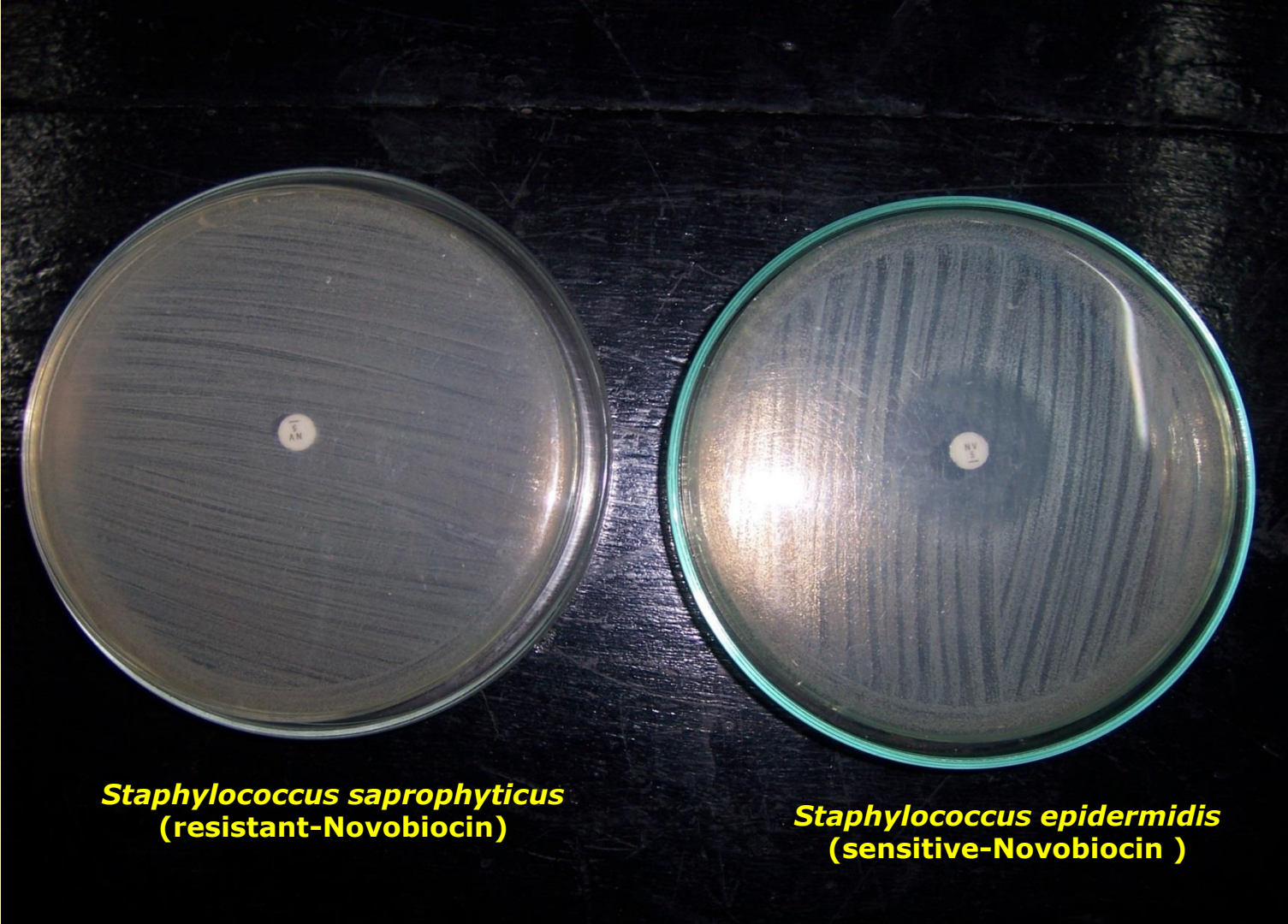
Differential Characteristics



Coagulase POS



NOVOBIOCIN TEST



Staphylococcus saprophyticus
(resistant-Novobiocin)

Staphylococcus epidermidis
(sensitive-Novobiocin)



[A]



[B]



[C]



