Urine Bench

Laboratory Examination of Urine

Most common organism causing UTI

- *E. coli (caused 60-90% of UTI)*
- *Klebsiella spp.*
- *Proteus spp.*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*
- *Enterobacter spp.*
- *Neisseria gonorrhoeae*
- *S. aureus*
- *S. saprophyticus*
- *Streptococci spp.*

Pathogens and commensals

Female patient? Pregnant? Ask of vaginal swab

Pathogens and commensals

Urine specimen	
Common pathogens	**commensal flora**
Neisseria gonorrhoeae	the urine is sterile except for the urethral mucosa which support the growth of microflora as:
E. coli and other Enterobacteriaceae	
Enterococcus spp	Diphtheroid bacilli
Staphylococcus aureus	*Lactobacillus* spp
Staph saprophyticus	Coagulase negative *Staphylococci*
Corynebacterium jeikeium	α Haemolytic *Streptococci*
Acinetobacter spp	Bacillus spp
Pseudomonas spp	Non pathogenic *Neisseria spp.*
Gardnerella vaginalis	Anaerobic cocci
β-haemolytic streptococci	Commensal *Mycobacterium*
Salmonella spp (early stage of infection)	Commensal *Mycoplasma spp.*
Parasites	
Schistosoma haemetobium	
Trichomonas vaginalis	

Urine specimen

- Normally urine is a sterile body fluid
- The bladder and urinary tract are sterile, the urethra may contain commensals
- Presence of bacteria in urine called bacteriuria
- Clean-catch midstream urine specimens that have more than 100,000 colonies of bacteria per mL of urine may be indicative of infection
- UTI occur more frequently in women than men due to the shortness of the female urethra

Types of Urine Specimens

1. First morining (preferably) Midstream “clean catch” specimen (MSU).

2. Catheterized specimen.

3. Suprapubic aspiration.

4. Pediatric specimen.

Collection of Urine Specimen

- The first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for lab examination.
- 10-20 ml specimen is needed.
- Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a ‘clean-catch’ specimen.
- Wash hands thoroughly before beginning the collection. **Clean the area very well** with water and soap.
- Label the container with the date, name and number of the patient, and the time of collection.

Transport of Urine Specimen

- Urine specimens should be delivered to the laboratory immediately with a request form.
- When immediate delivery is not possible, refrigerate the urine at 4–6 °C.
- When a delay in delivery of more than 2 hours (maximum 48 hrs) is anticipated, add boric acid preservative to the urine (no need to refrigerate).

Deterioration of Urine Specimen

The following changes occur when unpreserved urine is left at room temperature:

- Any bacteria in the urine will multiply so that the bacterial count will be unreliable.
- When the organisms are urease-producing, the ammonia released will increase the pH of the specimen which will result in the destruction of cells and casts. Bacteria will also break down any glucose which may be present.
- When white cells, red cells, and casts are present, these will begin to lyze especially in a concentrated specimen.
- The concentration of protein in the urine will be altered.
- When bilirubin is present this may be oxidized to biliverdin which will not be detected. Likewise, urobilinogen will not be detected because it will be oxidized to urobilin.

