MICROBIOLOGY PRACTICAL MATERIALS

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IDENTIFICATION OF BATERIA

1. STAPHYLOCOCCUS

Specimen: Localized pus from abscess.

Direct smear: Shows pus cells with gram positive cocci in clusters.

Culture: Plating done on

Culture media	Incubation	After 24 hr
Nutrient agar	Incubated at 37°c for 24 hr	Opaque golden yellow colonies
Blood agar		Opaque colonies with beta hemolysis
Mac conkey		Tiny pink colonies

Biochemical tests

Urease	Positive
Mannitol	Acid+;no gas

Smear from culture plate: gram positive cocci in clusters.

Coagulase test positive: Staphylococcus aureus

Antibiotic susceptibility pattern.

Phage typing - for epidemiology study

Other species:

Staphylococcus epidermidis - Opaque white colonies; coagulase test negative;
Mostly a commensal, but the most frequent organism isolated from infected indwelling prosthetic devices.

Staphylococcus albus - Opaque white colonies; coagulase test negative;
Opportunistic pathogens.

Staphylococcus citreus - lemon yellow colonies; coagulase negative;
Opportunistic pathogens

Clinical Significance:

Staphylococcus aureus-Food poisoning, Toxic shock syndrome, Staphylococcal Skin Scalded Syndrome, Pneumonia, Osteomyelitis, Skininfections, Meningitis, Acute bacterial endocarditis, UTI

Staphylococcus epidermidis-Mostly a commensal, but the most frequent organism isolated from

infected indwelling prosthetic devices, causes UTI, sepsis from IV line

Methicillin-resistant *Staphylococcus aureus* (MRSA)-These bacterial isolates are resistant to many antibiotics. In the community, most MRSA infections are skin infections. In medical facilities, MRSA causes life threatening blood stream infections, pneumonia and surgical site infections.

CASE HISTORY- 1

A group of six children under 8 years of age live in a semitropical country. Each of the children has several crusted weeping skin lesions of impetigo (pyoderma). The lesions are predominantly on the arms and faces. Which of the following microorganisms is a likely cause of the lesions?

(A) *Escherichia coli*

(B) *Chlamydia trachomatis*

(C) *Staphylococcus aureus*

(D) *Streptococcus pneumoniae*

(E) *Bacillus anthracis*

2. ESCHERICHIA COLI

Specimen: Mid stream urine specimen in sterile container from Patient X having fever with burning micturition.

Direct smear: Grams smear shows pus cells with gram negative rods.

Wet Mount : Centrifuged, Plenty of pus cells seen

Culture : After Incubation at 37°c for 24 hr

Culture media	**Cultural Charecteristics**
Nutrient agar	Non Mucoid, Convex, Greyish white colonies
Blood agar	Greyish white Non Mucoid Colonies
Mac conkey	Pink, Lactose fermenting colonies

Smear from colonies: Gram negative rods are seen

Biochemical tests:

Indole	Positive
Urease	Negative
Citrate	Negative
TSI	A/A Gas+ no H2S
MR	Positive
VP	Negative

Sugar reaction: Glucose, lactose, sucrose, maltose, mannitol, starch are fermented with acid and gas**.**

Antibiotic susceptibility testing.

Other tests: Agglutination with mono and polyvalent antisera to detect EPEC, ETECetc

Clinical Significance: Urinary tract Infections, Pyogenic infections, Septicemia, neonatal Meningitis Diarrhea (Enteropathogenic, Enterotoxigenic, Entero hemorrhagic, Enteroinvasive, enteroaggregative). Extended Spectrum Beta Lactamses (ESBL) producing isolates are resistance to 3rd generation cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, Cefpodoxime) and Monobactams (Aztreonam). These ESBLs are of clinical concern because they restrict therapeutic options causing treatment failures.

CASE HISTORY - 2

A 20-year-old college student goes to the student health center because of dysuria, frequency, and urgency on urination for 24 hours. She has recently become sexually active. On urinalysis, many polymorphonuclear cells are seen. The most likely organism responsible for these symptoms and signs is

(A) *Staphylococcus aureus*

(B) *Streptococcus agalactiae*

(C) *Gardnerella vaginalis*

(D) *Lactobacillus* species

(E) *Escherichia coli*

3. KLEBSIELLA

Specimen: Urine from patient X who complaints of fever,

Lower abdominal pain, increased frequency of micturition.

Direct smear: Grams smear shows pus cells with gram negative rods.

Wet Mount : Centrifuged, Plenty of pus cells seen

Culture : After Incubation at 37°c for 24 hr

Culture media	**Cultural Charecteristics**
Blood agar	Greyish white Colonies
Mac conkey	Pink, mucoid Lactose fermenting colonies

Smear from plate: Thick Gram negative bacilli seen, with some bacilli showing halo around it.

Biochemical tests:

Indole	Negative
Citrate	Positive
Urease	Positive
TSI	A/A;gas+;no H2S
Oxidase	Negative
Catalase	Positive
MR	Negative
VP	Positive

Sugar reactions: Glucose, lactose, sucrose, maltose, mannitol, starch are fermented with acid and gas**.**

Animal Pathogenicity Test Done – Mice intraperitoneal inoculation done & organisms were demonstrated in the peritoneal fluid

Antibiotic Susceptibility Testing And Klebocin Typing

Clinical Significance: Pneumonia caused by Klebsiella species frequently involves the necrotic destruction of alveolar spaces, formation of cavities, and the production of blood-tinged sputum. These bacteria also cause wound & soft-tissue infection, and UTIs. Extended Spectrum Beta Lactamses (ESBL) producing isolates are resistance to 3rd generation cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, Cefpodoxime) and Monobactams (Aztreonam). ESBL can pose a intimidating challenge with limited therapeutic options.

CASE HISTORY - 3

The patient is a 40 year-old male with multisystem failure secondary to bilaterial pneumonia. Three days before he Complained to physician with history fever, malaise, and vague respiratory symptoms. He was given amantadine for suspected influenza. The patients condition became progressively worse, with shortness of breath a fever to 40.5 ^{0}C, and he was admitted to an outside hospital 24 h prior to transfer to this hospital. A laboratory examination revealed liver and renal functions as normal. Therapy with Timentin (Ticarcillin + Clavulanic acid) and trimethoprim-sulfamethoxazole was begun. On admission, he underwent a bronchoscopic examination which revealed mildly inflamed airways containing thin, watery secretions. A Gram stain of bronchial washings was obtained which showed the presence of gram negative bacilli. On culturing in Nutrient agar, it showed mucoid grayish white colonies.

1.) Escherichia coli

2.) Pseudomonas aeruginosa

3.) Klebsiella pneumonia

4.) Streptococcus pneumonia

5.) Mycoplasma pnemoniae

4. PSEUDOMONAS

Specimen: Wound swab from patient X

Direct smear: Gram staining shows plenty of pus cells with Gram negative bacilli seen.

Culture: After Incubation @ 37°c for 24 hr

Culture media	**Colony Characteristics**
Nutrient agar	Opaque irregular colonies with earthy smell. Pseudomonas pyogenes produce green pigment
Blood agar plate	Opaque irregular colonies surrounded by zone of hemolysis.
Macconkey agar	Non lactose fermenting colonies

Smear from colony: Gram negative bacilli.

Hanging drop: Motile rods seen

Biochemical Tests:

Indole	Negative
Urease	Negative
Citrate	Positive
TSI	k/no change No gas/no H2S
Oxidase	Positve
Catalase	Positive

Sugar reaction: Glucose – is utilized oxidatively, form acid only;

Lactose; sucrose; maltose; mannitol – not fermented

Pyocin typing and Antibiotic susceptibility testing.

Clinical Significance: Pneumonia (Cystic fibrosis patient, Immunocompromised), Burns wound infection, bed sore infection, Skin and soft tissue infection, Urinary tract infection, Malignant otitis externa, Corneal ulcer for contact lens wearer or following trauma, Endocarditis for iv drug users, Septicemia.

Most common cause for nosocomial infection

CASEHISTORY - 4

A 37-year-old firefighter suffers smoke inhalation and is hospitalized for ventilatory support. He has a severe cough and begins to expectorate purulent sputum. Gram stain of his sputum specimen shows numerous polymorphonuclear cells and numerous gram-negative rods. Sputum culture grows numerous gram-negative rods that are oxidase-positive. They grow well at 42 °C. On clear agar medium they produce a blue-green color in the agar. The agar where the blue-green color is located fluoresces when exposed to ultraviolet light. The organism causing the patient's infection is

(A) *Burkholderia cepacia*

(B) *Klebsiella pneumoniae*

(C) *Escherichia coli*

(D) *Pseudomonas aeruginosa*

(E) *Burkholderia pseudomallei*

5. PROTEUS

Specimen: Urine of patient suffering from Urinary tract infection

Direct smear: Pus cells with gram negative bacilli

Culture: Plating done on

Culture media	Incubation	After 24 hr
Nutrient agar	Incubated at 37°c for 24 hr	Tiny colonies with swarming growth
Blood agar		
Mac conkey		Pale tiny non lactose fermenting colonies

Smear from colony : gram negative bacilli seen, exhibits Pleomorphism. Different morphological forms are seen in the same organisms.

Biochemical test:

	P.mirabilis	**P.vulgaris**
Indole	Negative	Positive
Urease	Positive	Positive
Citrate	Positive	Positive
TSI	Alk/acid	Alk/acid
	Gas+	Gas+
	H2S+	H2S+
Catalase	Positive	Positive
Oxidase	Negative	Negative

Hanging drop:motile gram negative bacilli seen

Sugar reactions:

Glu	Lactose	Sucrose	Maltose	Mannitol
Acid+	Not fermented	Not fermented	Acid+	Not fermented
Gas+			Gas+	

Antibiotic susceptibility pattern:

Amoxicillin Ciprofloxacin Cotrimoxazole Erythromycin Nalidicic acid Nitrofurantoin

Clinical significance: Urinary Calculi formation

CASE HISTORY- 5

A 37-year-old woman with a history of urinary tract infections comes to the emergency room with burning on urination along with frequency and urgency. She says her urine smells like ammonia. The cause of her urinary tract infection is likely to be

(A) *Enterobacter aerogenes*

(B) *Proteus mirabilis*

(C) *Citrobacter freundii*

(D) *Escherichia coli*

(E) *Serratia marcescens*

6. VIBRIO

Specimen: Rice water stools from patient suffering from acute watery diarrhea.

Direct smear: Gram staining shows Gram negative bacilli. Some are comma shaped.

