دانشگاه اصفهان
دانشکده علوم و فناوری های زیستی، گروه زیست شناسی سلولی مولکولی و میکروبیولوژی،
آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسی ۱

جمع آوری نمونه از موارد کلینیکی (خون،ادرار، مدفوع) و کشت، جداسازی و خالص سازی و بررسی خصوصیات ماکروسکوپی و میکروسکوپی و شناسایی باکتری های گرم مثبت از نمونه مجهول

تهیه کننده:سهیلا عباسی

Blood culture

Blood culture is different from culture of other samples in that the patient should be taken to laboratory or Media and other materials be taken to patient's bedside. The **blood should be collected aseptically** and introduced directly into the blood culture bottle.

Following points are to be considered for blood culture to be done appropriately

Skin Asepsis:

- The likelihood that a **positive blood culture represents infection rather than contamination** is, at least in part, a function of skin antisepsis at the time of collection of blood for culture. The recommended antiseptic preparations are **70% Ethanol**, followed by **Chlorohexidine**.

Methods of obtaining Blood for culture

- **Venipuncture** is the best method.
- Contamination rates for blood cultures obtained from **intravascular devices** is higher.

Number of blood cultures

- **Two - three blood cultures** within 24 hours from different sites and sittings **regardless of fever.**
- For suspected cases of sub-acute **bacterial endocarditis** (serious infection in endocardium of the heart) **several blood cultures** are needed

Volume of blood /culture

- In case of adult, 20-30ml
- For neonates, 1-2ml
- For children aged 1 month to 2 years, 2-3 ml
- For older children, 3-5 ml
- For adolescent, 10-20 ml

Media used for Blood culture

- Varieties of culture media are used. But **no one culture is sufficient** for isolation of all microorganisms.
- **Trypticase soy broth and Brain heart infusion broth** are most commonly used.
- These are supplemented with various additives by different companies which will be discussed later on.
- However, in **Isolator (lytic) method** no culture media is needed other than for subculture.

Blood to Broth Ratio

- Human blood contains a number of substances (e.g., **complement, lysozyme, and phagocytic WBCs**) cells **capable of inhibiting microbial growth.**
- Moreover, **Some of the patients are receiving antimicrobial agents** at the time blood for cultures is obtained.
- Thus, to optimize the diagnostic yield, **blood should be diluted adequately in the culture broth to minimize** the effect of these substances.
- **Dilution 1:10 was done previously.** However in ongoing methods, due to content of additives, **1:5 dilution is adequate; may be even lower.**

Neutralization and inactivation of Antimicrobials

For patients already receiving antimicrobial agents, several manufacturers have marketed products designed to counteract the potential inhibitory effect on growth. This is called **Antimicrobial Removal Device (ARD).**

- **BACTEC** (BD Diagnostic Instrument Systems, Sparks, MD) **uses antibiotic absorbing 'Resin'.**
- **BacT/Alert FAN media** (bioMerieux, Inc.) uses **'activated charcoal'.** FAN means **Fastidious Antibiotic Neutralization.**
- In isolator (Lytic) system, **Heparin** (anticoagulant) and **Saponin** (lytic agent) is present. In our institution (BIHSH), **SPS** (sodium polyanethol sulfonate 0.05%) is added which **counteract the inhibitory effects of blood** and some antibiotics specially **aminoglycosides.**

Atmosphere of Incubation

- **Traditional two-bottle blood culture** systems have included **one for aerobic** bottle and **one for anaerobic**.
- However, during the **past two decades**, the proportion of bacteremias due to obligate **anaerobes has decreased** substantially.
- In fact, **several recent studies** of adults and children have concluded that the **routine use of anaerobic blood culture bottles is not necessary** and have recommended that these bottles be used only selectively for patients **who are at high risk for bacteremia due to anaerobes.**

13

Length of incubation of blood cultures

- In routine circumstances, **5-day incubation** is sufficient for detecting the majority of pathogens.
- For **endocarditis and brucellosis longer period** may be needed. However, longer incubation rarely give positive result but increases rate of contamination.

Length of incubation of blood cultures

- In routine circumstances, **5-day incubation** is sufficient for detecting the majority of pathogens.
- For **endocarditis and brucellosis longer period** may be needed. However, longer incubation rarely give positive result but increases rate of contamination.

Parameters that may be useful in interpreting results include

- the identity of the microorganism,
- the presence of more than one blood culture positive for the same microorganism,
- and the presence of the same microorganism as that found in the blood from another normally sterile site.

- Microorganisms that almost always (>90% of isolates) represent true infection when isolated from the blood include:
  - *Staphylococcus aureus,*
  - *Escherichia coli* and other Enterobacteriaceae,
  - *P. aeruginosa,* S. *pneumoniae* and *Candida albicans.*
- Isolates from blood that rarely «5% of isolates) represent true infection include
  - *Corynebacterium* species,
  - *Bacillus* species,
  - and *Propionibacterium acnes.*

32

- **Coagulase-negative Staphylococci** are particularly problematic, not only because they are so ubiquitous, but also because 12%-15% of the blood isolates are pathogens rather than contaminants
- A useful interpretive concept is the number of culture sets found to be positive vs. the number obtained.
- If most or all cultures in a series are positive, regardless of the microorganism recovered, the probability that the organism is clinically important is high.

Of course, it is the physician who must ultimately make find the final judgment, taking into account not only the laboratory findings but also the clinical presentation.

33

Limitations of Blood Cultures

- Blood cultures, as described herein, currently represent the "gold standard" for diagnosis of septicemia.
- Nonetheless, they have limitations. Positive results require hours to days of incubation.
- No one culture medium or system in use has been shown to be best suited to the detection of all potential bloodstream pathogens.
- Some microorganisms grow poorly, or not at all, in conventional blood culture media and systems.

What is Culture urine test?

- A Culture urine test is performed to detect and identify the presence of bacteria and yeast in the urine which may be the cause of urinary tract infection

Why is Culture urine test done?

The Culture urine test is performed:

- To detect and diagnose a urinary tract infection (UTI) caused by bacteria or yeast in the presence of symptoms like
  - Painful or difficult urination with burning sensation
  - Increased urination frequency
  - Pain and pressure in the Lower abdomen (belly) and back
  - Undiagnosed fever or chills
  - Tiredness
- To screen for urinary tract infections in the first trimester of pregnancy

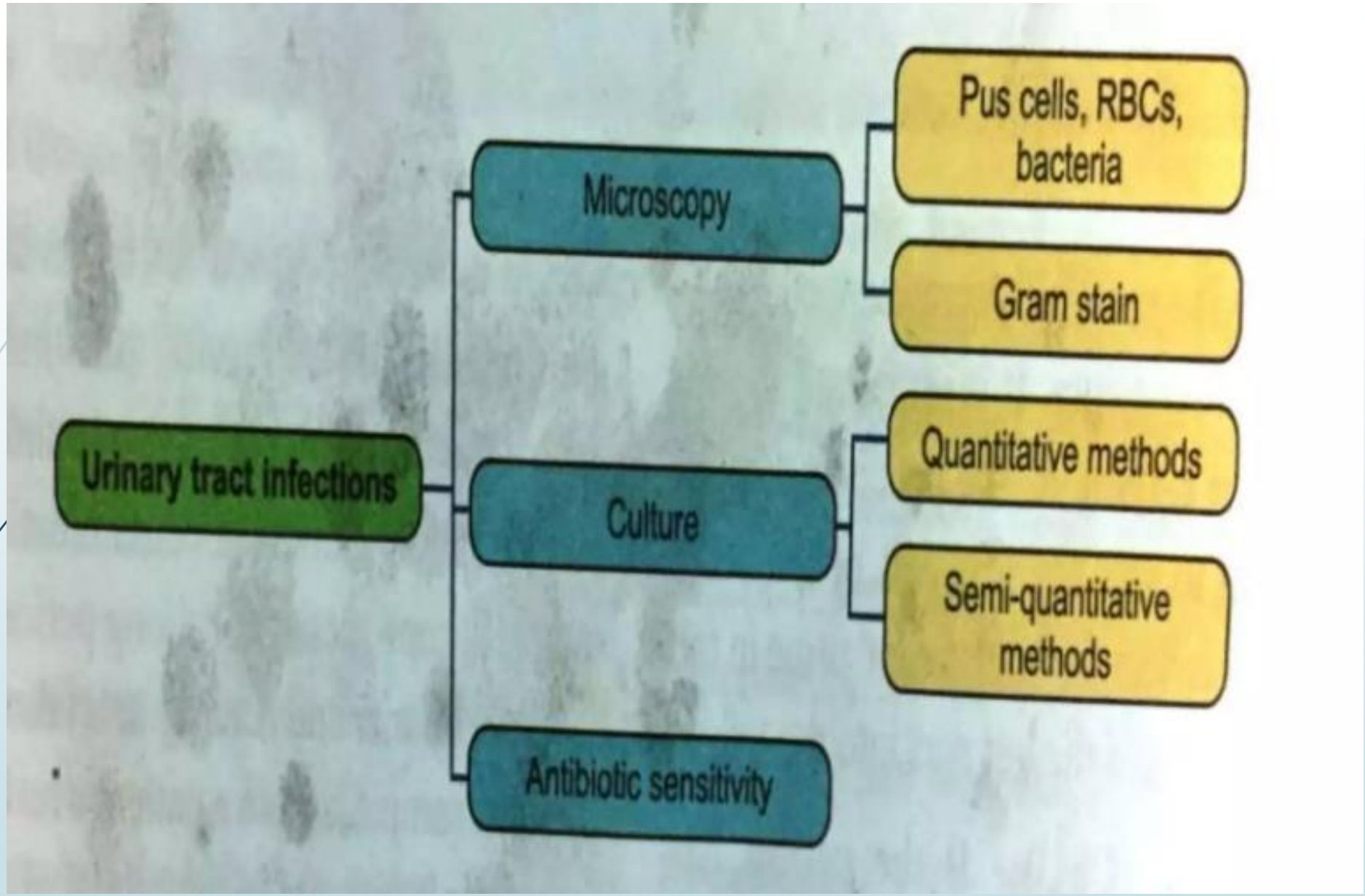
How is Culture urine test done?