Lab Examination of Urine

- **Urine Analysis:**

✓ Macroscopic examination

✓ Biochemical Analysis

✓ Microscopic examination

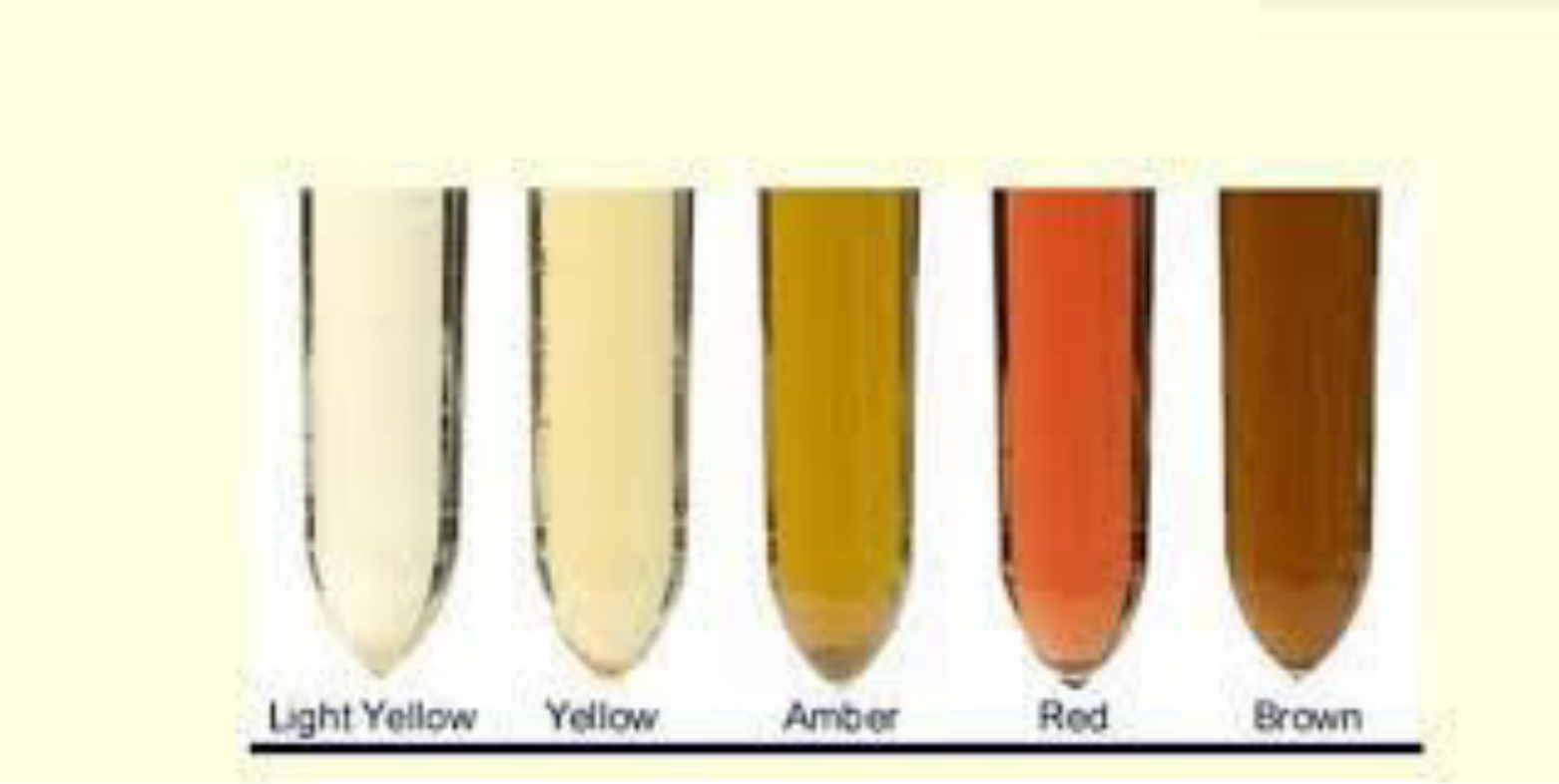
- **Urine Culture**
- **Biochemical Reactions**

Macroscopic Examination

- ✓ Report **color** and **clarity** (clear or turbid) of the urine specimen.
- ✓ Normal freshly passed urine is clear and pale yellow to yellow in color.
- ✓ Sometimes color changes is due to certain foods, herbs, or drugs like Vit.

Appearance	Possible Cause
Cloudy (usually with bad odor)	Bacterial UTI
Red + Cloudy	- Urinary Bilharzia - Bacterial UTI
Brown + Cloudy	- Malaria - Intravascular Disease
Yellow-brown or Green-brown	- Acute viral hepatitis - Obstructive jaundice
Yellow-orange	- Haemolysis - Hepatocellular jaundice
Milky-white	Bancroftian filariasis