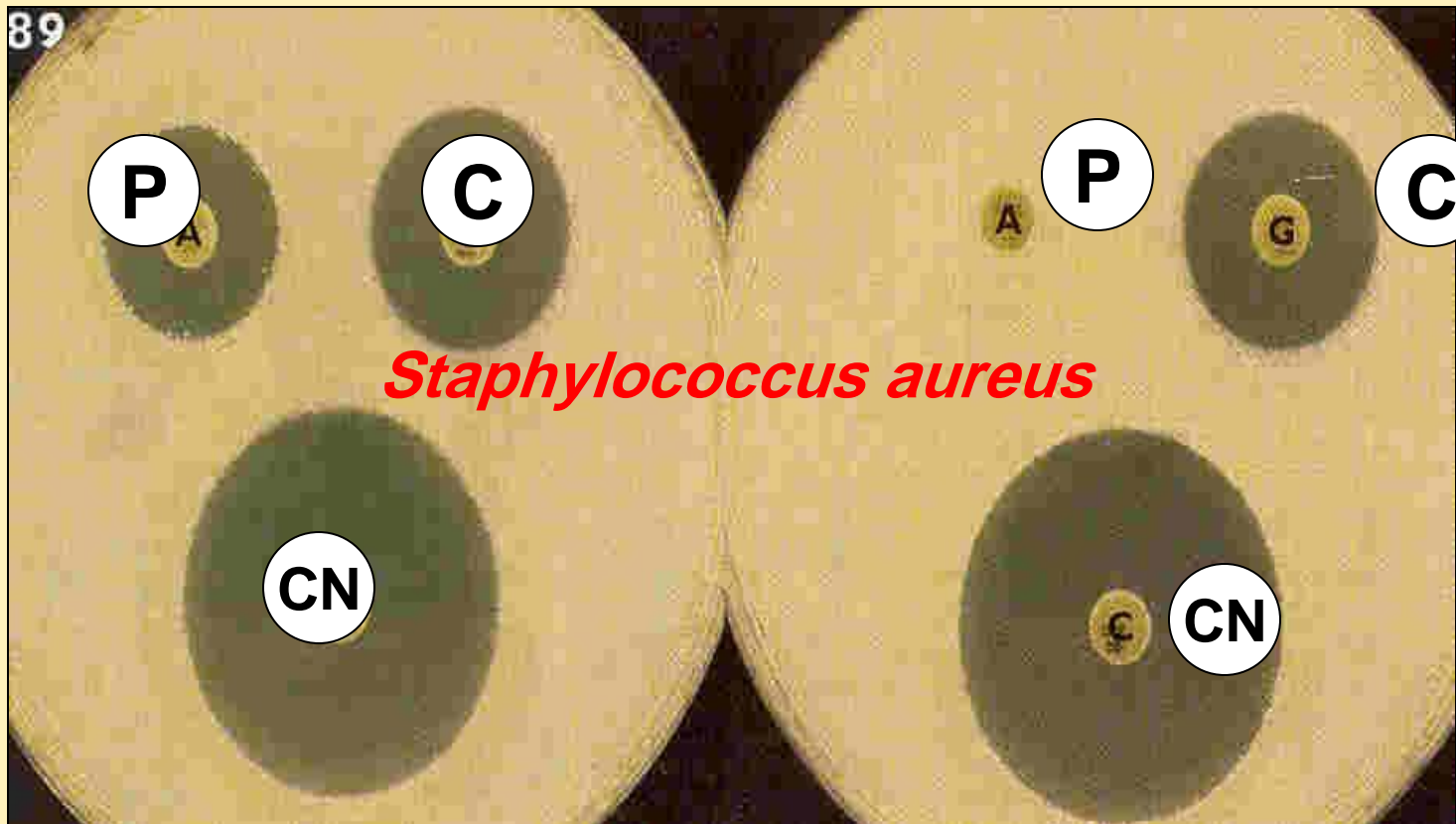
[D]



[E]

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

R (Resistant) ; S (Sensitive)