- Culture urine test is done on a urine sample.
- The patient can collect the urine sample by itself.
- The sample can also be collected by inserting catheter into urethra to avoid contamination.

What does result of Culture urine test mean?

- Negative results indicate:
  - No bacterial colonies to less than 1,00,000 Colony Forming Units/ml

- Positive results indicate:
  - Bacterial colonies more than 1,00,000 Colony forming Units/ml
  - Patient undergoing antibiotic treatment: More than 1,000 Colony forming units/ml
  - For gram-positive bacteria like *Staphylococcus aureus*: Single colony


Urinary tract infections
Microscopy
Pus cells, RBCs, bacteria
Gram stain
Culture
Quantitative methods
Semi-quantitative methods
Antibiotic sensitivity

MICROBIOLOGY.....

- Microbiology is the study of living organisms that are invisible to the naked eye, such as .....
- Bacteria
- Virus
- Fungi

Microbiological Culture

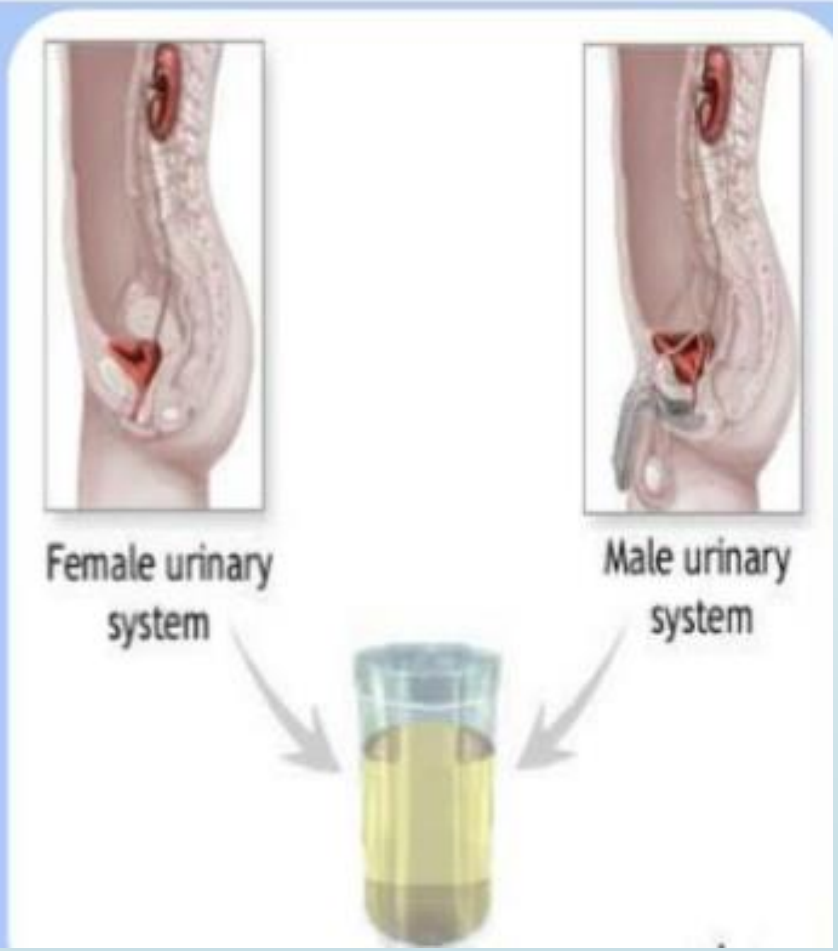
- It is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.
- Microbial cultures are used to determine the type of organisms and its abundance in the sample being tested or both

SPECIMEN COLLECTION

- The urine collected in a wide mouthed container from patients
- A mid stream specimen is the most ideal for processing
- Female patients passes urine with a labia separated and mid stream sample is collected

COLLECTION AND PRESERVATION OF URINE SPECIMENS

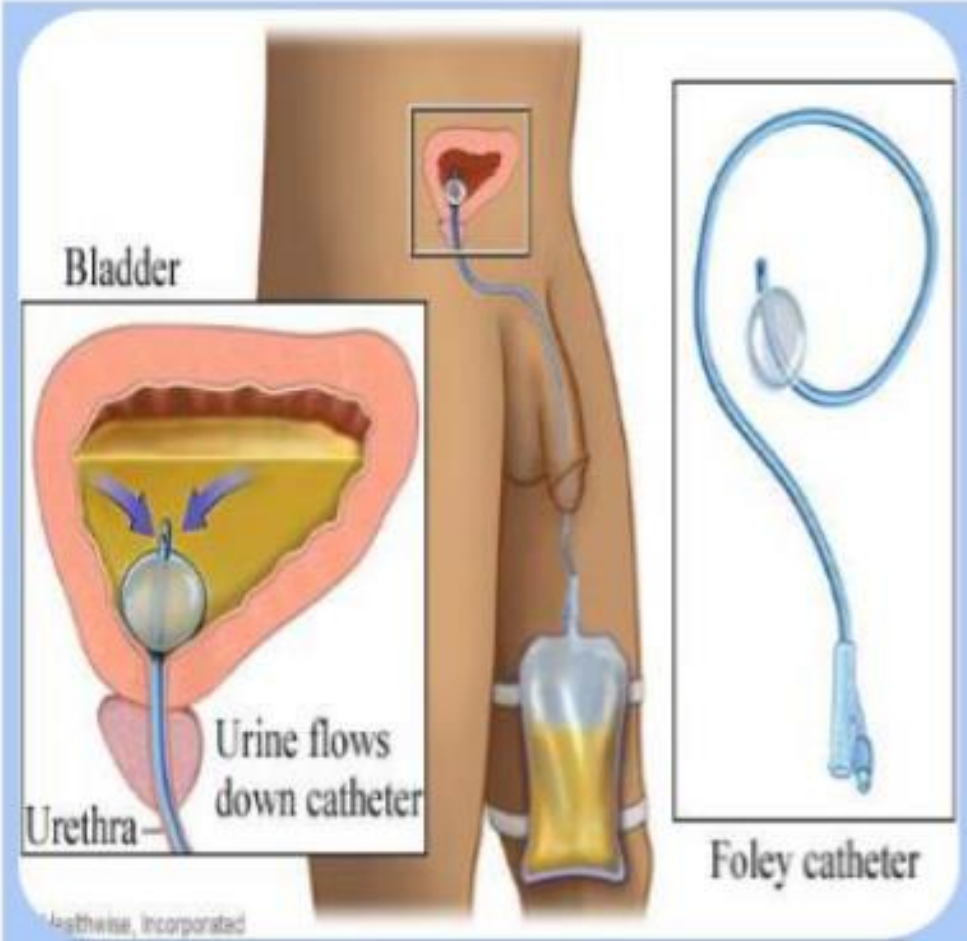
- Urine collected in sterile specimen container must be processed within 2 hours, or refrigerated and processed within 24 hours
- Urine collected in sterile specimen container with borate preservative should be processed within 24 hours (no refrigeration required)



COLLECTING URINE IN INFANTS AND YOUNG CHILDREN DIFFERS FROM ADULTS

- In infants, a urinary collection bag (plastic bag with an adhesive seal on one end) is attached over the labia in girls or a boy's penis to collect the specimen.

CATHETERIZED URINE



- Another method is the catheterized urine specimen in which a lubricated catheter (thin rubber tube) is inserted through the urethra (tube-like structure in which urine is expelled from the bladder) into the bladder. This avoids contamination from the urethra or external genitalia.