Hanging Drop Preparation : Darting motility seen

Culture: Transport media – enrichment media such as alkaline water or

Monsur's med media or cary blair media is used to preserve Sample for long periods.

Media	**Colony nature -** After Incubation @ 37°c for 24 hr
Nutrient agar	Circular transparent water drop colonies
Mac conkey	Circular transport non lactose fermenting colonies

Special Media - Thiosulphate Citrate Bile salt sucrose Mdium (TCBS) - Yellow circular colonies

Smear from colony: Gram negative bacilli, some are comma shaped ;
Hanging drop: Motile rods seen.

Biochemical test:

Indole	Positive
Urease	Negative
Citrate	Negative
TSI	Acid/acid;no gas;noH2S
Oxidase	Positive
Catalase	Positive
Cholera red reaction	Positive
Polymyxin sensitivity	

Sugar reaction:

Glucose	Lactose	Sucrose	Maltose	Mannitol	Mannose	Arabinose
Acid+	--	Acid+	Acid+	Acid+	Acid+	--

Other tests:

High titre sera agglutination for O group + serotypes (Ogawa, Inaba, Hikojima)

Chick cell agglutination to differ the EI tor and classical.

Antibiotic susceptibility testing.

Clinical Significance: Severe Watery diarrhea (Classically with Rice water stools)

CASE HISTORY - 6

An 18-year-old woman in rural Bangladesh develops profuse (8 L/d) diarrhea. She has no symptoms other than the diarrhea and the manifestations of the fluid and electrolyte loss caused by the diarrhea. The most likely cause of her diarrhea is

(A) *Campylobacter jejuni*

(B) Enterotoxigenic *Escherichia coli*

(C) *Salmonella* Typhimurium

(D) *Vibrio cholerae*

(E) *Shigella dysenteriae*

7. Salmonella typhi

Specimen : Blood sample from patient X suffering from high grade fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : Specimen inoculated immediately at bed side of patient in to Ox bile or BHI broth medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli seen.

Hanging Drop: Motile rod seen.

BIOCHEMICAL TESTS

Organism	Indole	Urease	Catalase	Oxidase	Tsi	Citrate
S.typhi	Negative	Negative	Positive	Negative	K/A;No Gas; Speck of H2S+	Negative

Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.typhi	Acid + ; no gas	---------	----------	Acid + ; no gas	Acid + ; no gas	Acid + ; no gas	----------

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: **Poly O, Typhi H, O9**

New Taxonomy : **Salmonella enterica enterica typhi**

Clinical Significance : Enteric Fever, Step ladder fever, have soft Palpable spleen, may have rose spots. It may lead on to intestinal perforation, hemorrhage & circulatory collapse

CASE HISTORY - 7

A 27-year-old woman is admitted to the hospital because of fever, with increasing anorexia, headache, weakness, and altered mental status of 2 days' duration. She works for an airline as a cabin attendant, flying between the Indian subcontinent and other places in Southeast Asia and the West Coast of the United States. Ten days prior to admission she had a diarrheal illness that lasted for about 36 hours. She has been constipated for the last 3 days. Her temperature is 39 °C, heart rate 68/min, blood pressure 120/80 mm Hg, and respirations 18/ min. She knows who she is and where she is but does not know the date. She is picking at the bedclothes. Rose spots are seen on the trunk. The remainder of the physical examination is normal. Blood cultures are done and an intravenous line is placed. The most likely cause of her illness is

(A) Enterotoxigenic *Escherichia coli (ETEC)*

(B) *Shigella sonnei*

(C) *Salmonella enterica* subspecies *enterica* serotype Typhimurium (*Salmonella* Typhimurium)

(D) Salmonella *enterica* subspecies *enteric* serotype Typhi (*Salmonella* Typhi)

(E) Enteroinvasive *Escherichia coli (EIEC)*

8. Salmonella paratyphi A

Specimen : Blood sample from patient x suffering from fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : specimen inoculated immediately at bed side of patient in to ox bile medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli Seen.
Hanging Drop: Motile Rod Seen.

Biochemical Tests

Organism	Indole	Urease	Catalase	Oxidase	TSI	Citrate
S.Paratyphi A	Negative	Negative	Positive	Negative	K/A; Gas +; No H2s	Negative

Sugar Reactions :

Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.Paratyphi A	Acid + ; Gas +	----------	----------	Acid + ; Gas +	Acid + ; Gas +	----------	Acid + ; Gas +

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: **Poly O, O2**

Clinical Significance: Paratyphoid Fever, even lead on to frank septicemia with supparative complications

9. Salmonella paratyphi B

Specimen : Blood sample from patient X suffering from fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : Specimen inoculated immediately at bed side of patient in to Ox bile medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli seen.

Hanging Drop: Motile rod seen.

BIOCHEMICAL TESTS

Organism	Indole	Urease	Catalase	Oxidase	TSI	Citrate
S.Paratyphi B	Negative	Negative	Positive	Negative	K/A; Gas +; H2s +	Positive

Sugar Reactions

Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.paratyphi B	Acid + ; gas +	----------	----------	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: Poly O, O4

Clinical Significance: Paratyphoid Fever, even lead on to frank septicemia with supparative complications

LIST OF BACTERIAL ORGANISMS FOR IDENTIFICATION

1. **Staphylococcus Aureus**
2. **Escherichia coli**
3. **Klebsiella**
4. **Pseudomonas**
5. **Proteus**
6. **Vibrio**
7. **Salmonella typhi**
8. **Salmonella paratyphi A**
9. **Salmonella paratyphi B**

MYCOLOGY

Mucor sp.

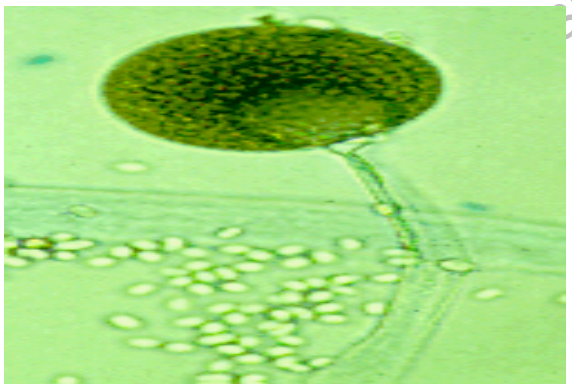
Macroscopic: Colonies are very fast growing, cottony to fluffy, white, becoming dark-grey, with the development of sporangia.

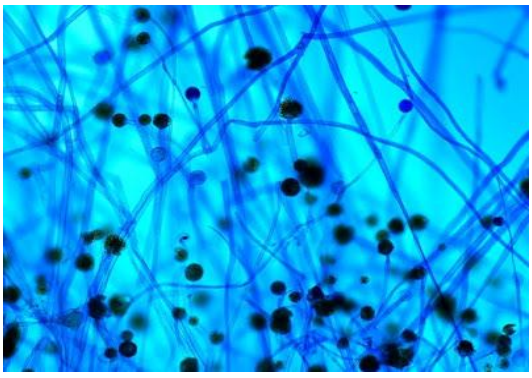
Microscopic:

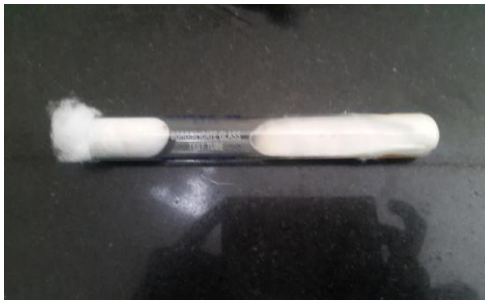
- Broad ,irregular, aseptate hyaline hyphae seen.
- The sporangiophores are long, straight with irregular branching.
- Sporangiospores enlarge at distal end into collemullae
- Branching sporangiophores with collamulla supporting sporangia are filled with sporangiospores
- The sporangia are globose
- No rhizoids are seen

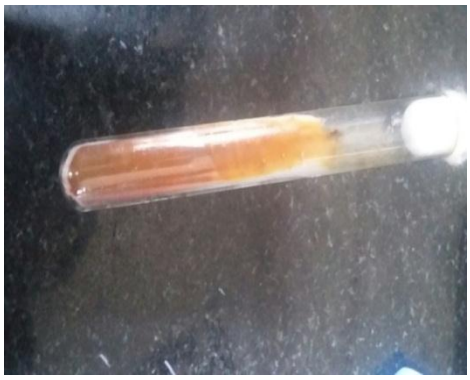
Clinical Significance:

Mainly in patients with uncontrolled diabetes or trauma can cause opportunistic, and often spreading infections known as mucormycosis.









Rhizopus sp.

Macroscopic: Colonies growth is rapid, with cotton texture. Salt and Pepper appearance. Colony appearing white initially, turns grey to yellowish brown in time.

Reverse is white to Pale.

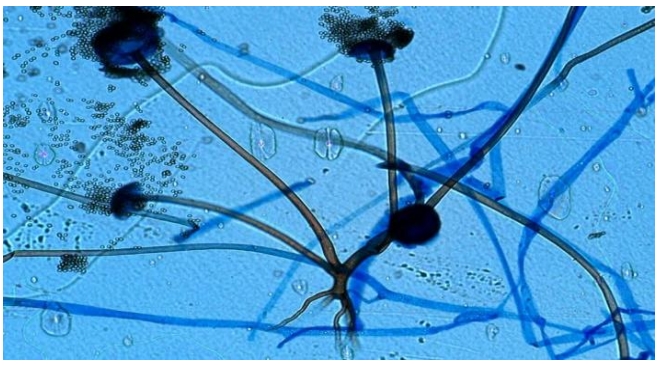
Microscopic:

- Hyphae or non septate or sparsely septate.
- Sporangiophore are unbranched.
- Rhizoids present.
- Collumullae hemispherical.
- Apophyses absent.
- Hyaline or brown coloured round to ovoid Sporangiospores.

Clinical Significance:

Caused by Diabetes and Immunosuppression, can cause Zygomycosis is an angio invasive disease. It can be of several types mucocutaneous, rhinocerebral, genitourinary, gastrointestinal, pulmonary, and disseminated infections.