Antibiotic sensitivity test: Agar diffusion method



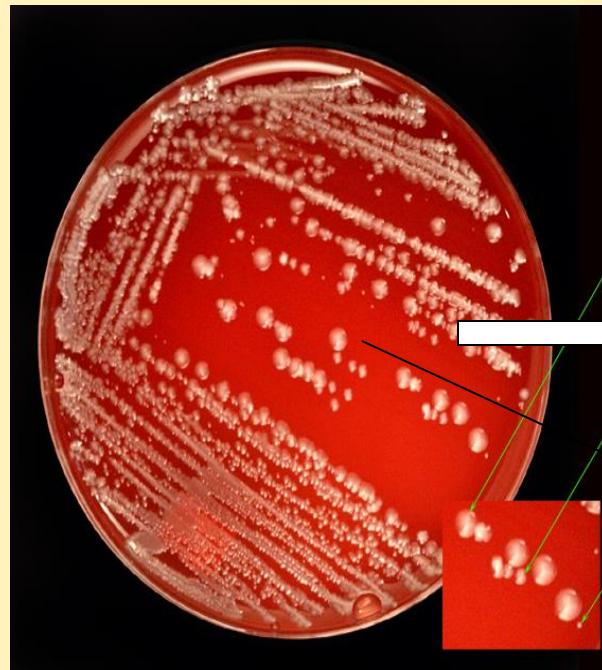
Case 1

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.

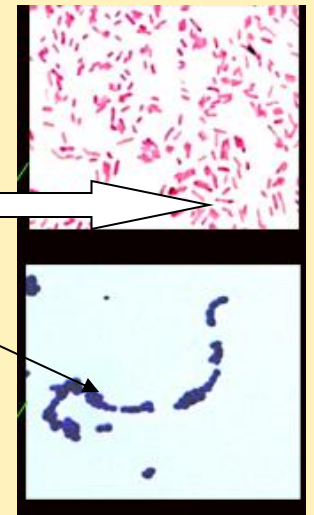
- Identify the colonies on the blood agar plates and photographs. The photographs show the results of the Gram stain of each colony type.
- Large colonies are Gram.....and small colonies are Gram.....



CLED plate



Blood agar



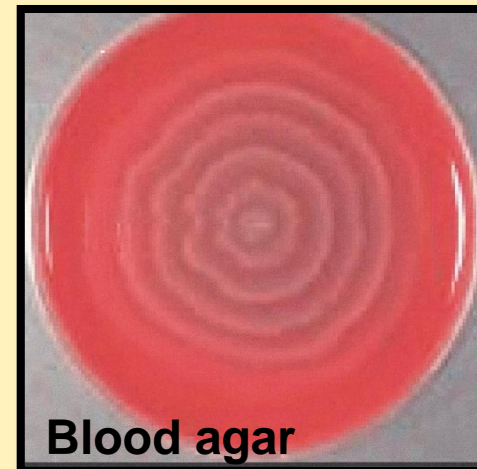
Gram stain

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

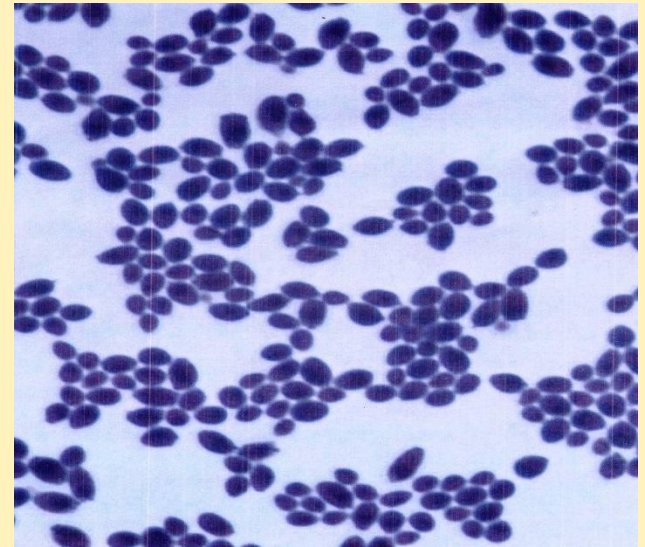
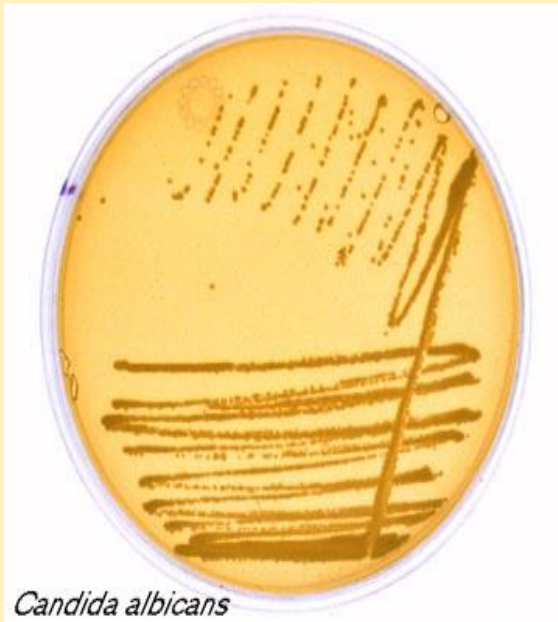