SENDING THE SPECIMEN TO LABORATORY

- If delivery of the urine specimen to the laboratory within one hour of collection is not possible, it should be refrigerated. The health care provider should be informed of any antibiotics currently or recently taken.

DIAGNOSIS OF URINARY TRAC INFECTION

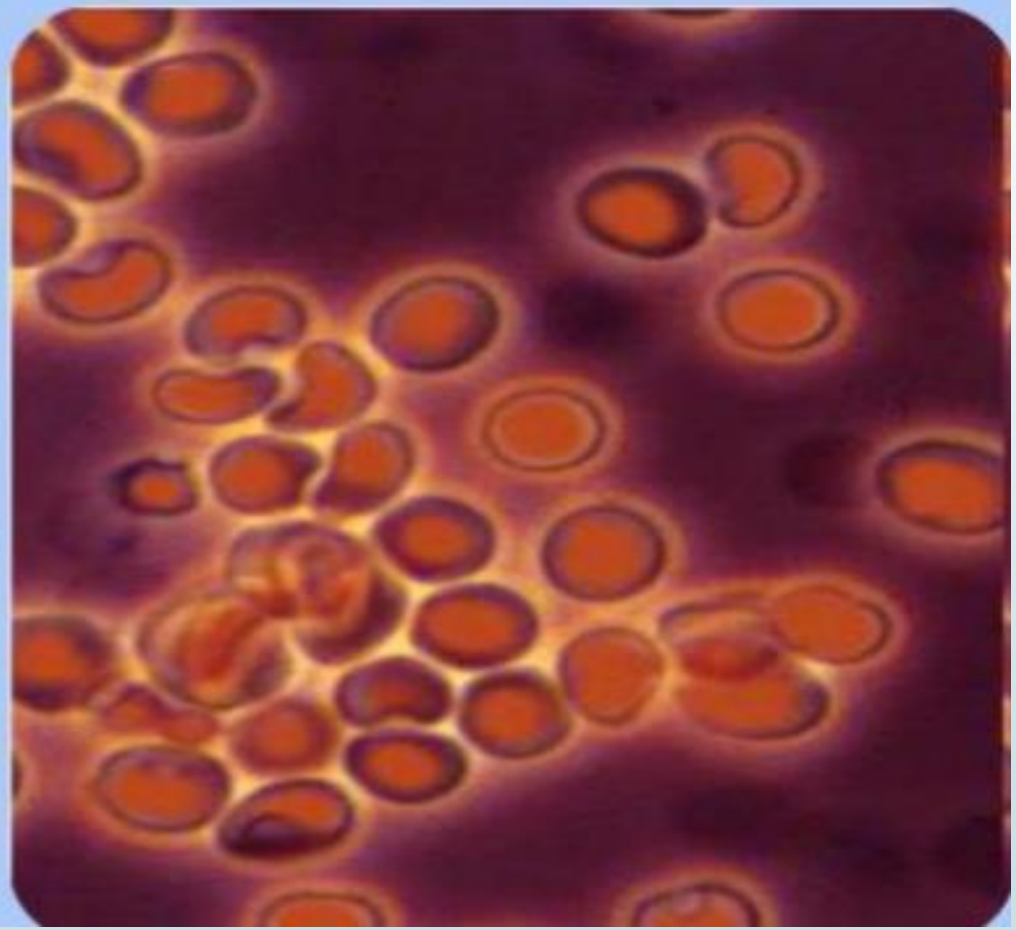
Step 1

Microscopy of Urine for detection of Pyuria, Leucocytes should be found in numbers of at least as great as 10^4 / ml before the pyuria is established

Dr.T.V.Rao MD 18

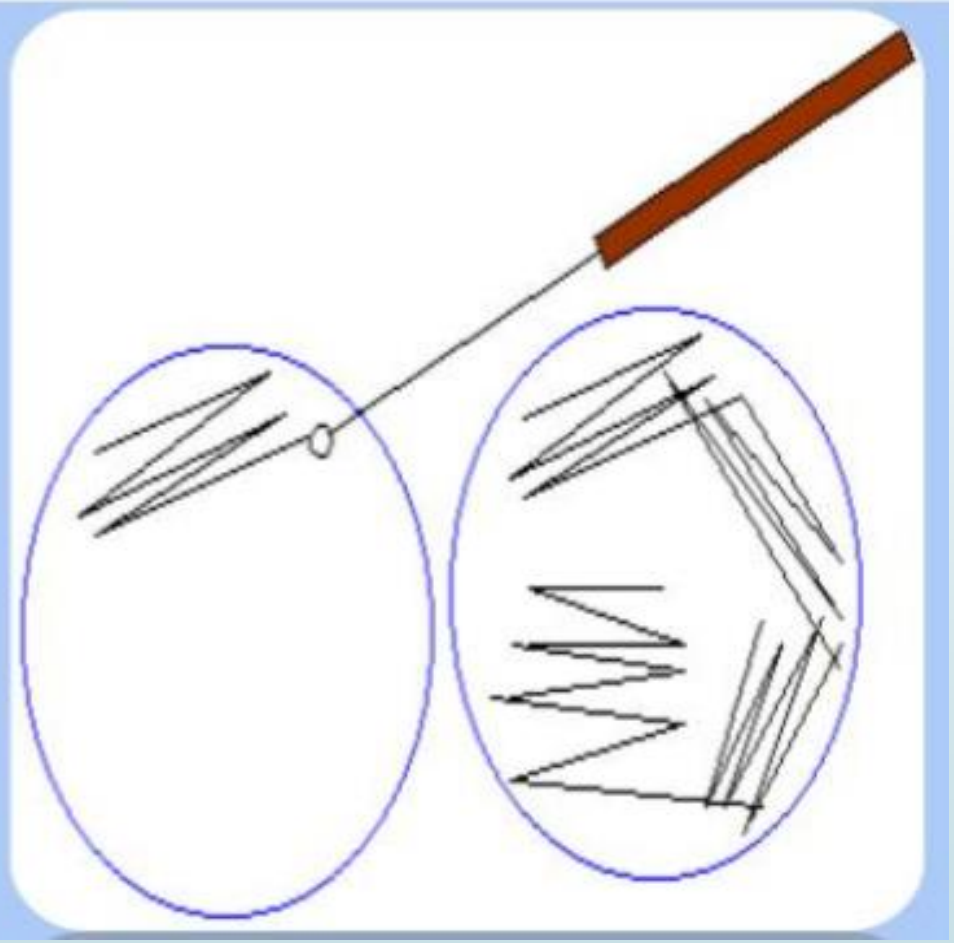
WET FILM EXAMINATION OF URINE

- All wet films to be examined with high power (x 40) objective.
- Prepare the drop of urine after mixing the urine without centrifugation
- Transfer 0.05 ml on the middle of the microscope slide and cover slip is applied.
- The prepared specimen show a small excess of fluid along the edges of the cover slip.
- A approximate finding of 1 leukocyte / 7 high power fields corresponds to presence of pyuria.