Obverse **Reverse**



Rhizopus Microscopic – Rhizoid Present

Aspergillus fumigatus

Macroscopic: Dark green velvety colonies are seen

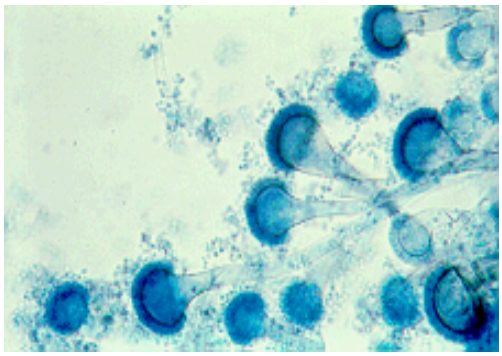
Reverse white to Tan

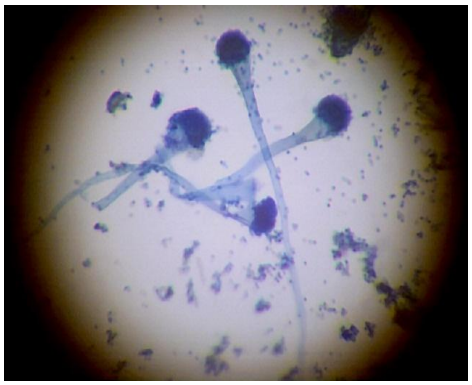
Microscopic:

- Broad, hyaline septate hyphae seen
- Conidiophores are smooth walled and are light green or brown in colour
- At the teriminal end of conidiophores, flask shaped vesicle is seen
- Uniseriate phialides are present at the upper half ($2/3^{rd}$) of the vesicle
- Each phialide bears a chain of conidia

Clinical Significance:

It can cause Allergic broncho pulmonary aspergillosis, Aspergilloma (fungal ball infection developing in a preexisting cavity), Invasive Pulmonary Aspergillosis. In immunocompromised can cause Disseminated Aspergillosis.





Conidial head of *A. fumigatus*
(Note: uniseriate row of phialides on the upper two thirds of the vesicle).

Culture of *Aspergillus fumigatus. Obverse* ***Reverse***

Aspergillus niger

Macroscopic : Coarse black granules present against creamy colony

Reverse white to yellow.

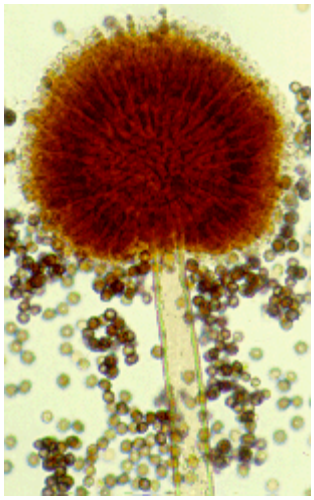
Microscopic:

- Broad , hyaline septate hyphae
- Conidiophores are wide with a brown tint in upper half
- Vesicles are spherical
- Phialides are biseriate
- The conidia are globose and are jet black

Clinical Significance:

It causes aspergillus infection affecting otitis externa, (Swimmer's Ear) , a chronic local inflammation which is characterized by itching, pain, scaling.

Culture of *Aspergillus niger*. *Obverse* ***Reverse***



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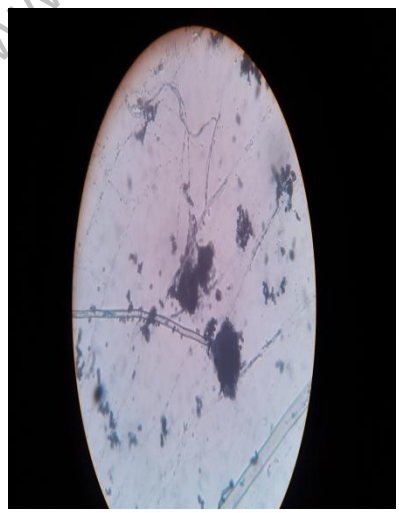
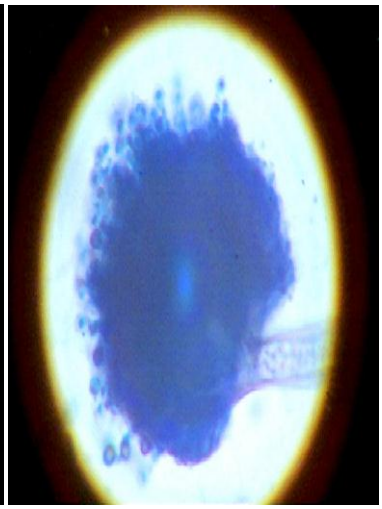




Note: Conidial head of *A. niger*. conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.

Aspergillus flavus

Macroscopic: Yello to Yellow green colonies seen

Reverse goldish to red brown.

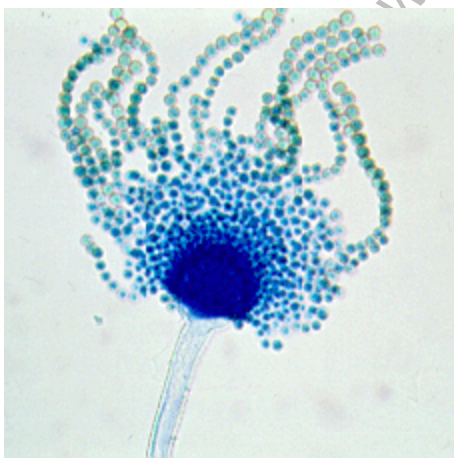
Microscopic:

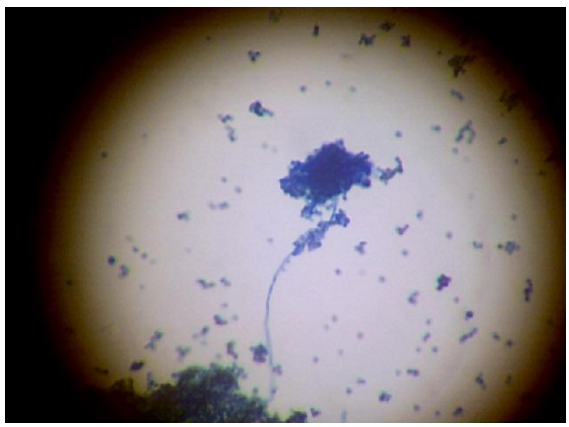
- Broad , hyaline septate hyphae
- Conidiophores are thick walled , hyaline
- Vesicles are large and globose
- Phialides are biseriate and present over the entire surface of the vesicle
- The conidia are unicellular & globose

Clinical Significance:

It occurs in immunocompromised host, can cause Allergic broncho pulmonary aspergillosis. Less commonly it causes Invasive Pulmonary Aspergillosis

Culture of *Aspergillus flavus*. Obverse **Reverse**





Conidial head of *A. flavus*.

Note: conidial heads with both uniseriate and biseriate arrangement of phialides may be present over the entire surface.

Candida Species

Macroscopic: Creamy white moist or pasty colonies

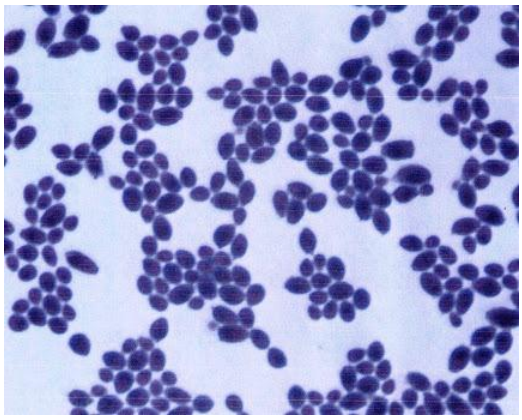
Microscopic: Oval Gram Positive budding Yeast Cells, Pseudohyphae are constricted at the ends and remain attached like links of sausages. Hyaline are septate

Clinical Significance:

It most commonly involves mucous membranes oral cavity (Oral Candidiasis), vulva and vagina (Vulvo vaginal candidiasis). It also causes cutaneous candidiasis. Disseminated candidiasis is caused in immunocompromised individuals (eg., HIV, Organ transplantation, Neoplastic debilitating patients)

Germ Tube Test - It helps to differentiate Candida albicans from non albicans group Germ tube is atrue hyphal structure and therefore does not have constriction characterize of pseudohyphae. Formation of Germ tube is present in Candida albicans

Culture of Candida albicans- Obverse **ReversE**



Candida – Microscopic – Budding Yeast Cells

OSPE

A. CULTURE MEDIA

1. Identify the culture media?

2. What type of media is this?

3. List two main ingredients of this medium?

4. List four organisms grown in this media?

5. How is this media sterilized?

A. CULTURE MEDIA

1.Nutrient Agar

2.Simple media (basal media)

3.Peptone water, 1%Meat extract, 2% Agar

4.

a. Staphylococcus aureus,
b. Pseudomonas aeruginosa
c. Escherichia coli
d. Klebsiella pneumoniae

5.Autoclaving

B. CULTURE MEDIA

1.Identify the culture media?

2.What type of media is this?

3.List two main ingredients of this medium?

4.List four organisms grown in this media?

5.How is this media sterilized?

B. CULTURE MEDIA

1.Blood Agar

2.Enriched media

3. 5% blood and nutrient agar

4.

a. Streptococcus pyogenes,
b. Streptococcus pneumonia,
c. Neisseria spp,
d. Vibrio cholera

5.Medium is prepared by adding sterile blood to sterile nutrient agar that has been melted and cooled to 50°C

C. CULTURE MEDIA

1. Identify the culture media?

2. What type of media is this?

3. List two main ingredients of this medium?

4. List four organisms grown in this media?

5. How is this media sterilized?

C.CULTURE MEDIA

1.Mac Conkey medium

2.Differential media or Indicator medium

3.Lactose, Peptone, Agar, Neutral red & Taurocholate.

4.

a) Pseudomonas aeruginosa
b) Escherichia coli
c) Klebsiella pneumoniae
d) Salmonella typhi

5.Autoclaving

1. INSTRUMENT

1. Identify the jar?

2. What is the use of this jar?

3. List four organism which can be grown by using this jar?

4. Enumerate two disease caused by these organisms?

5. Chemical indicator used for verifying the required condition in the jar?

1. INSTRUMENT

1. McIntosh and Filde's anaerobic jar

2. For cultivation of anaerobic organism by achieving anaerobiosis.

3.Clostridium tetani, C. perfringens, C. botulinum, C. septicum

4. C. tetani causes Tetanus.
   C. perfringens causes gas gangrene.

5.Reduced methylene blue, it remains colorless anaerobically but turns blue on exposure to oxygen.