Examples of urine color



Biochemical Analysis

Urine dipstick

Glucose

Bilirubin

Ketones

Specific Gravity

Blood

pH

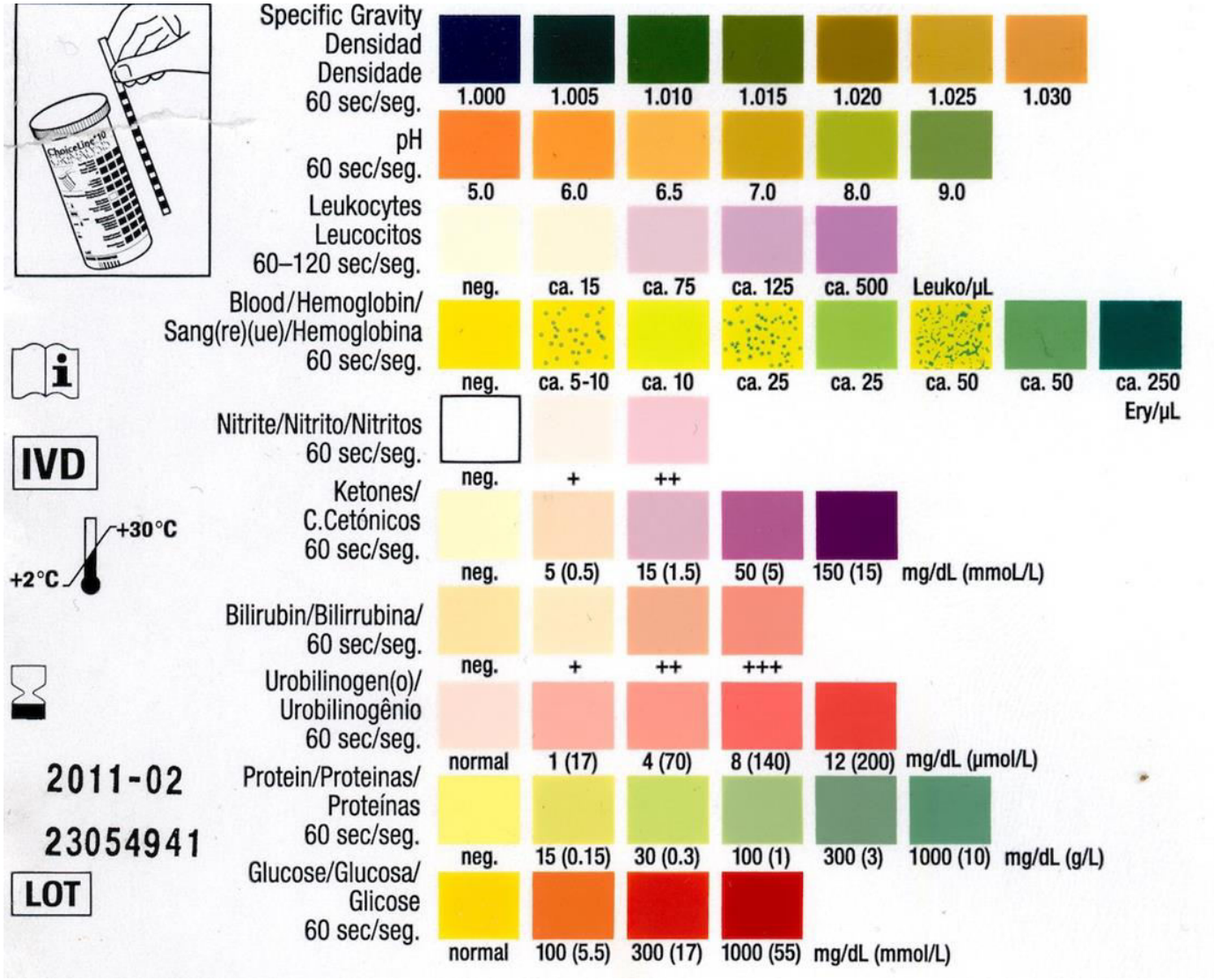
Protein

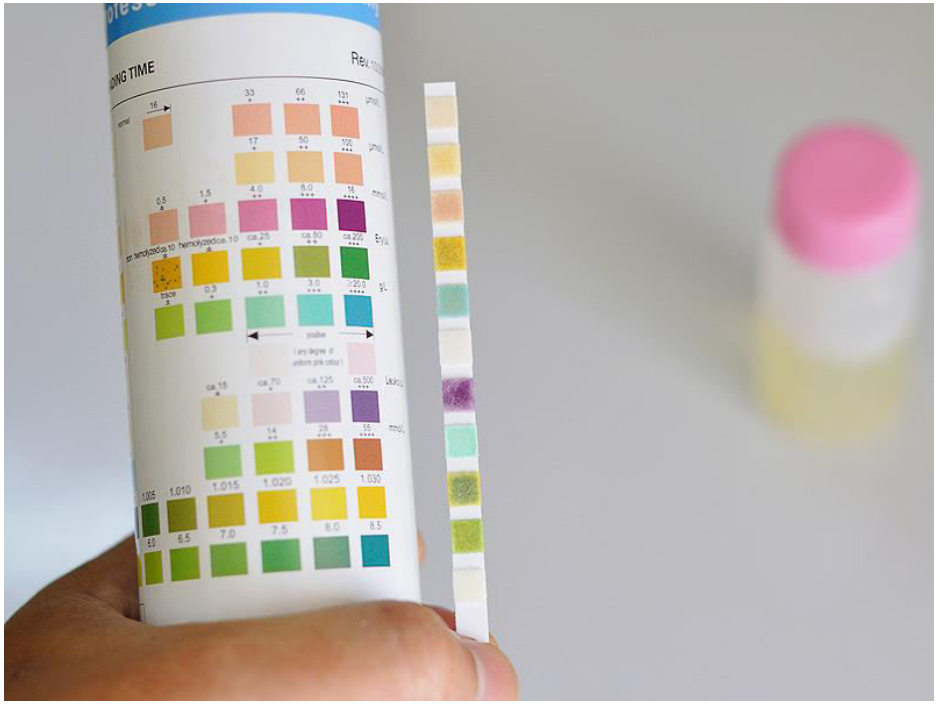
Urobilinogen

Nitrite

Leukocyte Esterase

Dipstick Test




NG TIME

1. Specific Gravity (SG)

- Reflect the concentration of the urine (amount of substances dissolved in urine).
- **Range of 1,001 to 1,040.**
- ***Increase SG:*** Dehydration (due to fever, vomiting, diarrhea), Diabetes Mellitus, and decreased fluid intake (urine volume↓ and SG↑).
- ***Decrease SG:*** diabetes insipidus, renal failure, increased fluid intake (urine volume↑ and SG ↓).

2. PH

- Reaction reflects ability of kidney to maintain normal hydrogen ion concentration.
- Normal urine PH= 4.6-8
- ***Acidic urine*** might be caused by: Ketosis-diabetes, starvation, fever, systemic acidosis, UTI (E.coli), acidification therapy.
- ***Alkaline urine*** might be caused by: strict vegetarian, systemic alkalosis, UTI (Proteus), alkalization therapy.

3. Blood

- A positive dipstick for blood in the urine indicates either hematuria, hemoglobinuria, or myoglobinuria.
- **Hematuria** can be distinguished from hemoglobinuria and myoglobinuria by microscopic examination of the centrifuged urine.
- The presence of a large number of erythrocytes establishes the diagnosis of hematuria.

4. Protein

- Healthy adults excrete 80 to 150 mg of protein in the urine daily
- **Normal protein level in a random urine test should be (0-20 mg/dl).**
- **Proteinuria** is found in most bacterial urinary tract infections.
- Other causes include: glomerulonephritis, nephrotic syndrome, hypertension.
- Normally, urine protein is about 30% albumin, 30% serum globulins, and 40% tissue proteins.

5. Glucose & 6. Ketones

- Urine testing for glucose and ketones is useful in screening patients for diabetes mellitus
- A serum glucose of about 180 mg/dL; above this level, glucose will be detected in the urine (**glucosuria**).