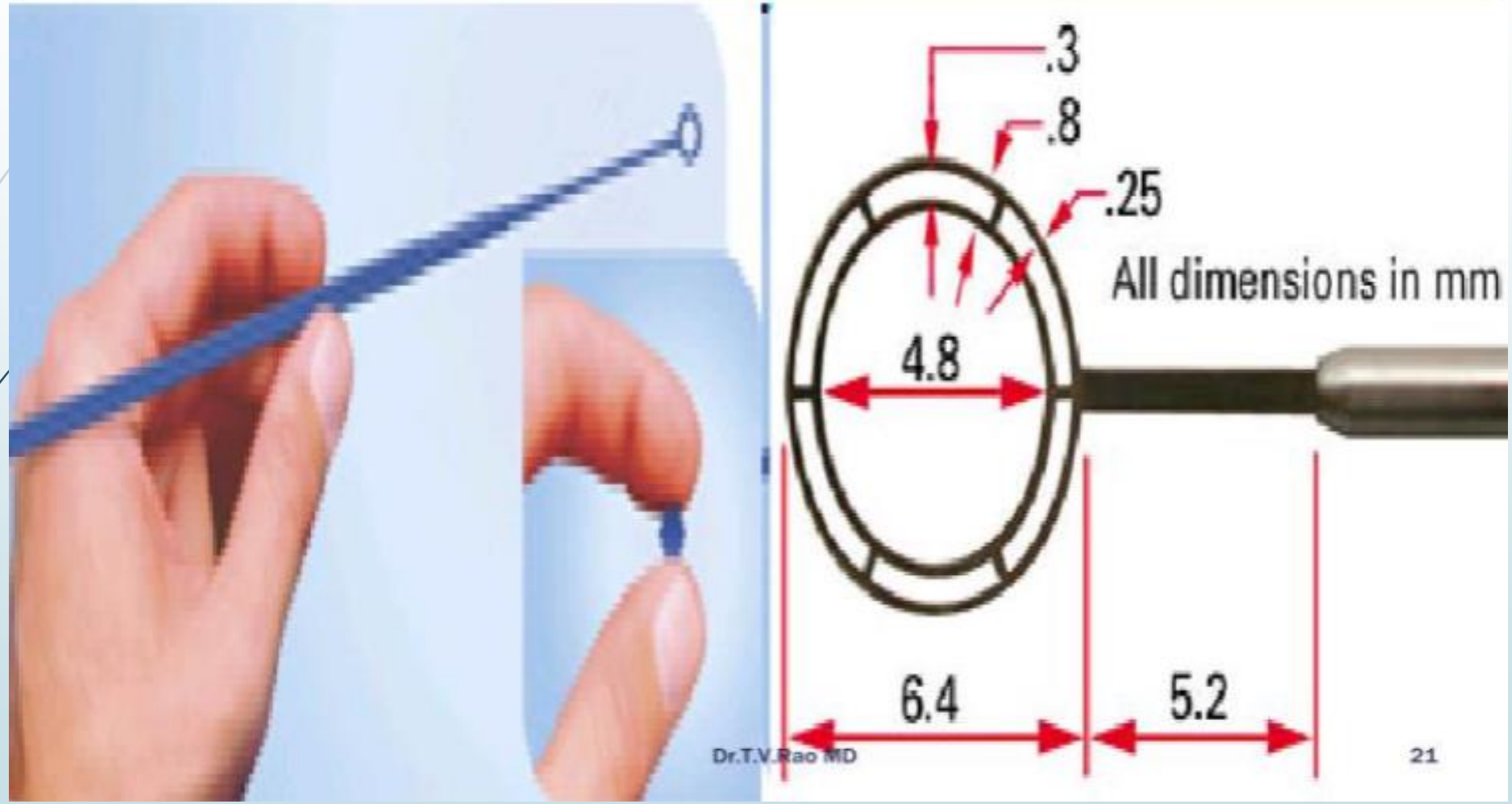


INOCULATING THE CULTURE PLATE WI URINE

- Plate: provide large surface for isolation and observation of colonies
- Using a sterile loop or a sterile swab streak your sample on the petri plate
- Important let your sterilized loop cool before you pick up your sample

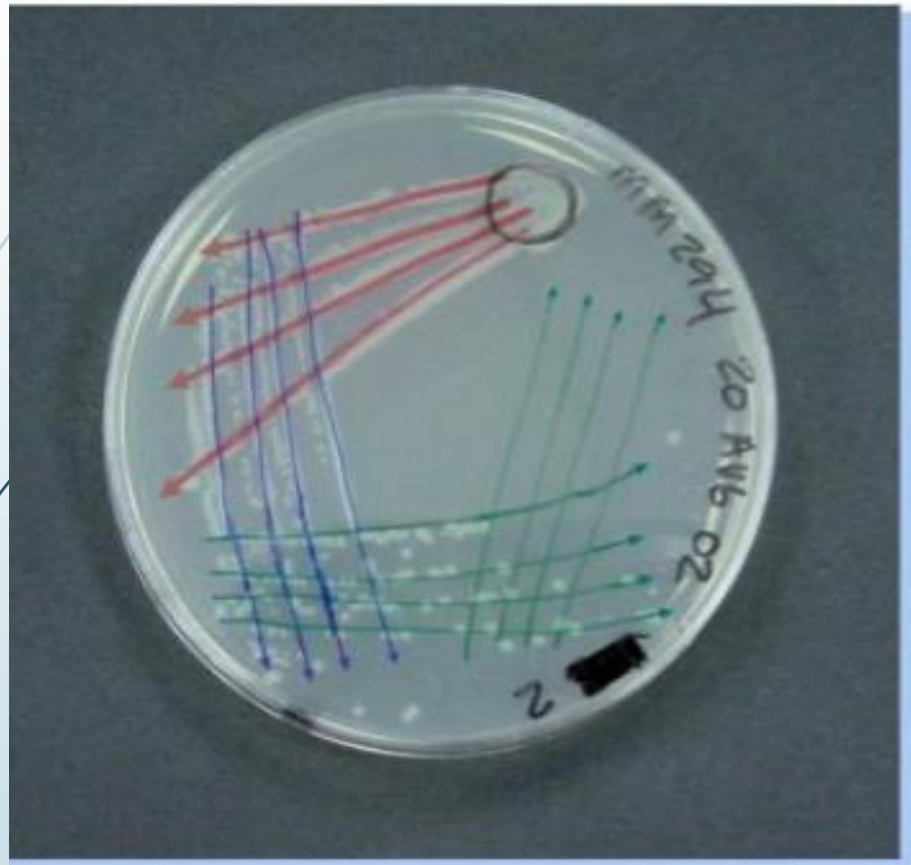


SELECTION OF LOOP FOR SEMIQUANTITATIVE METHOD



تهیه کننده:سهیلا عباسی

SPECIMEN INOCULATIONS



- All cultures processed by Semiquantitative method a loop of standard dimension of approximately known volume is inoculated into selected culture plate
- In general a loop of SWG – 28 with a diameter of 3.26 mm internal diameter which can hold a drop of water or urine 0.004 ml.
- After inoculation the culture plates are incubated at 37^{0}c extending to > 18 hours are read
- The colony counts are made, as each colony corropsdes to number of viable bacteria per ml of urine

Dr.T.V.Rao MD 22

CULTURING OF URINE FOR ISOLATIO OF BACTERIAL PATHOGENS

- **Semiquantitative culture**

Select the Media

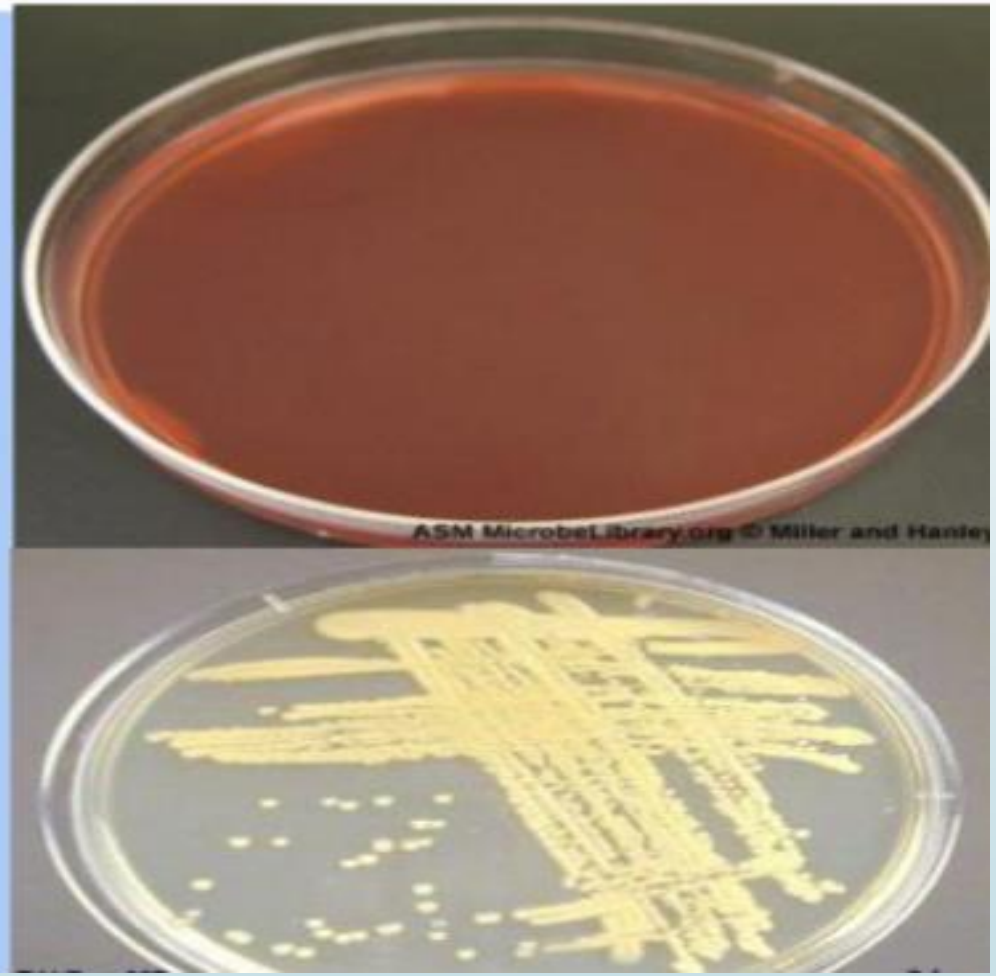
For common isolates

Mac Conkey's agar

helps in differentiation of Lactose fermenting organisms from non lactose fermenting pathogens