2. INSTRUMENT

1. Identify the given object

2. What is it used for and what is the type of test done by using this object?

3. What is the antigen used in the test?

4. What is the disease diagnosed by the test done using this object?

5. What is the causative agent of the disease diagnosed by using this object?

2.INSTRUMENT

1.VDRL Rotator

2.It is used for doing VDRL test.
VDRL (Venereal Disease Research Laboratory) test is a slide flocculation test.

3.Cardiolipin antigen

4.Syphilis

5.Treponema pallidum.

3. INSTRUMENT

1. What is this instrument ?

2. What are the instruments that can be sterilized using this ?

3. What is the ideal temperature and pressure ?

4. What is the Sterility check used ?

5. What is this type of sterilization ?

3.INSTRUMENT

1. Autoclave

2. Dressing, instruments,laboratory ware, media and pharmaceutical products

3. 121°C for 15 minutes at 15 lbs

4. Spores of Bacillus stearothermophilus

5. Steam under pressure

4. INSTRUMENT

1. What is this instrument?

2. What are the instruments to be sterilized?

3. Ideal temperature and holding time?

4. Sterility check used.

5. What is this type of sterilization?

.

4.INSTRUMENT

1. Hot air oven

2. Glassware, forceps, scissors, glass syringes,swabs and pharmaceutical products'

3. 160°C for 1 hour.

4. Spores of nontoxigenicstrain of Clostridium tetani or Bacillus subtilus

5. Dry heat sterilization

CASE 1

A 23 year old female gives a 2 day H/O fever, frequency, dysuria and mild haematuria. She also complains of suprapubic pain, but there is no vaginal discharge. There is no relevant previous history and examination is unremarkable.

1. What is the probable diagnosis?

2. List four common organisms causing this infection

3. What is the relevant microbiological investigation?

4. Name the media

5. Describe the colonies

6. Gram stain of the organism isolated showed Gram negative bacilli
   Identify the organism from the given biochemical reactions?

CASE 1

A 23 year old female gives a 2 day H/O fever, frequency, dysuria and mild haematuria. She also complains of suprapubic pain, but there is no vaginal discharge. There is no relevant previous history and examination is unremarkable.

1. Urinary tract infection

2. Proteus, Escherichia coli, Klebsiella, Staphylococcus saprophyticus

3. Urine culture & sensitivity

4. MacConkey agar.

5. MacConkey agar- Lactose Fermenting colonies

6. Gram stain of the organism isolated showed Gram negative bacilli
   Identify the organism from the given biochemical reactions?
   Indole: positive
   TSI: A/A with gas, no H_2S
   Urease: Negative
   Citrate: Not utilized
   Sugars:
   Glucose- - Fermented with acid & gas production
   Lactose- - Fermented with acid & gas production
   Sucrose-- Fermented with acid & gas production
   Maltose-- Fermented with acid & gas production
   Mannitol-- Fermented with acid & gas production

   The organism is identified as Escherichia coli.

CASE 2

A 14 year old boy gives H/O fever, headache and abdominal pain for the past 10 days. O/E he is toxic with coated tongue and hepatosplenomegaly.

1. List two infective causes of fever?

2. Write the relevant microbiological tests done to detect Enteric fever?

3. Identify the given diagnostic test.

4. Name the antigens used

5. What is significant titre?

CASE 2

A 14 year old boy gives H/O fever, headache and abdominal pain for the past 10 days. O/E he is toxic with coated tongue and hepatosplenomegaly.

1.Enteric fever,Tuberculosis,Malaria.

2.Blood Culture,Widal test,Stool Culture and Urine Culture.

3.Widal test – Tube agglutination test.

4. O antigen
   H antigen
   AH antigen
   BH antigen

5.O agglutinin - 1:100 dilution or more.

H agglutinin – 1:200 dilution or more.

CASE 3

A 35 year old male with burns over both the arms gives an H/O discharge of pus from the wound.

1. Name three bacteria commonly isolated from burns wound infection?

2. What is the relevant microbiological investigation required in this case?

3. Name the media?

4. Describe the colonies?

5. Gram stain of the organism isolated showed Gram negative bacilli
   Identify the organism from the given biochemical reactions

CASE 3

A 35 year old male with burns over both the arms gives an H/O discharge of pus from the wound.

1. Pseudomonas aeruginosa
   Staphylococcus aureus
   Proteus species

2. Pus for culture and sensitivity.

3. Nutrient Agar
   Mac Conkey agar

4. Nutrient agar - Greenish pigmented colonies
   Mac Conkey agar - Non lactose fermenting colonies.

5. Indole- Negative
   TSI- K/K
   Urease- Negative
   Citrate- Utilised
   Sugars- Glucose: no acid, no gas
   Lactose : no acid, no gas
   Sucrose: no acid, no gas
   Maltose: no acid, no gas
   Mannitol: no acid, no gas

The organism is identified as Pseudomonas aeruginosa.

CASE 4

A 40 year old male with a H/O discharge of pus from a wound in the leg. O/E a sinus is seen which is attached to the bone.

1. Name three bacteria commonly isolated in osteomyelitis?

2. What are the revelant microbiological investigation required to diagnose this condition?

3. Name the media?

4. Describe the colonies?

5. Gram stain of the organism isolated showed Gram positive cocci
Identify the organism from the given biochemical reactions?

CASE 4

A 40 year old male with a H/O discharge of pus from a wound in the leg. O/E a sinus is seen which is attached to the bone.

1. Staphylococcus aureus , Proteus, Pseudomonas aeruginosa.

2. Pus for culture and sensitivity.

3. Nutrient agar media.

4. Golden yellow pigmented colonies.

5. Urease : Positive.
   Mannitol is fermented with acid production without gas.
   Slide coagulase test : Positive.

   The organism is identified as Staphylococcus aureus.

CASE -5

A 23 year old female gives a 2 day H/O fever, frequency, dysuria and mild haematuria. She also complains of suprapubic pain, but there is no vaginal discharge. There is no relevant previous history and examination is unremarkable.

1. What is the probable diagnosis?

2. List four common organisms causing this infection

3. What is the relevant microbiological investigation?

4. Name the media

5. Describe the colonies

6. Gram stain of the organism isolated showed Gram negative bacilli
   Identify the organism from the given biochemical reactions?

CASE -5

A 23 year old female gives a 2 day H/O fever, frequency, dysuria and mild haematuria. She also complains of suprapubic pain, but there is no vaginal discharge. There is no relevant previous history and examination is unremarkable.

1. Urinary tract infection

2. Proteus, Escherichia coli, Klebsiella, Staphylococcus saprophyticus

3. Urine culture & sensitivity

4. MacConkey agar.

5. MacConkey agar-Non Lactose Fermenting colonies

6. Indole: negative
   TSI: K/A with gas, Abundant H_2S
   Urease: positive
   Citrate: Not utilized
   Sugars:
   Glucose- - Fermented with acid & gas production
   Lactose- -Not Fermented
   Sucrose—Not Fermented
   Maltose—Not Fermented
   Mannitol—Not Fermented

The organism is identified as Proteus mirabilis

SMEAR PREPARATION

The preparation of a smear is required for many laboratory procedures, including the Gram-staining. The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure.

Materials Required:

Clean glass slides , Inoculating loops or needles , Normal saline or Sterile water , Glass marking pencil, Specimen (may be Broth culture, Urine, Sputum , pus, swab,etc.,)

Procedure:

1. Take a clean, grease free glass slide. Wash the glass slide with fine sand soap , rinse it well, dry it thoroughly. Label your slide with the glass marking pencil.
2. Place one loopful of bacterial growth in the center of a clean slide.
3. If working from a solid medium, add one drop (and only one drop) of Normal Saline / Sterile water to the slide. If using a broth medium, do not add the water.
4. Now, with the inoculating loop, mix the specimen with the Normal Saline/ Sterile water completely and spread the mixture out to cover about half of the total slide area.
5. Place the slide on a slide warmer and wait for it to air dry.
6. Dried smear is then fixed by passing it three times through the flame with the film facing downwards. . The smear is now ready for the staining procedure.

BIOMEDICAL WASTE MANAGEMENT

1. What is biomedical / hospital wastes?

Any wastes generated while providing healthcare,performing research &undertaking investigation or related procedures on human beings or animals in hospitals/laboratories or in any health care setup.

2. What are the types of biomedical wastes?

1.Infectious wastes - placenta,body fluids, laboratory samples, cultures, sharp wastes (forms only 10% of total waste)

2.Non infectious hazardous wastes – chemicals,radioactive substances, pharmacological wastes.

3. What are the objectives of biomedical waste management?
   - To prevent harm resulting from biomedical wastes.
   - To minimize waste volumes.
   - To retrieve reusable material.
   - To ensure safe &economical disposal.

4. What are the colour coding & types of container for disposal of biomedical wastes?

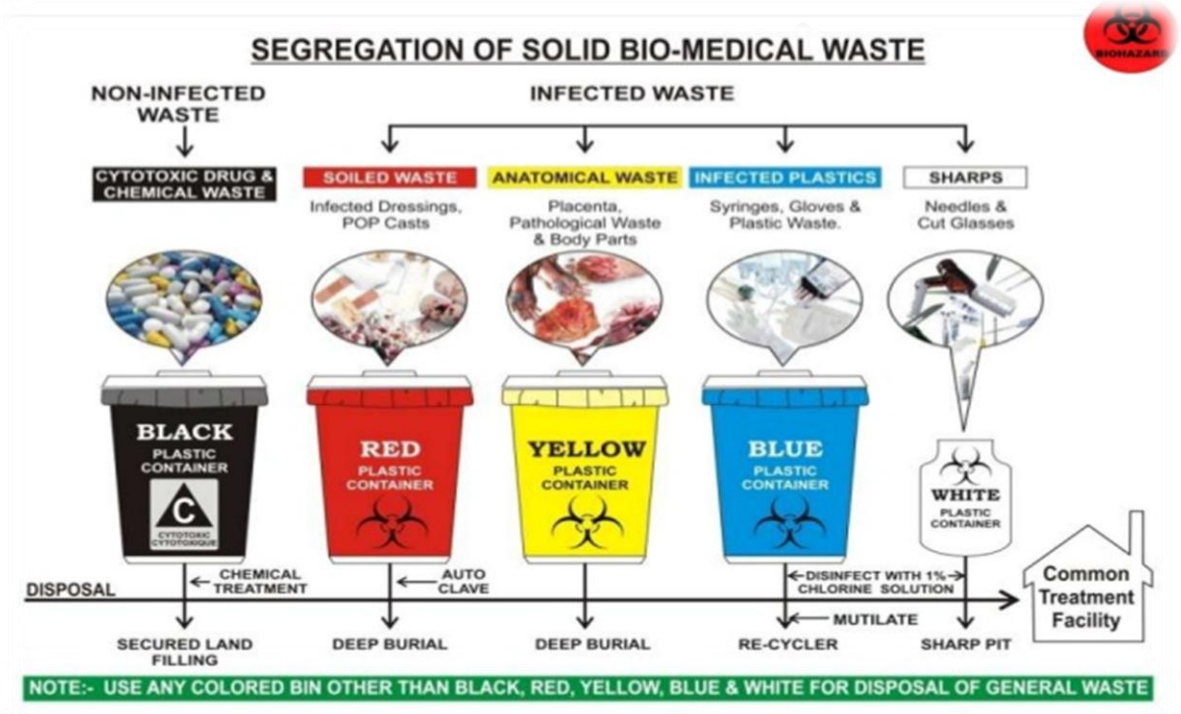
COLOUR CODING	TYPES OF CONTAINER	WASTE CATEGORY
1.YELLOW	PLASTIC BAG	HUMAN ANATOMICAL WASTES. DISCARDED MEDICINE. CYTOTOXIC DRUGS.
2.RED	DISINFECTED CONTAINER PLASTIC BAG	MICROBIOLOGICAL, BIOTECHNOLOGICAL WASTES. SOILED WASTES. SOLID WASTES.
3.BLUE / WHITE	PUNCTURE PROOF CONTAINER	WASTE SHARPS
3.BLACK	PLASTIC BAG BIODEGRADABLE	INCINERATOR ASH CHEMICAL WASTES HOUSEHOLD WASTES