- **Normal glucose level in a random urine test should be (0-15 mg/dl).**
- *Ketones are not normally found in the urine* but will appear (**ketonuria**) when the carbohydrate supplies in the body are depleted and body fat breakdown occurs.

7. Nitrite

- Nitrites are not normally found in the urine, and its presence is strongly suggestive of bacteriuria.
- Many species of gram-negative bacteria can convert nitrate (normally present in urine) to nitrite, *e.g. E. coli*, *Proteus species, and Klebsiella species* if the organisms are present in the urine in sufficient concentration. When first morning urine is tested, about 80–90% of UTI caused by nitrate-reducing pathogens can be detected.
- The test is negative when the infection is caused by pathogens that do not reduce nitrate such as *Enterococcus faecalis, Pseudomonas species, Staphylococcus species and Candida organisms, or* when as previously mentioned the bacteria are too few in the urine.
- Occasionally the nitrite test is negative because nitrate is lacking in the urine due to the person being on a diet lacking in vegetables.

8. Leukocyte Esterase (LE)

- This enzyme is specific for polymorphonuclear neutrophils (pus cells). It detects the enzyme from both active and lyzed WBCs.
- LE testing is an alternative method of detecting **pyuria** when:

1. It is not possible to examine fresh urine microscopically for WBCs.
2. When the urine is not fresh and likely to contain mostly lyzed WBCs.

- False negative strip test results can occur when the urine contains boric acid or excessive amounts of protein (500 mg/100 ml) or glucose (2 g/100 ml).
- The major cause of false-positive leukocyte esterase tests is specimen contamination.

9. Bilirubin & 10. Urobilinogen

- Normal urine contains no bilirubin and only very small amounts of urobilinogen.
- The normal level of bilirubin is 0-1.2 mg/dl.
- There are many causes of bilirubin in urine:

1) Blockage of bile ducts due to gall stones
2) Infection
3) Decreased conjugation.