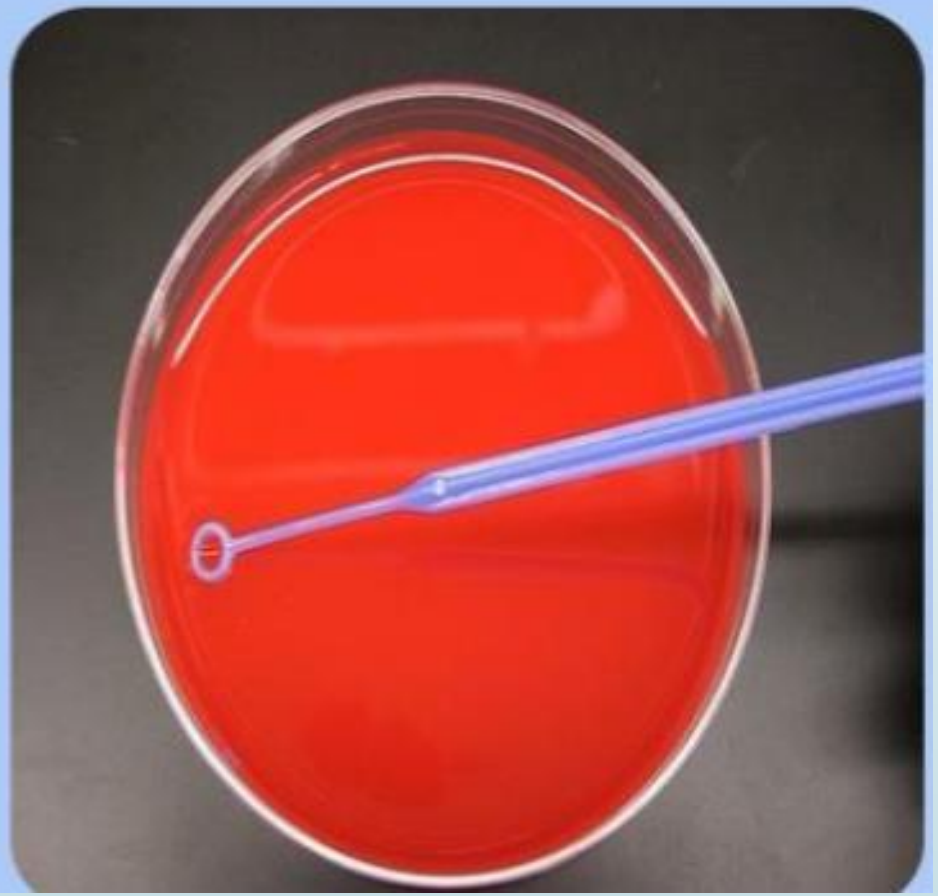
CHOOSING MEDIA TO SUIT MICROORGANISMS IS IMPORTANT

- **MacConkey agar** utilized as selective differential agar for gram-negative bacteria,
- **Colistin nalidixic acid agar** as selective agar for gram-positive bacteria, and chocolate agar for fastidious gram-negative bacteria (*Haemophilus*)



INOCULATION OF URINE

- Inoculation of urine for quantitative culture (colony forming units→cfu's) performed with a calibrated 0.001 mL and 0.01 mL plastic or wire loop
- Sheep blood agar (SBA) utilized for quantitative urine culture



CULTURE MEDIA FOR ISOLATIOI

CLED MEDIUM

It is also an excellent universal culture medium owing to its wide spectrum of nutrients, lack of inhibitors and the fact that it allows a certain degree or differentiation between the colonies. It contains lactose as a reactive compound which, when degraded to acid, causes bromothymol blue to change its colour to yellow. Alkalinization produces a deep blue colouration. The lack of electrolytes suppresses the swarming of Proteus

Laboratories which have difficulty in availability of blood agar, CLED medium is opted

ENRICHED CULTURE MEDIA FOR ISOLATION

- **Blood agar**

  helps in isolation of fastidious, extracting strains May extended incubation for isolation of pathogens for more than 48 hours with added atmosphere of 5 – 10 % co_2

READING THE CULTURE PLATES

- A true infection in the absence of prior antibiotic therapy the number of bacteria is likely to be at least 10^5 or more.
- Contaminated specimens present with colony counts $<10^4$, however even less than 10^3
- On several occasions the colonies are diverse species
- Several studies prove counts $>10^4$ to be considered as presence of Urinary tract infection with the **supporting clinical history**
- On some occasions more than one pathogen is isolated but should be processed for all practical purposes

  eg E.coli along with Streptococcus fecalis

On few occasions even counts 10^3 are proved significant

IDENTIFICATION OF GRAM + ORGANISMS

- All colonies identified morphologically as Staphylococcus to be characterized as

Staphylococcus aureus

Staphylococcus *saprophyticus*

Staphylococcus *epidermidis*

Enterococci - fecal group of organisms

Petri dish with blood agar substrate

Dr.T.V.Rao MD

Bacterial colonies growing on blood agar substrate 29

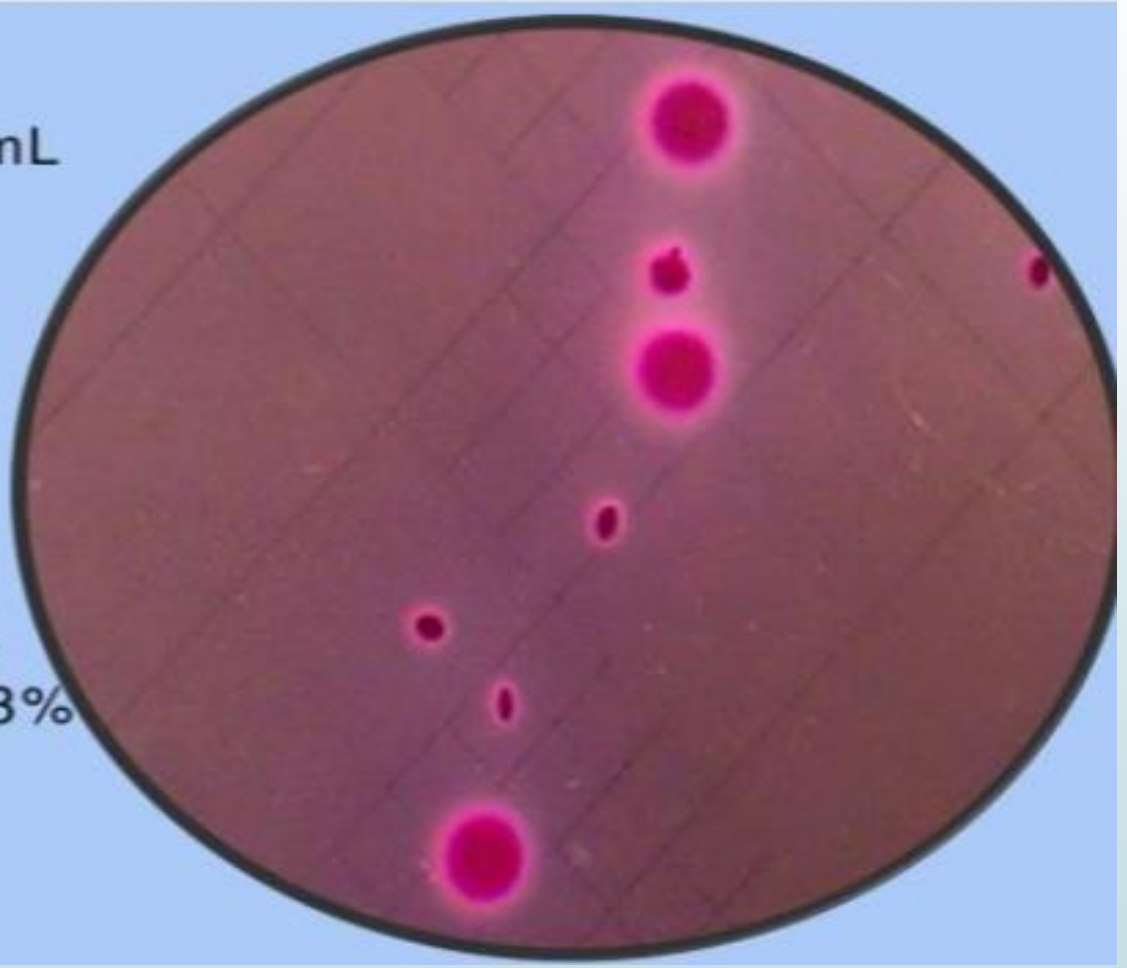
COUNTING THE COLONIES

- With 0.001 ml loop, 1 colony on SBA equivalent to 1,000 cfu's per mL of urine
- With 0.01 ml loop, 1 colony on SBA equivalent to 100 cfu's per mL of urine