5.What are the steps of biomedical wastes management?

- Reduction.
- Segregation at the point of generation of waste
- Storage.
- Transportation.
- Treatment.

6.What are the methods of biomedical wastes treatment?(any 4)

- ✓ Chemical disinfections.
- ✓ Deep burial.
- ✓ Incineration.
- ✓ Autoclaving.
- ✓ Microwaving.



VENIPUNCTURE

Procedure for Venipuncture:

1. Clean your hands with soap and water or gel cleanser. Ask the patient to state his/her name. Determine if the test to be obtained has any special requirements.
2. Explain the procedure to the patient. Position the arm for venipuncture; support the arm on a firm surface; the arm should be in a downward position. The **median cubital** and **cephalic veins** are most commonly used for venipuncture
3. The patient can make a fist, but should not pump the hand open and closed. Apply tourniquet Palpate the vein. Release the tourniquet and assemble appropriate equipment.
4. Wear gloves, Cleanse site with approved disinfectant. Allow the disinfectant to air-dry to avoid hemolysis of the specimen.
5. Re-apply tourniquet about 3-4 inches above puncture site, donot palpate the vein, insert needle, bevel-side up, at about a 30° angle, and collect specimens
6. Once sufficient blood has been collected, release the tourniquet BEFORE withdrawing the needle. Some guidelines suggest removing the tourniquet as soon as blood flow is established, and always before it has been in place for two minutes or more.
7. Withdraw the needle gently and apply gentle pressure to the site with a clean gauze or dry cotton-wool ball. Ask the patient to hold the gauze or cotton wool in place, with the arm extended and raised. Ask the patient NOT to bend the arm, because doing so causes a haematoma.
8. Apply direct pressure to stop bleeding at puncture site. After about 2 minutes, check the puncture site to verify that bleeding has stopped. Apply bandage if appropriate. Thank the patient for his/her cooperation.
9. Label specimen(s) in the presence of the patient including all the information that is required by your facility.

4.HANDWASHING METHOD

SEROLOGY

Anti Streptolysin O (ASO) Test:

Aim:

To determine the presence of anti streptolysin O antibodies in the given serum

Principle:

It is a rapid latex agglutination test for the qualitative and semi-quantitative determination of anti-streptolysin-O antibodies (ASO) in serum. When the latex reagent is mixed with a serum containing ASO antibody, agglutination occurs. Sera having titers more than 200 IU/ml will be considered as positive.

Procedure:

1. Using a disposable pipette place one drop of each undiluted sample into its identified circle of the slide. Deliver one drop of positive and negative control into its identified circle.
2. Mix the ASO latex reagent by gently shaking. Add one drop of reagent to each control and sample.
3. Thoroughly mix each sample with reagent within the full area of the circle.
4. Slowly rock the slide for exactly two (2) minutes and observe for agglutination under a high intensity light.
5. Record results.

Result / Interpretation:

A test sample is considered to contain ASO antibodies in excess of 200 IU/ml when agglutination (clumping) is observed when compared to the result of the negative control (uniform suspension)

Rapid Plasma Reagin (RPR) Test:

Aim:

To detect IgM and IgG antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes.

Principle

The rapid plasma reagin (RPR) test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis.RPR antigen is mixed with unheated or heated (to inactivate complement) serum or with unheated plasma on a plastic-coated card.

If antibodies are present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card. If antibodies are not present in the test serum, the test mixture is uniformly gray.

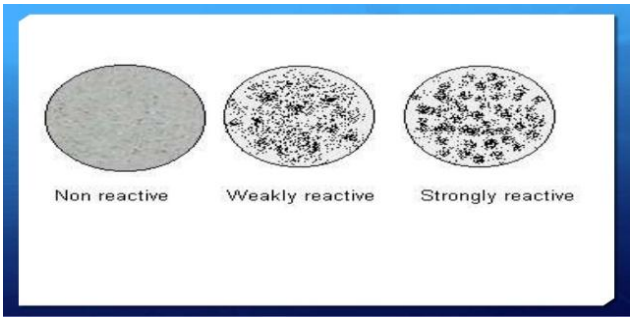
Procedure:

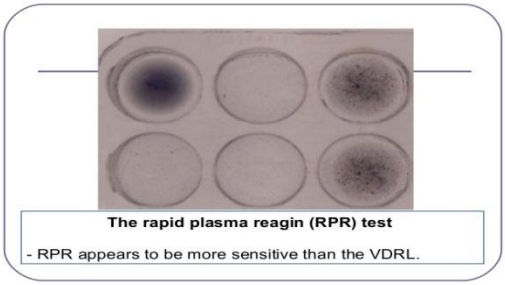
1. Using disposable serum dispensers or droppers, dispense one drop (0.05 ml) of serum or plasma sample onto a circle on the test card. Also add one drop of positive control and Negative control in the respective circle.
2. Spread the sample smoothly across the circle area.
3. After mixing the antigen solution by swirling, add one drop of the antigen suspension to each sample / control testing area. Do not stir or spread the antigen.
4. Place the card on an VDRL rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes
5. Immediately read results macroscopically in the “wet” state under a high intensity light source.

Interpretation of RPR Test

1. Non-reactive (NR) – smooth suspension, no clumping or slight roughness
2. Reactive (R) – any degree of clumping

If the test is negative, but the physician still suspects syphilis infection is present, the more specific treponemal tests (FTA-ABS, TPHA, TPI) should be performed because false positives can occur in RPR. False positives can occur in RPR. RPR is sensitive and used as screening test.





RHEUMATOID FACTOR

Aim:

To detect rheumatoid factor in the given serum qualitatively.

Principle:

Rheumatoid factors (RF) are antibodies directed against the Fc fragment of human and animal IgG, which acts as antigen.. The RF reagent is a suspension of polystyrene latex particles sensitized with specially prepared human IgG. The reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen.

When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs. The RF latex reagent sensitivity has been adjusted to detect a minimum of 8 IU/mL of rheumatoid factors according to the WHO International Standard without previous sample dilution

Procedure:

1. .Place one drop RF Positive and Negative Control in field 1 & 2. Using pipettes, place one drop of the undiluted specimens on successive fields.
2. Gently resuspend the RF Latex Reagent and add one drop to each test field. Use pipette/Stir Stick to spread reaction mixture over entire test field.
3. Rotate the slide manually or with a mechanical rotator at 80-100 rpm for 2 minutes and read immediately under direct light.
4. Presence of agglutination of the latex particle is a positive result (see figure 1). Agglutination indicates a RF concentration of equal or more than 8 IU/ml. Sera with positive agglutination should be run again with the Quantitative Test.

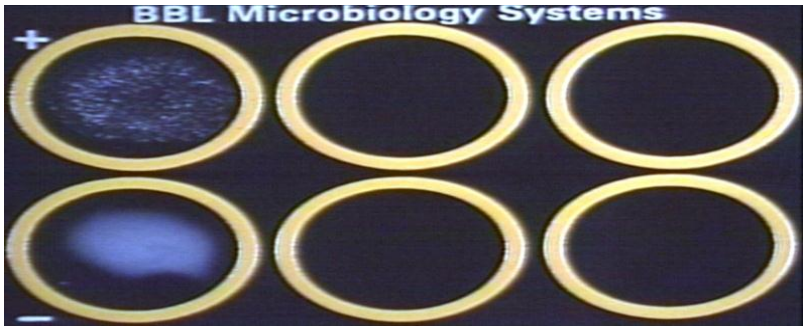
Result / Interpretation:

Negative Result: A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the RF Negative Control.

Positive Result: A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the RF Negative and Positive Controls

Positive result Signifies Rheumatoid Arthritis

Positive



Negative

C- REACTIVE PROTEIN

Aim:

To detect the presence of C-Reactive protein in the given serum

Principle:

CRP is one of the Acute phase protein, which is considered to be a sensitive indicator of inflammation. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles. The use of the CRP test to measure the effectiveness of therapy is of great clinical significance in cases such as rheumatoid arthritis and also other inflammation, bacterial and viral infection.

Procedure:

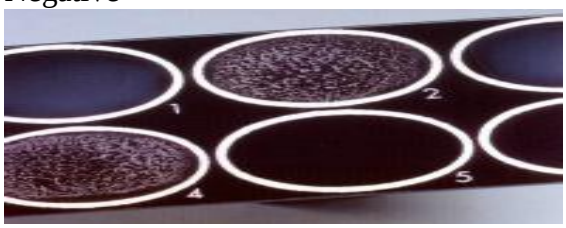
1. Gently shake the CRP latex vial to disperse and suspend latex particles. Positive and negative controls should be tested with each series of test.
2. Using the disposable pipette provided, place one drop of test serum onto a circle on the slide. Use a separate disposable pipette for each test serum.
3. Deliver one drop of CRP Latex to each circle that contains specimens on the slide. Spread the resulting mixture by using the paddle end of the pipette.
4. Gently tilt and rotate slide by hand for two (2) minutes. Observe for macroscopic clumping using the indirect oblique light source. Compare the reaction of the test serum to the CRP positive and negative control sera.