- A) What is the likely this pathogen?
- B) How would you confirm the identity of this pathogen?
- C) What is the role of this organism in forming stones?

Candida albicans

**Growth on Sabouraud's
Dextrose Media**

***Candida albicans* on blood agar;**



1. CHLAMYDOSPORE TEST.

CULTURE OF *CANDIDA ALBICANS* ON CORN MEAL
AGAR SHOWING CHLAMYDOSPORE



Candida albicans: identification tests

- Chlamydospore

- Germ tube test

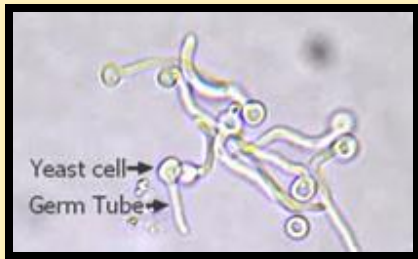


2. GERM TUBE TEST

USE:

FOR IDENTIFICATION OF *CANDIDA ALBICANS*

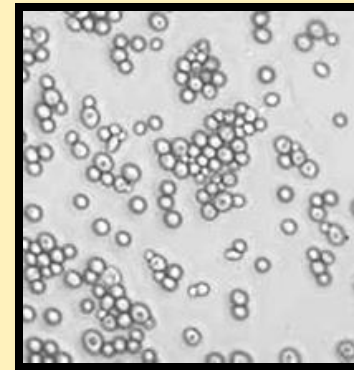
POSITIVE TEST



+Ve

CANDIDA ALBICANS

NEGATIVE TEST

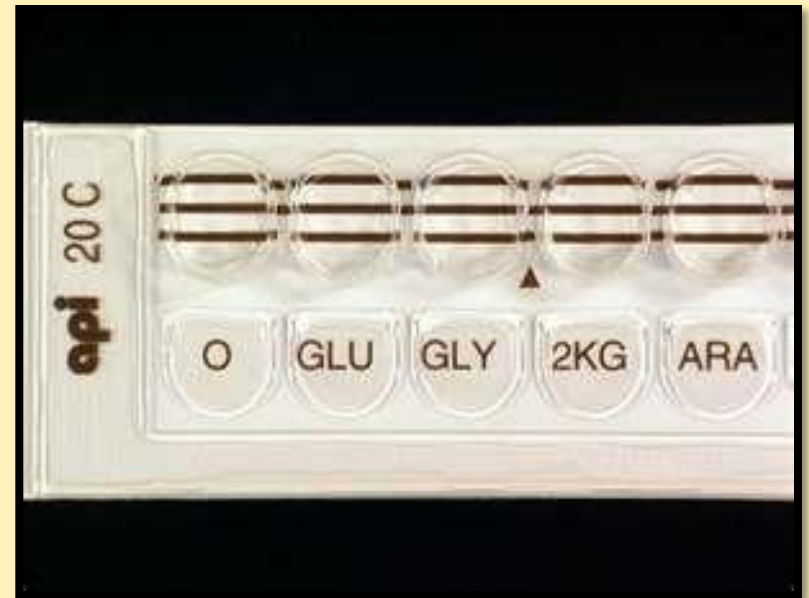


-Ve

OTHER CANDIDAS

3.API 20C KIT (ANALYTICAL PROFILE INDEX)

FOR IDENTIFICATION OF YEAST BY ASSIMILATION



Schistosoma haematobium



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

discussion

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

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