Rao MD 30

INTERPRETATION OF ENTEROBACTERIACEAE

- A single species of ***Enterobacteriaceae*** recovered at >10^5 cfu's/mL urine: with patients symptomatic for urinary tract infection, 95% probability of true bacteriuria
- A single species of ***Enterobacteriaceae*** recovered at 10^4-10^5 cfu's/mL urine: with patients symptomatic for urinary tract infection, 33% probability of true bacteriuria

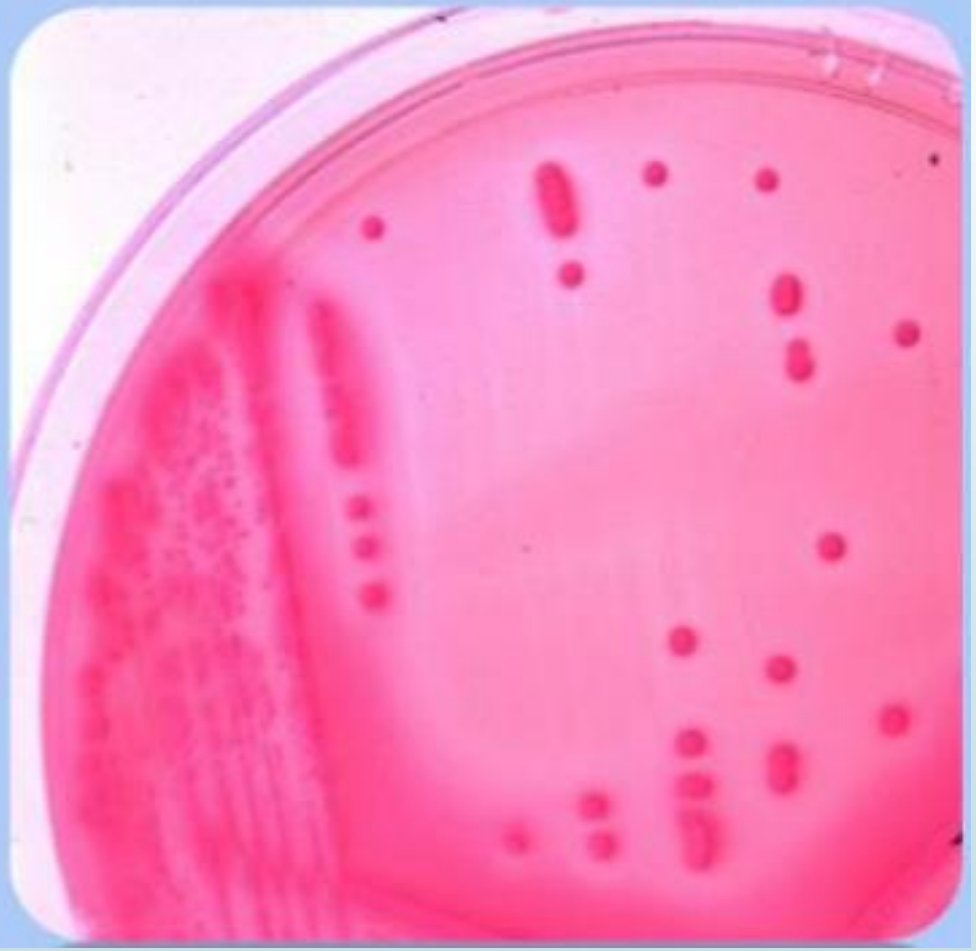


INTERPRETATION OF ENTEROBACTERIACEAE

- A single species of ***Enterobacteriaceae*** recovered at $>10^5$ cfu's/mL urine: with patients symptomatic for urinary tract infection, 95% probability of true bacteriuria
- A single species of ***Enterobacteriaceae*** recovered at 10^4-10^5 cfu's/mL urine: with patients symptomatic for urinary tract infection, 33% probability of true bacteriuria

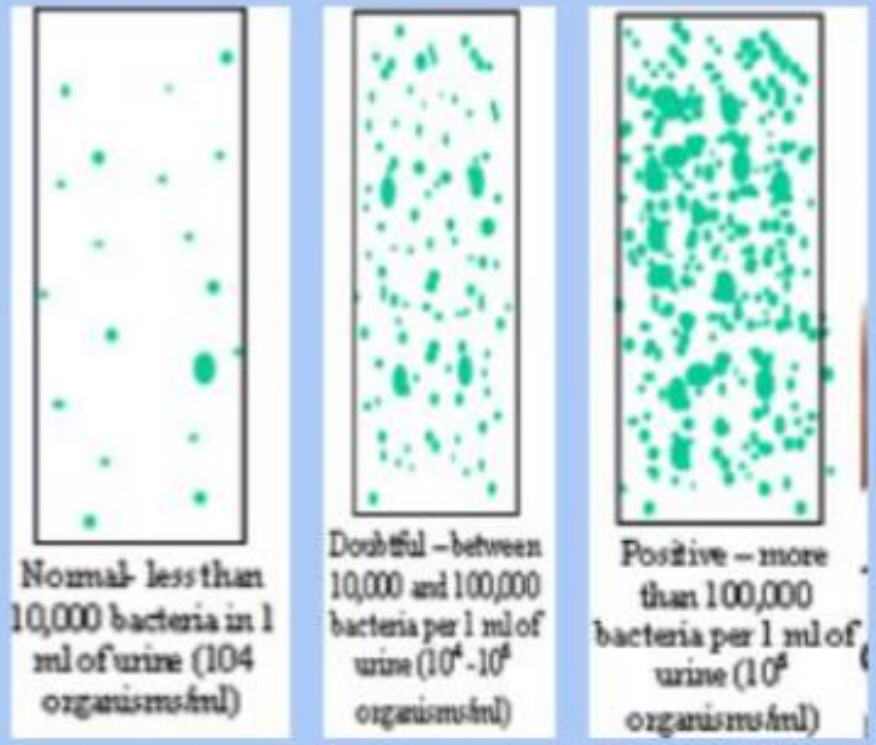
WHAT IS SIGNIFICANT BACTERIURIA

- Significant bacteriuria in an asymptomatic patient is 100,000 or more colonies per milliliter of urine from a midstream, clean-catch specimen; yet, a colony count of 200 *Escherichia coli* per ml may be significant in a midstream male void or catheterized female. About 95% of all positive UTI cultures will produce essentially pure cultures if urine is collected carefully and the media inoculated promptly.



CONCEPT OF SIGNIFICANT BACTERIURIA

- Up to 10^4/ml considered normal i.e. Insignificant
- 10^5/ml and above considered to be Significant
- Concept valid only for voided specimen of urine
- Exceptions - slow growing organisms, patient on antibiotic therapy, diuretic therapy

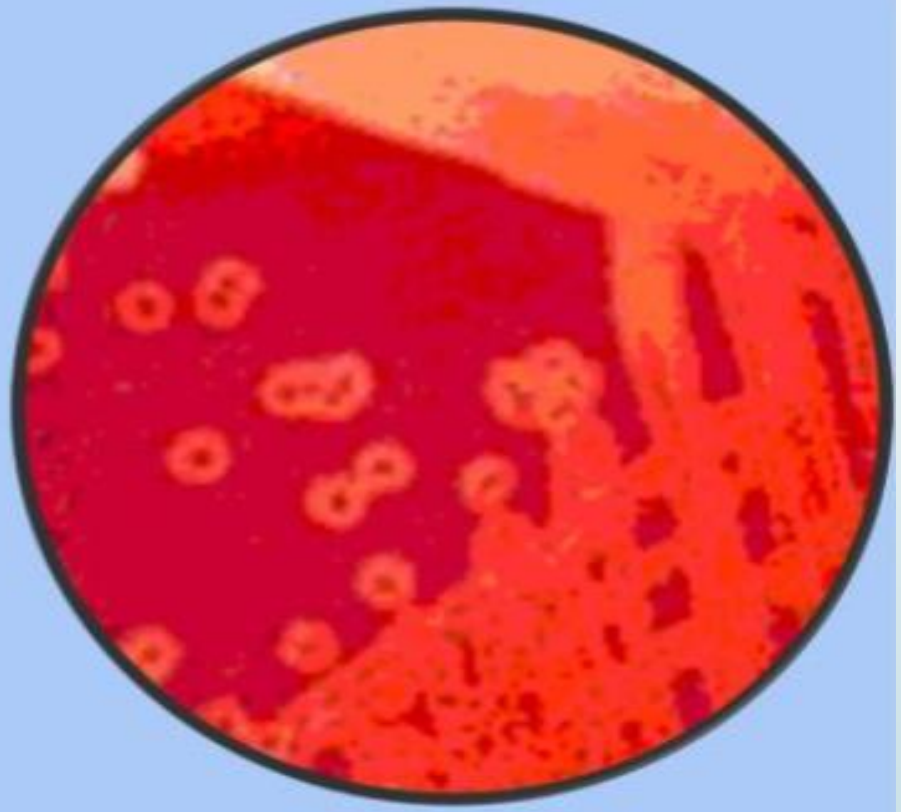


WHAT CAN BE A SIGNIFICANT COUNT

- A single species of *Enterobacteriaceae* recovered at 10^4-10^5 cfu's/mL urine: with patients symptomatic for urinary tract infection, 33% probability of true bacteriuria