Result / Interpretation:

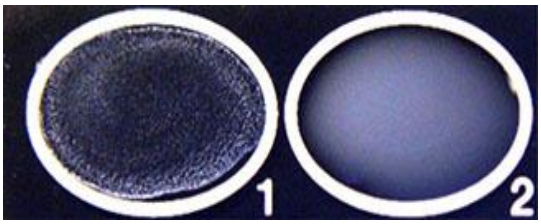
Positive Result: Agglutination
Negative Result: Smooth milky suspension

Since negative results may be caused by CRP antigen excess, the test should be repeated using a diluted serum sample in case prozone effect is suspected.
Positive result signifies that CRP >10mg/L.
It is used ininfection or inflammation. Increased levels observed in Acute rheumatic fever and in Rheumatoid arthritis.

Negative



Positive



Positive Negative

HEPATITIS B SURFACE ANTIGEN

Aim:

To detect the prescence of Hepatitis B Surface antigen in given serum or plasma.

Principle:

One step test for HBsAg utilizes the principle of Immunochromatography, a unique two site immunoassay on a membrane. As the test sample flows through the membrane assembly of the test device, the colored monoclonal anti-HBsAg-colloidal gold conjugate complexes with the HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane leading to formation of a pink-purple colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any move further on the membrane and are subsequently immobilized by the anti-rabbit antiserum coated on the membrane at the control region, forming a pink-purple band. This control band serves to validate the test results.

Procedure:

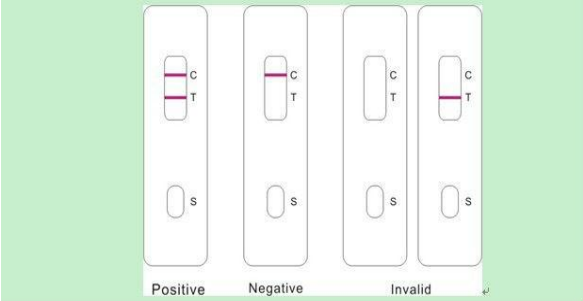
1. Label the card with patient name or identification number.
2. Use the disposable pipette, dispense about 2~3 drops sample in a vertical position into the sample well on the card.
3. Wait for coloured bands to appear. Read within 15-20 minutes. Do not read results after 30 minutes.

Result / Interpretation:

Negative : Only one red line in the Control (C) area, with no coloured line in the Test (T) area indicates a negative result.

Positive : Two red lines, one in the Test (T) area and one in the Control (C) area indicate a positive result.

The test should be considered invalid if neither the test band nor the control band appear. Repeat the test with a new device.



Negative Positive

Enzyme-linked immunosorbent assay (ELISA)

Aim:

To detect the presence of antibody against the specifeic antigen is present in the given serum.

Principle:

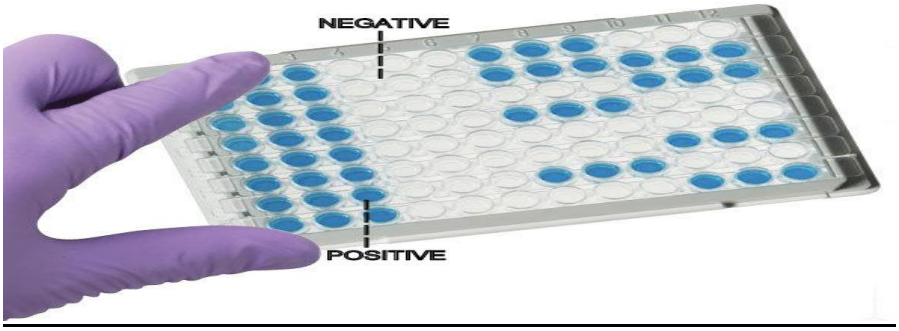
This testing method is a type of immunoassay. It is based on the principle that antibodies will bind to very specific antigens to form antigen-antibody complexes, and enzyme-linked antigens or antibodies can be used to detect and measure these complexes.

Procedure:

To detect or measure an antibody in a person's blood, a known antigen is attached to a solid surface. A solution containing the patient sample is added. If the patient's sample contains antibody, it will bind to the antigen. A second antibody (against human antibodies) that is labeled with an enzyme is then added. If the enzyme-linked antibody binds to human antibodies, the enzyme will create a detectable change that indicates the presence and amount of the antibody in the patient sample.

Result / Interpretation:

Color should develop in positive wells on addition of substrate within 30 minutes (yellow or orange, for pNPP or OPD, respectively). Absorbance may be read directly in a microplate reader (at 405 nm or 450 nm, for pNPP or OPD, respectively) or the reaction may be stopped with 50 µl per well of the appropriate stopping reagent and absorbance read later (at 405 nm or 492 nm, for pNPP or OPD, respectively).



HCV TEST

AIM:

To detect HCV antibody in the given serum sample

PRINCIPLE OF THE TEST

Immunochromatographic rapid test cassette for qualitative detection of antibody against HCV in serum

The HCV-Card Rapid Test (Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with recombinant HCV antigen on the test line region of the card. During testing, the serum or plasma specimen reacts with the recombinant HCVantigen coated colloidal gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with another recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

TEST PROCEDURE

1.When you are ready to begin testing, open the sealed pouch by tearing from the pouch.

2.Draw 0.2ml (about 4 drops) sample into the pipette, and dispense it into the sample well.

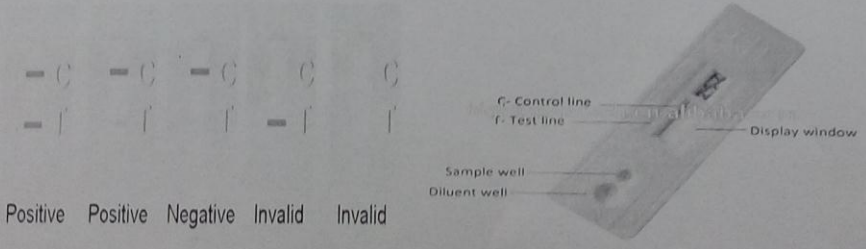
3.Wait 10-20 minutes and read results. Do not read results after 30 minutes.

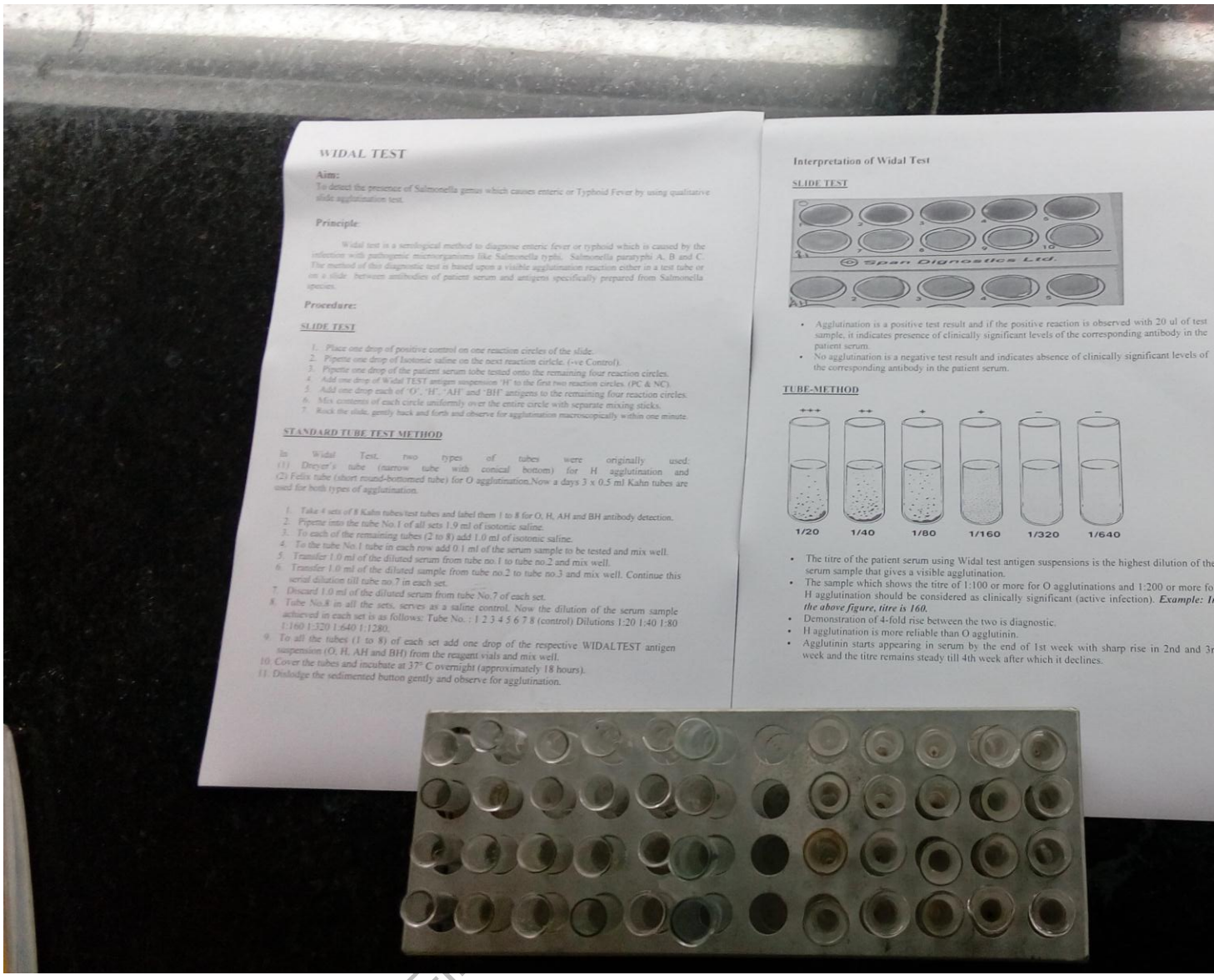
INTERPRETATION

Negative: Only one colored band appears on the control region. No apparent band on the test region.

Positive: In addition to a pink colored control band, a distinct pink colored band will also appear in the test region.

Invalid: A total absence of color in both regions is an indication of procedure error and/or that test reagent deterioration has occurred





WIDAL TEST

Aim:
To detect the presence of Salmonella genus which causes enteric or Typhoid Fever by using qualitative slide agglutination test.