- Presence of bilirubin and urobilinogen in urine usually indicate of liver diseases (ex: liver hepatitis, cirrhosis)

Microscopic Examination

A- Wet preparation to look for:

1. Cells: - WBCs
   - RBCs
   - Epithelial cells
2. Bacteria
3. Yeast
4. Parasites
5. Casts
6. Crystals

- **A normal urine microscopy contains few epithelial cells, occasional RBC's, few crystals.**

How to make Wet Preparation?

1. Aseptically transfer about 10 ml of well mixed urine to a labelled conical tube.
2. Centrifuge at 500–1000 rpm for 5 minutes. Pour the supernatant fluid by completely inverting the tube.
3. Remix the sediment by tapping the bottom of the tube.
4. Transfer one drop of the *well-mixed* sediment to a slide and cover with cover glass.
5. Examine the preparation microscopically using the 10 and 40 objective.

Microscopic Examination

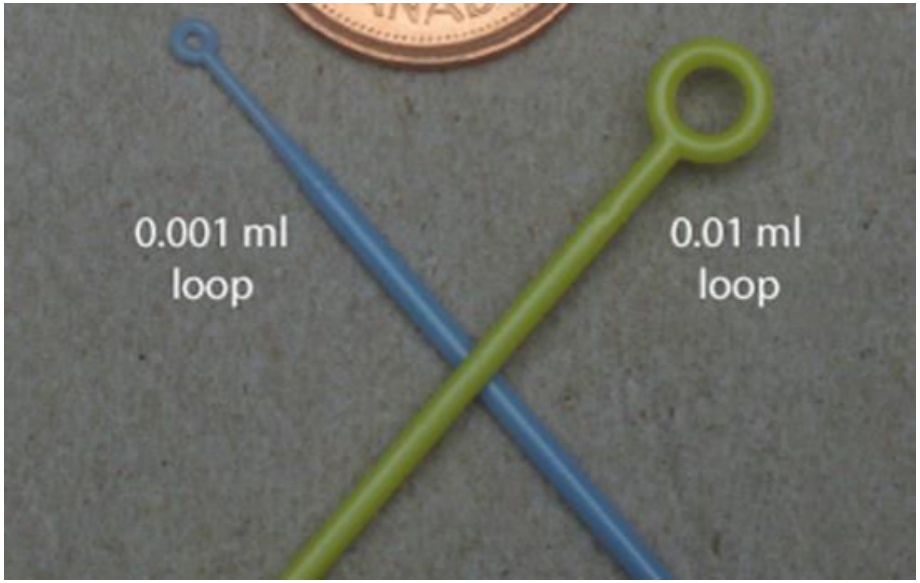
B- Gram stained smear

Transfer a drop of the urine sediment to a slide and spread it to make a thin smear, allow to air dry or heat fix. Stain it by Gram stain

Urine culture

Calibrated Loop Method

- With a 0.001 ml loop, 1 colony = 1,000 cfu/ml urine and With a 0.01 ml loop, 1 colony = 100 cfu/ml urine.



Culturing procedure

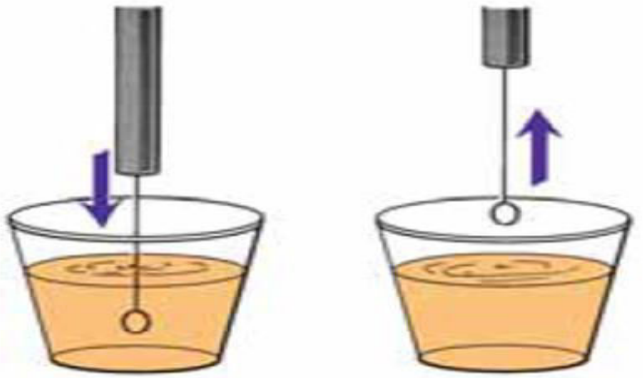


Figure 57-3 Method for inserting a calibrated loop into urine to ensure that the proper amount of specimen adheres to the loop.