GRAM POSITIVES AND FUNGI THE COUNTS MAY BE <10^5

- Gram-positive, fungal, and fastidious uropathogens often present in lower numbers (10^4-10^5 cfu's/mL urine)
- Urethral commensals recovered at <10^4 cfu's/mL urine

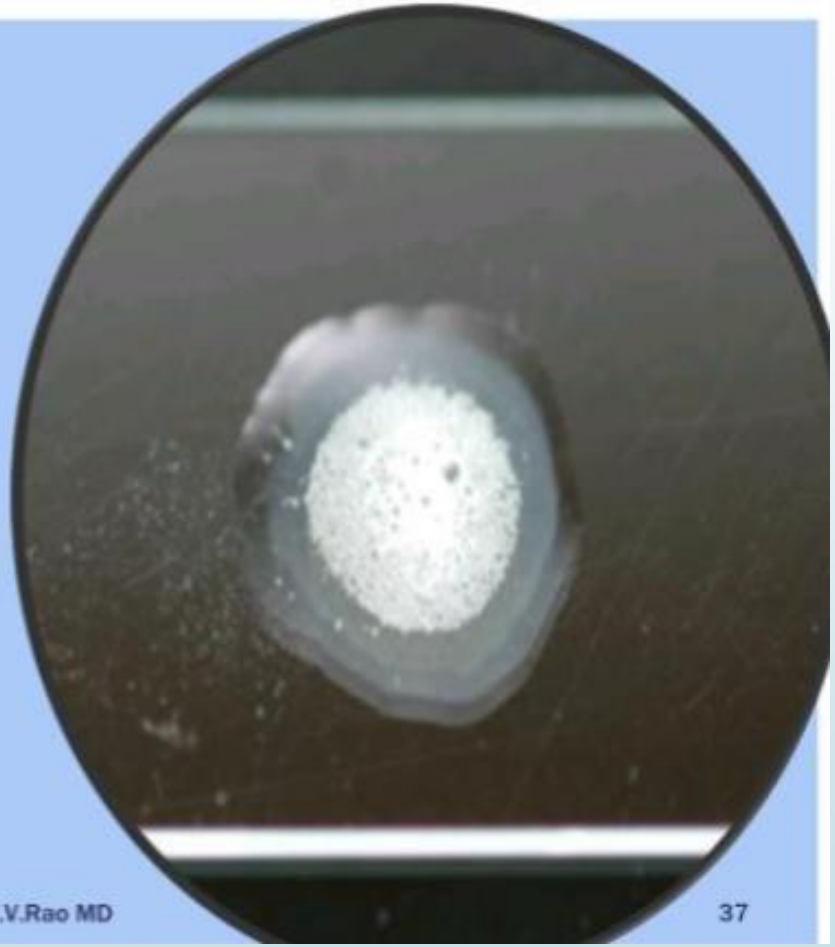


Dr.T.V.Rao MD 36

IDENTIFICATION OF ISOLATES

GRAM + ISOLATES

- The minimal tests to differentiate Gram + cocci include
  - **1 Catalase**
  - **2 Coagulase test**
  - 3 Bile esculin testing
  - 4 Bacitracin in Streptococcus isolates



Dr.T.V.Rao MD 37

BIOCHEMICAL TESTS IN GRAM - VE BACILLI

- Catalase test
- Oxidase test
- Nitrite reduction test
- Indole test
- Methyl red test
- V P test
- Citrate test
- Decarboxylation tests
  Lysine, ornithine, Arginine tests

Dr.T.V.Rao MD 38

Stool Culture

❖ *Aim of the test*

- Detect bacterial pathogenic organisms in the stool; diagnose typhoid fever, enteric fever, bacillary dysentery.

❖ *Types of specimen*

- Stool or rectal swab or stool (fresh random) in fecal transport system.

❖ *Criteria of specimen rejection*

- specimen contaminated with urine.
- residual soap, or disinfectants.
- Specimens received in grossly leaking transport containers.
- dry specimens.
- specimens submitted in fixative or additives.

Pre specimen processing

❖ Patient preparing

- Instruct the patient on how the specimen should be collected and transferred to the container; provide him/her with sticks and containers.

❖ Specimen collection

- A single stool specimen cannot be used to rule out bacteria as a cause of diarrhea.
- More than two specimens should only be submitted from patients for whom there is a high degree of suspicion.

❖ ***Who will collect the specimen***

- The patient. If stool is unobtainable, nursing staff or physician will collect fecal swab.

❖ ***Quantity of specimen***

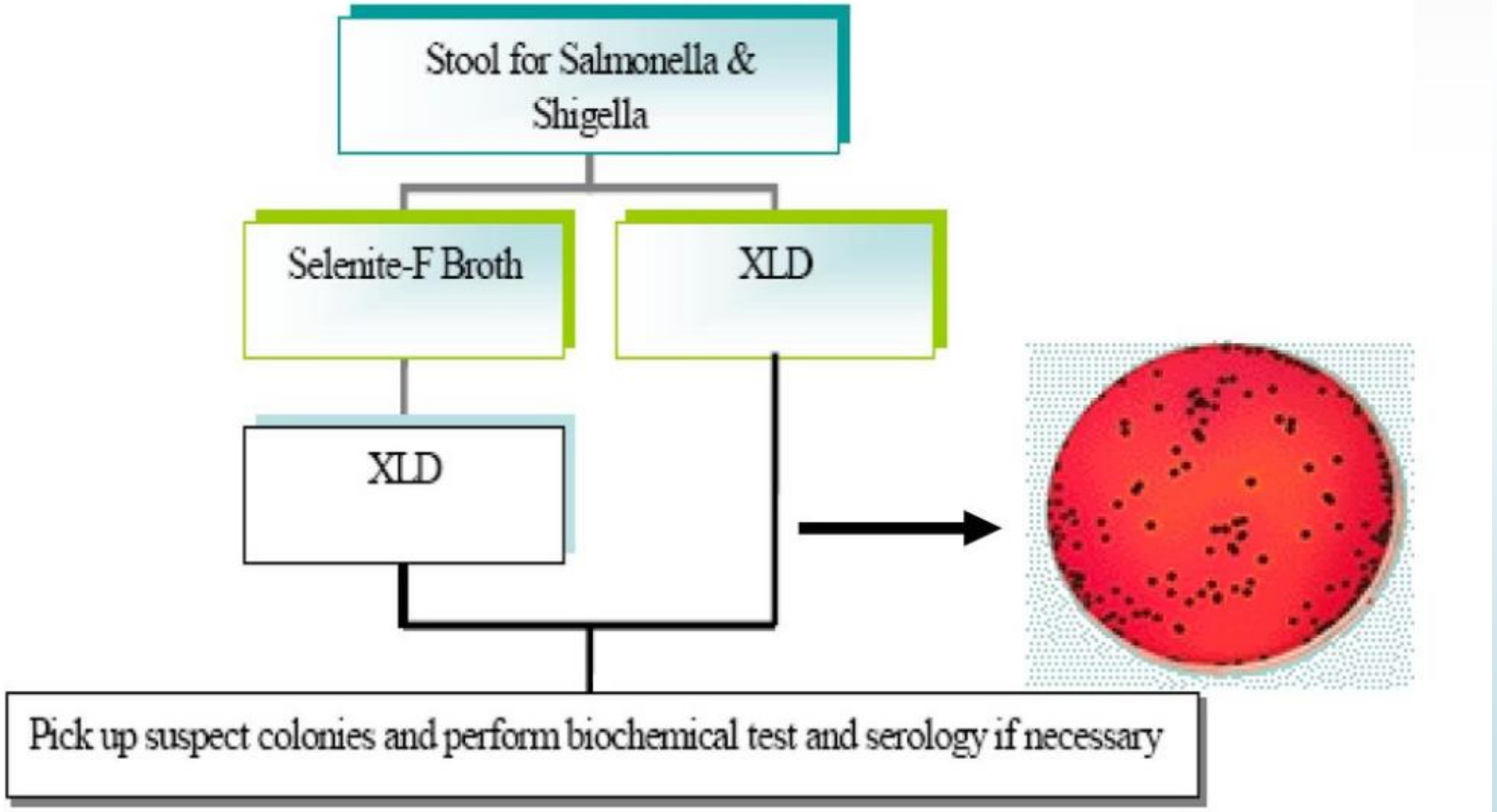
- The specimen should contain at least 5 g of faeces

❖ ***Time relapse before processing the sample***

- Stool samples should be examined and cultured as soon as possible after collection. As the stool specimen cools, the drop in pH will inhibit the growth of most *Shigella spp*. and some *Salmonella spp*.