Principle:

Widal test is a serological method to diagnose enteric fever or typhoid which is caused by the infection with pathogenic microorganisms like Salmonella typhi, Salmonella paratyphi A, B and C. The method of this diagnostic test is based upon a visible agglutination reaction either in a test tube or on a slide between antibodies of patient serum and antigens specifically prepared from Salmonella species.

Procedure:

SLIDE TEST

1. Place one drop of positive control on one reaction circles of the slide.
2. Pipette one drop of Isotonic saline on the next reaction circle. (-ve Control).
3. Pipette one drop of the patient serum tobe tested onto the remaining four reaction circles.
4. Add one drop of Widal TEST antigen suspension 'H' to the first two reaction circles. (PC & NC).
5. Add one drop each of 'O', 'H', 'AH' and 'BH' antigens to the remaining four reaction circles.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide, gently back and forth and observe for agglutination macroscopically within one minute.

STANDARD TUBE TEST METHOD

In Widal Test, two types of tubes were originally used: (1) Dreyer's tube (narrow tube with conical bottom) for H agglutination and (2) Felix tube (short round-bottomed tube) for O agglutination.Now a days 3 x 0.5 ml Kahn tubes are used for both types of agglutination.

1. Take 4 sets of 8 Kahn tubes/test tubes and label them 1 to 8 for O, H, AH and BH antibody detection.
2. Pipette into the tube No.1 of all sets 1.9 ml of isotonic saline.
3. To each of the remaining tubes (2 to 8) add 1.0 ml of isotonic saline.
4. To the tube No.1 tube in each row add 0.1 ml of the serum sample to be tested and mix well.
5. Transfer 1.0 ml of the diluted serum from tube no.1 to tube no.2 and mix well.
6. Transfer 1.0 ml of the diluted sample from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7 in each set.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Tube No.8 in all the sets, serves as a saline control. Now the dilution of the serum sample achieved in each set is as follows: Tube No. : 1 2 3 4 5 6 7 8 (control) Dilutions 1:20 1:40 1:80 1:160 1:320 1:640 1:1280.
9. To all the tubes (1 to 8) of each set add one drop of the respective WIDALTEST antigen suspension (O, H, AH and BH) from the reagent vials and mix well.
10. Cover the tubes and incubate at 37° C overnight (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

Interpretation of Widal Test

SLIDE TEST

- Agglutination is a positive test result and if the positive reaction is observed with 20 ul of test sample, it indicates presence of clinically significant levels of the corresponding antibody in the patient serum.
- No agglutination is a negative test result and indicates absence of clinically significant levels of the corresponding antibody in the patient serum.

TUBE-METHOD

- The titre of the patient serum using Widal test antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.
- The sample which shows the titre of 1:100 or more for O agglutinations and 1:200 or more fo H agglutination should be considered as clinically significant (active infection). ***Example: I the above figure, titre is 160.***
- Demonstration of 4-fold rise between the two is diagnostic.
- H agglutination is more reliable than O agglutinin.
- Agglutinin starts appearing in serum by the end of 1st week with sharp rise in 2nd and 3r week and the titre remains steady till 4th week after which it declines.

SPOTTERS

NUTRIENT AGAR

- It is a simple medium.
- It is prepared by adding 2% agar to nutrient broth.
- It is sterilized by autoclaving.

BLOOD AGAR

- It is an enriched medium.
- It is also a differential medium since the degree of hemolysis caused by hemolysin is assessed to differentiate among Gram positive colonies.
- It is used for growing fastidious organisms like Streptococci, Pneumococci, Hemophilus influenzae.
- It is prepared by adding sterile sheep blood to sterile nutrient agar that has been melted and cooled to 50 degree cent

CHOCOLATE AGAR

- It is an enriched medium.
- It is prepared by adding 10% sterile sheep blood to sterile molten nutrient agar at 75 degree centigrade.
- It is opaque and chocolate in colour.
- It is useful for the isolation of fastidious organisms like Hemophilus influenza, Neisseria meningitidis.

TCBS MEDIUM

- It is a selective medium.
- It contains thiosulphate, citrate, bile salts, sucrose with bromothymol blue as an indicator.
- It is useful for isolation of vibrio cholerae which produces yellow coloured colonies due to fermentation of sucrose.

MAC CONKEY AGAR

- It is a differential medium.
- It consists of peptone, lactose, agar, neutral red and sodium taurocholate.
- It is used to differentiate lactose fermenting colonies (pink) and non lactose fermenting colonies (colourless or pale).
- It is sterilized by autoclaving.

ROBERTSONS COOKED MEAT MEDIUM

- It is an anaerobic medium.
- It contains glucose broth with minced meat pieces with 1 cm layer of sterile liquid paraffin at the top.
- It is used for growing anaerobic organisms like Clostridium tetani.

BACTERIOLOGICAL LOOP

- It is usually made of nichrome.
- It is used to transfer and streak clinical specimens onto culture medium.
- It is sterilized by heating red hot in flame.

MC INTOSH AND FILDES JAR

- It is used for anaerobic culture.
- It is made up of glass or stainless steel jar with a lid.
- The lid has an inlet and outlet.
- On the underside of the lid is the catalyst, consisting of alumina pellets coated with palladium.
- Reduced methylene blue is used as an indicator.

PETRI DISH

- It is a shallow flat bottomed circular clear glass container with lid.
- It is usually 90mm in diameter.

- Melted agar medium solidified in a petri dish provides a large surface area for the culture of bacteria.
- It is sterilized by hot air oven.

DREYERS TUBE

- It is a narrow tube with a conical bottom.
- It is used for H antigen agglutination in Widal test.
- Loose fluffy cotton wool clumps seen in positive agglutination test.

FELIX TUBE

- It is a short round bottom tube.
- It is used for O antigen agglutination in Widal test.

- Disc like pattern with granular deposits seen at the bottom of the tube in positive agglutination test.

STERILE SYRINGE

- It is used to collect specimens like blood, body fluids and pus.
- It should be used only once and to be disposed.
- It is sterilized by gamma radiation or ethylene oxide gas.

UNIVERSAL CONTAINER

- It is a screw capped glass bottle used to collect specimens like urine, blood etc.
- It has a capacity of 30 ml.
- It is sterilized by hot air oven.

PASTEUR PIPETTE

- It is used to deliver solutions or reagents in various diagnostic procedures.
- It is sterilized by hot air oven

DURHAMS TUBE

- It is a small tube to detect gas formation in sugar fermentation reactions.
- Formation of air bubbles inside the tube indicates gas production.

STERILE TEST TUBE

- It is used to collect specimens like blood, urine, body fluids.
- It is also used for keeping sterile swab.
- It is sterilized by hot air oven.

STERILE SWAB

- It is made up of absorbent cotton.
- It is used to collect specimens from throat, wounds and ear.
- It is used for making lawn culture for antibiotic sensitivity test.
- It is sterilized by hot air oven.

MICROTITRE PLATE

- It is a polystyrene plate.
- It contains 96 wells (8 rows and 12 columns).
- Wells are coated with antigen or antibody.
- It is used for ELISA test.

CONICAL FLASK

- It is made up of borosilicate glass.
- It is of different capacities (eg., 250ml, 500ml, 1000ml).
- It is used as container for media.
- It is sterilized by hot air oven.

VDRL ROTATOR

- It is used in VDRL test.
- It is used for uniform mixing of antigen and antibody.
- It rotates at 180 rotations per minute.

ANTIBIOGRAM

- Mueller Hinton agar is commonly used.
- Antibiotic discs are placed on Mueller Hinton agar and incubated at 37 º C for 18-24 hrs.
- Diameter of zone of inhibition is measured and it is interpreted as sensitive or resistant by comparing the zone size using the zone size interpretative chart.

UREASE MEDIUM WITHOUT REACTION

- Christensens urease medium is used to determine the ability of an organism to produce urease.
- Phenol red is used as an indicator.
- Yellow colour indicates negative test, eg. Escherichia coli.

UREASE MEDIUM WITH REACTION

- Christensens urease medium is used to determine the ability of an organism to produce urease.
- Phenol red is used as an indicator.
- Pink colour indicates positive test. eg. Kelbsiella pneumoniae, Proteus vulgaris.

CITRATE MEDIUM WITH REACTION

- Simmons citrate medium is used to determine the ability of an organism to utilise citrate as the sole source of carbon for its growth.
- Bromothymol blue is used as an indicator.
- Blue colour indicates positive test. eg., Klebsiella pneumoniae, Citrobacter.

CITRATE MEDIUM WITHOUT REACTION

- Simmons citrate medium is used to determine the ability of an organism to utilise citrate as sole source of carbon for its growth.
- Bromothymol blue is used as an indicator.
- Green colour indicates negative test. eg. Escherichia coli.

TSI MEDIUM

- It is a triple sugar iron medium.
- It is a differential medium.
- It is in the form of slant and butt which is of equal size in the test tube.
- It contains three carbohydrates- 10% glucose, 1% sucrose, 1% lactose.
- It contains ferric salts to detect Hydrogen sulphide production.
- It is sterilized by autoclave.

INDOLE TEST - POSITIVE

- It is used to determine the ability of an organism to produce indole from tryptophan
- Formation of red coloured ring on adding kovacs reagent indicates positive test. eg. Escherichia coli, Proteus vulgaris.

INDOLE TEST- NEGATIVE

- It is used to determine the ability of an organism to produce indole from tryptophan
- Absence of red coloured ring on adding kovacs reagent indicates negative test. eg. Klebsiella pneumoniae.

TSI MEDIUM- K/NO CHANGE

- It contains alkaline slant and alkaline butt (Pink / Pink in colour).
- It indicates glucose, lactose and sucrose are not fermented .
- Eg., Pseudomonas aeruginosa.

TSI MEDIUM- A/A

- It contains acid slant and acid butt (yellow / yellow in colour).
- It indicates fermentation of glucose, lactose and sucrose.
- Eg. Escherichia coli, Klebsiella pneumoniae.

TSI MEDIUM –K/A

- It contains alkaline slant and acid butt (pink / yellow in colour).
- It indicates that glucose is only fermented.
- Eg. Shigella, Salmonella.

TSI MEDIUM- K/A WITH H2S

- It contains alkaline slant and acid butt (pink/yellow in colour) with abundant hydrogen sulphide production.
- Hydrogen sulphide production is detected by blackening of the medium.
- Eg. Proteus vulgaris, Salmonella typhi para B.