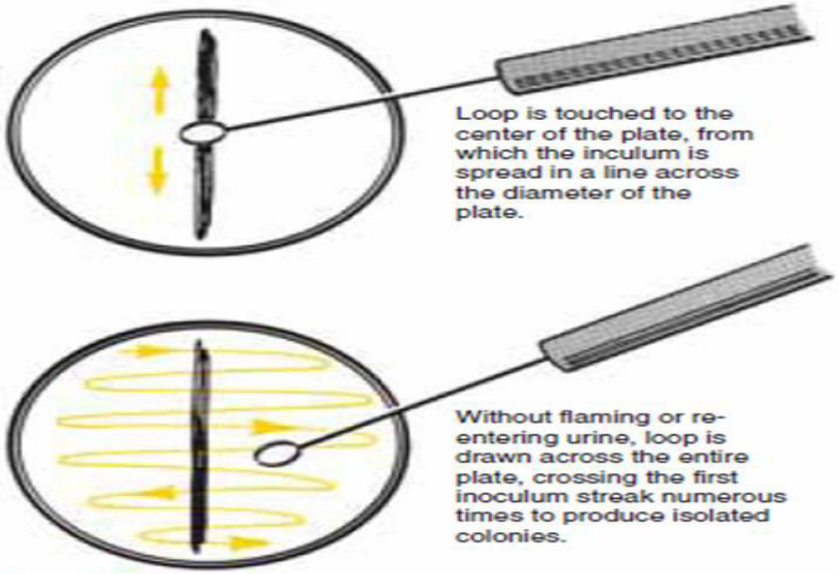


Figure 57-4 Method for streaking with calibrated urine loop to produce isolated colonies and countable colony-forming units.

Reporting Bacterial Numbers

- **Not significant:** $<10^4$ organisms/ml.
- **Doubtful significance:** 10^4–10^5/ml (suggest repeat specimen)
- **Significant bacteriuria:** $>10^5$/ml perform full ID and susceptibility testing.

Culture the specimen

Media used:

- CLED Agar
- MacConkey Agar
- Blood Agar

CLED

- CLED agar is widely used to isolate urinary pathogens
- Allows the growth of both Gram +ve and Gram –ve bacteria
- Gram –ve: LF>> yellow colonies, NLF>> blue (colorless) colonies
- Prevent *Proteus spp.* from swarmming

MAC

- is a culture medium designed to selectively grow Gram-negative bacteria and differentiate them for lactose fermentation

Blood Agar

Specimen Result

Turn around time:

- Wet mount results should be available 1 hour after specimen receipt.
- Isolation of a possible pathogen can be expected after 2-3 days.
- Negative culture will be reported out 1-2 days after the receipt of the specimen.

Automated Microbiology Method

- **Microscan:** for **Id of bacteria**
  Antibiotic sensitivity
- Ready Kit
- Make suspension
- Fill wells: Red for G +ve & Blue for G-ve
- Run the machine & get the report.


Baxter
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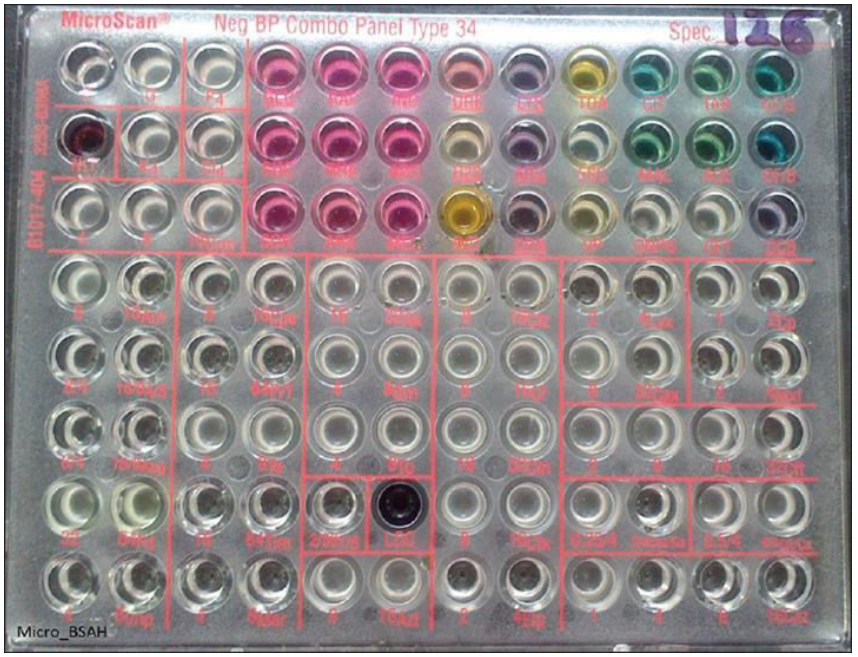
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