Routine Stool Culture, *Salmonella & Shigella*

- ***Media***
  - Selenite-F broth or tetrathionate.
  - SSA, XLD and HEA.
- ***Reagents***
  - API 20 E Kit.
  - Salmonella and Shigella antiserum (polyvalent and monovalent).


Stool for Salmonella & Shigella
Selenite-F Broth
XLD
XLD
Pick up suspect colonies and perform biochemical test and serology if necessary

Selenite-F broth

- ***Selenite Broth*** (Selenite-F Broth) is used as an enrichment medium for the isolation of Salmonella from feces, urine, water, foods and other materials.
- ***Sodium selenite*** inhibits the growth of gram-positive and many gram-negative bacteria including *Eenterococci* and *Coliforms*, whereas the salmonellae are not affected.
- Sodium selenite is highly toxic at near-neutral pH.

- ***Buffer salts*** are present to help maintain the pH which may rise as the toxicity decreases . A rise in pH decreases selective activity of Selenite.
- A fermentable carbohydrate (***lactose***) is also present to provide acid to neutralise the alkali produced when the selenite is reduced by bacteria.

❖ ***Tetrathionate Broth***

- Tetrathionate Broth base, with added iodine-iodide solution, is used as a selective enrichment medium for the isolation of Salmonella from feces, urine, foods and other materials.

component of (XLD) Agar

- Xylose
- Lysine
- Lactose
- Sucrose
- Sodium chloride
- Phenol red
- Sodium desoxycholate inhibits contaminating Gram-positive flora
- Sodium thiosulphate
- Ferric ammonium sulphate
- Agar

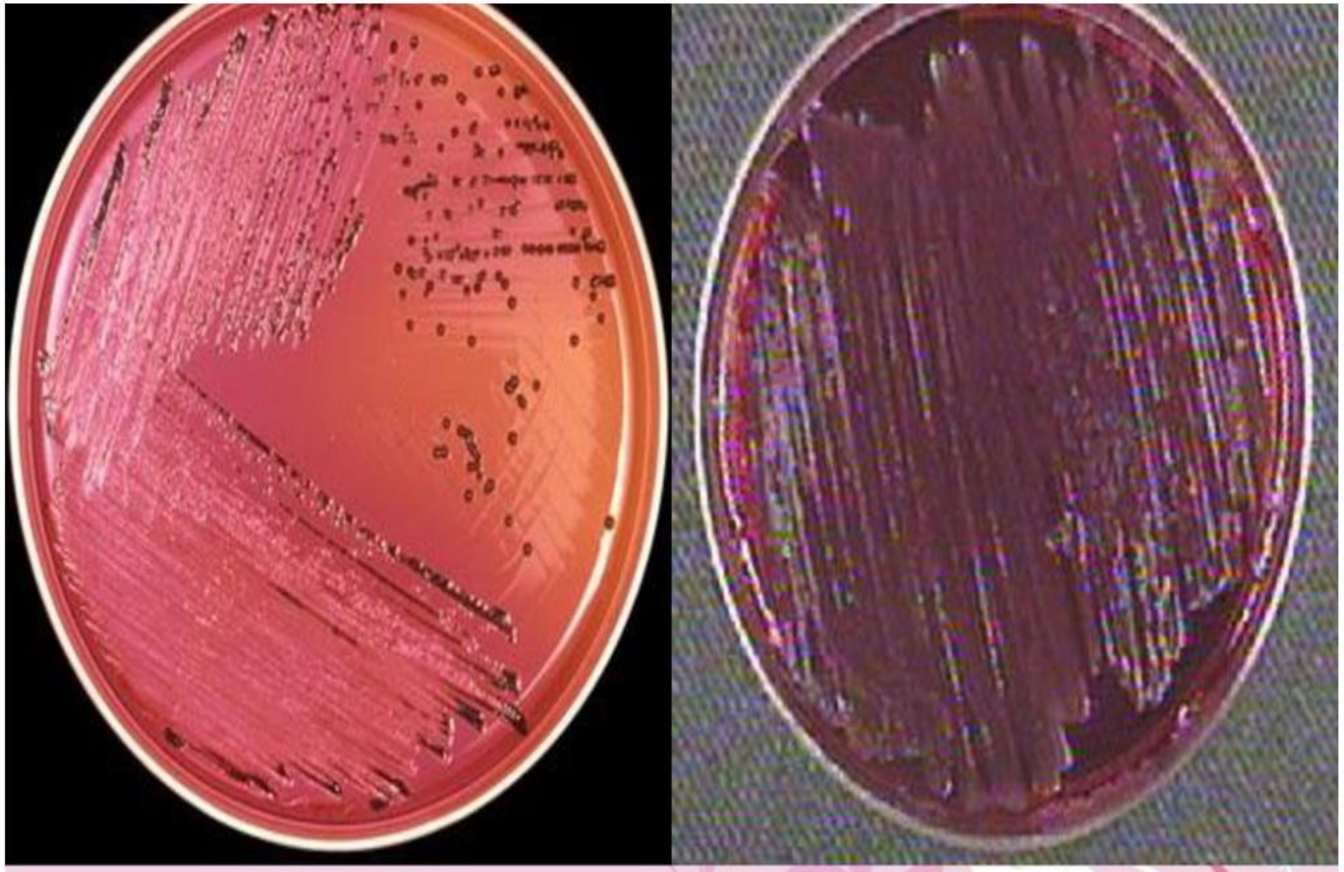
Xylose Lysine Desoxycholate (XLD) Agar

- A selective and differential medium for the recovery of Salmonella and Shigella species.
- It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red.
- Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow.
- Most enteric organisms except Shigella ferment xylose to produce acid.
- Salmonella also decarboxylate lysine which keeps the pH neutral or slightly alkaline.

- At this pH Salmonella species can produce hydrogen sulphide from the reduction of thiosulphate.
- This is indicated by ferric ammonium citrate producing black or black-centred colonies.
- Other Enterobacteria such as *E. coli* will ferment the lactose and sucrose present in the medium to an extent that will prevent pH reversion by decarboxylation and acidify the medium turning it yellow.

Results

Organism	Color of colony
Salmonella	Red colonies, black centre
Shigella	Red colonies
E. coli	Yellow
Proteus	Red colonies, black centre



Salmonella on XLD agar

Shigella on XLD agar

تهیه کننده:سهیلا عباسی

Stool Culture

Salmonella Shigella Agar (SSA)

- SS Agar and *Salmonella Shigella* Agar are moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella* and *Shigella*.

SS Agar Plate
(Salmonella–Shigella Agar)

Component of SSA

- ***Bile salts, brilliant green and Sodium citrates:*** inhibit Gram-positive bacteria, most coliform bacteria. Differentiation of enteric organisms is achieved by the incorporation of ***lactose*** in the medium.
- ***Sodium thiosulfate and Ferric citrate*** allow the detection of the H_2S producing bacteria such as *Proteus* and some strains of *Salmonella*, as they produce colonies with black centers
- ***Neutral Red*** is the pH indicator.

- Organisms that ***ferment lactose*** produce acid which, in the presence of the ***neutral red indicator***, results in the ***formation of red colonies. Lactose nonfermenters form colorless colonies***.
- The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

A .*Klebsiella pneumoniae*
B .*Escherichia coli*
Klebsiella pneumoniae & Escherichia coli are positive for acid production from fermentation of the carbohydrate(s) present .
C :*Salmonella sp.*
D :*Proteus mirabilis*
Both *Salmonella sp. & Proteus mirabilis* product hydrogen sulfide .
E :*Pseudomona aeruginosa*
The *Pseudomonas* colonies are nearly colorless .

Hektoen Enteric Agar

- ***Bile salts and Acid Fuchsin :*** These substances inhibit gram-positive organisms but also can be toxic for some gram-negative strains.
- ***lactose, sucrose as carbohydrates*.**
- Sodium Chloride: maintains the osmotic balance of the medium
- ***Ferric ammonium citrate and sodium thiosulfate*** in the medium enable the detection of hydrogen sulfide production.
- ***Bromothymol Blue and Acid Fuchsin*** are added as the pH indicator. The indicator bromothymol blue changes its color to yellow and acid fuchsin would changes color from yellow to orange- red when acid is formed.

Results

Organisms	Colony Color
Salmonella & Shigella	Blue to green-blue
Escherichia coli	Yellow to salmon

Additional Information

- Indications for stool culture include:
- Bloody diarrhea
- Fever
- ***Tenesmus (is the constant feeling of the need to empty the bowel, accompanied by pain, and cramping)***
- Severe or persistent symptoms
- Recent travel to a third world country
- Known exposure to a bacterial agent
- Presence of fecal leukocytes

با سپاس از توجه شما

تهیه کننده:سهیلا عباسی