LACTOSE FERMENTING COLONIES ON MAC CONKEY AGAR

- Lactose fermenting colonies are seen as pink coloured colonies in Mac Conkey agar.
- Eg. Escherichia coli, Klebsiella pneumoniae.

NON LACTOSE FERMENTING COLONIES ON MAC CONKEY AGAR

- Colourless colonies are seen in Mac Conkey agar.
- Eg.Salmonella, Shigella, Proteus, Vibrio, Pseudomonas.

PSEUDOMONAS IN NUTRIENT AGAR

- Bluish green pigment produced in nutrient agar.
- Pigment diffuses into the medium.
- Pigments produced by Pseudomonas are pyocyanin(bluish green), pyoverdin (greenish yellow), pyorubin (red), and pyomelanin (brown).

STAPHYLOCOCCUS IN NUTRIENT AGAR.

- Golden yellow pigmented colonies produced in nutrient agar.
- Pigment does not diffuse into the medium.

- The pigment is enhanced by incorporation of `1% glycerol monoacetate or milk in the medium
- The pigment is considered to be carotenoid.

CLOSTRIDIUM TETANI

- Slender Gram positive bacilli with spherical terminal spore having characteristic drum stick appearance.
- It is an anaerobe which causes tetanus.

ACID FAST BACILLI

- Slender and pink coloured rod shaped bacilli seen against blue back ground.

- Eg. Mycobacterium tuberculosis.

SUGAR MEDIA WITHOUT REACTION

- It is a liquid medium used for sugar fermentation reactions.
- This medium contains peptone, sodium chloride, water, bromothymol blue and any one sugar (glucose, lactose, sucrose, maltose etc) and durhams tube.
- Due to non fermentation of sugars pH is not altered and colour remains blue.

SUGAR MEDIA WITH ACID

- It is a liquid medium used for sugar fermentation reactions.

- This medium contains peptone, sodium chloride, water, bromothymol blue and any one sugar (glucose, lactose, sucrose, maltose etc) and durhams tube.
- Due to fermentation of sugars pH is altered to acidic side and colour has changed from blue to yellow

SUGAR MEDIA WITH ACID AND GAS

- It is a liquid biochemical medium used for sugar fermentation reactions.
- This medium contains peptone, sodium chloride, water, bromothymol blue and any one sugar (glucose, lactose, sucrose, maltose etc) and durhams tube.
- Due to fermentation of sugars pH is altered to acidic side and colour has changed from blue to yellow.
- Gas production is seen as air bubbles in durhams tube.

GRAM POSITIVE COCCI IN CLUSTERS

- Spherical in shape and violet in colour.
- Cocci are found in grape like clusters.
- Eg. Staphylococcus aureus.

GRAM NEGATIVE BACILLI

- Pink in colour and rod shaped.
- Scattered in arrangement.
- Eg. Escherichia coli.

CANDIDA ALBICANS

- Gram positive budding yeast cells.
- Germ tube test is positive.
- It is an opportunistic fungi causing oral thrush.

MUCOR

- It has non septate hyphae.
- Sphorangiophores are sympodially branched.
- Sporangia are globose and brown to grey in colour and columella are ovoid and contain sporangiospores.
- Rhizoids are absent.

RHIZOPUS

- Rhizoids with four to eight radial branches are seen.
- Sporangiophores are seen singly and are unbranched and may be yellowish brown to dark brown in colour.
- Sporangia may be gray beige to black in colour.
- Sporangia contains sporangiospores.

TEANIA- SCOLEX

- Commonly called head.
- It is globular or quadrate in outline.
- It has four circular suckers.
- May or may not provided with rostellum and double row of hooklets.

TEANIA- PROGLOTTIDS

- It is an individual segment comprising the complete unit of tapeworm.
- According to its sexual maturity, a segment may be immature (reproductive organs are not differentiated) , mature (reproductive organs appeared) and gravid (uterus filled with eggs).
- The common genital pore is situated marginally near the posterior end.
- The testes are numerous and the number of ovary is two.

FASCIOLA HEPATICA.

- It is commonly known a liver fluke.
- It is large leaf shaped fluke.
- It contains two suckers- oral and ventral.

ENTEROBIUS VERMICULARIS.

- It is commonly known as pin worm, thread worm or seat worm.
- It is more or less spindle shaped and resembles a short piece of thread.
- In both male and female, a pair of cervical alae is present.
- Double- bulb oesophagus is a characteristic feature.

HYDATID CYST

- It is the larval stage of Echinococcus granulosus.
- It contains many brood capsules and small protoscolices.
- Each protoscolex contains a scolex which represents the future head of the adult worm.
- The cyst wall of hydatid cyst consist of two layers- outer cuticular layer, which is a hyaline laminated membrane and inner germinal layer.

- Hydatid cyst contains hydatid fluid which is secreted by germinal layer.

ASCARIS LUMBRICOIDES

- Common name – Round worm.
- It is the largest intestinal nematode.
- Adult worms live in the lumen of small intestine.
- It is light brown or pink in colour.
- In shape it is rounded and tapers at both ends, the anterior end being thinner than posterior.
- Infection in man is known as ascariasis.

TAPEWORM

- They are long , segmented and tape like hence known as tapeworm.
- They are flattened dorsoventrally.
- Sexes are not separate, that is hermaphrodite (monoecious).
- Adult worm contains head or scolex, neck and strobili consisting of proglottids.

- Head end contains suckers, often with hooks.
- Alimentary canal and body cavity are absent.

ENTEROBIUS VERMICULARIS

- It is commonly known as pinworm, thread worm, seat worm.
- Adult worms (gravid females) live in the caecum and vermiform appendix.
- It is small, more or less spindle-shaped and white in colour.
- In both male and female a pair of cervical alae is present at the anterior end.
- Double –bulb oesophagus is a characteristic feature of this nematode.

CERCARIA

- It is the final stage of larval development of trematodes in the mollusc. It possess a body and tail.
- According to the nature of tail different names are given. Eg Fork tailed (as in Schistosomes).

TUBERCULIN SYRINGE

- Tuberculin Syringe is a small syringe with fine needle that hold upto one half to one cubic centimeter of fluid.
- It is used to administer antigen under the skin and perform tuberculin test.
- It is sterilized by gamma radiation.

DEPRESSION SLIDE

- It is used for hanging drop preparation.
- It is used to demonstrate motility of the organism.

VDRL SLIDE

- It has 12 concavities.
- It is used to perform VDRL test to diagnose syphilis.
- It is a slide flocculation test.

ASPERGILLUS FLAVUS

- It has hyaline septate hyphae.
- Conidiophores are thick walled, hyaline and coarsely roughened.
- Vesicles are large and globose.

- They are produced over most of the vesicle.
- Conidia are unicellular and typically globose.

ASPERGILLUS NIGER

- It has hyaline septate hyphae.
- Phialides are biseriate covering entire vesicle, form radial head.
- Conidiophores are wide hyaline changes into brown tint .
- Vesicles are spherical.
- Conidia are black in colour.

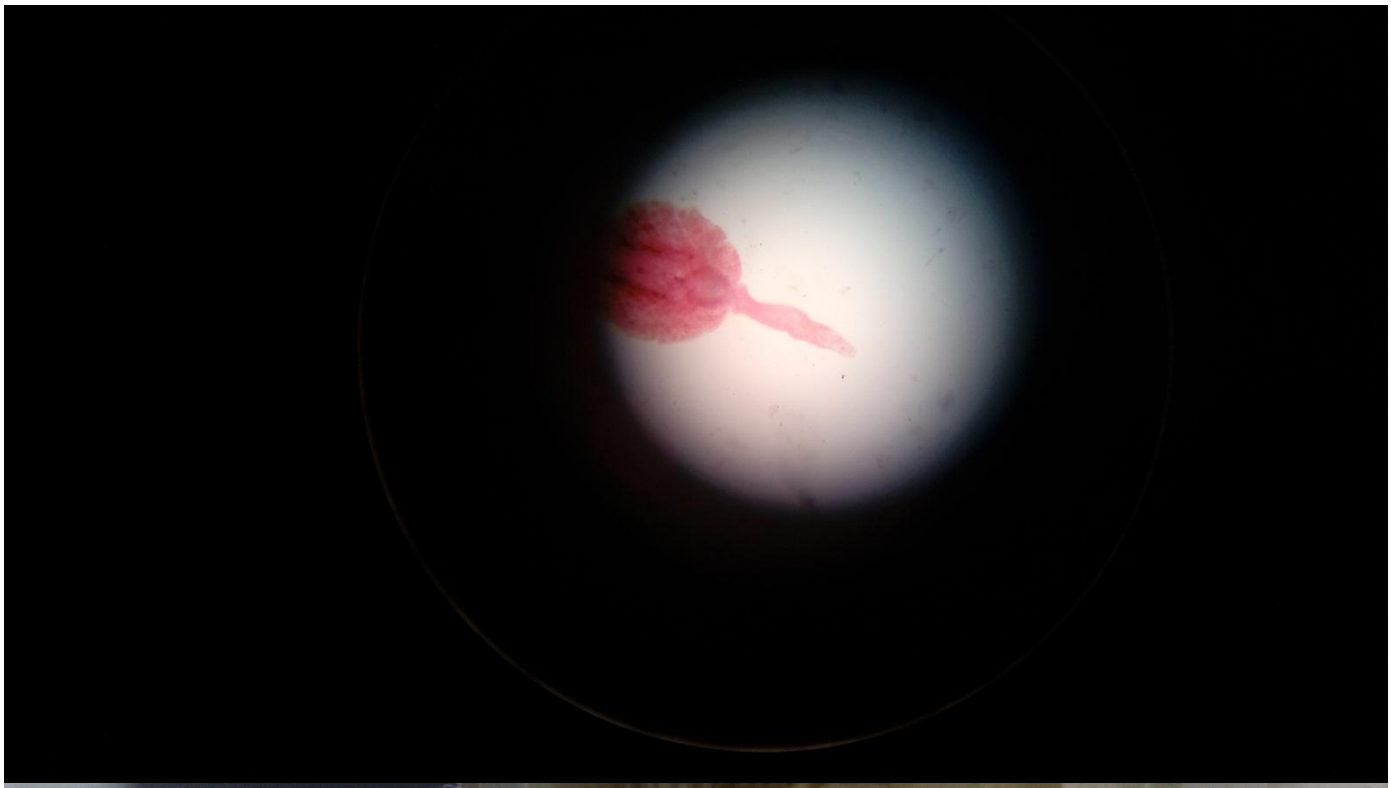
ASPERGILLUS FUMIGATUS

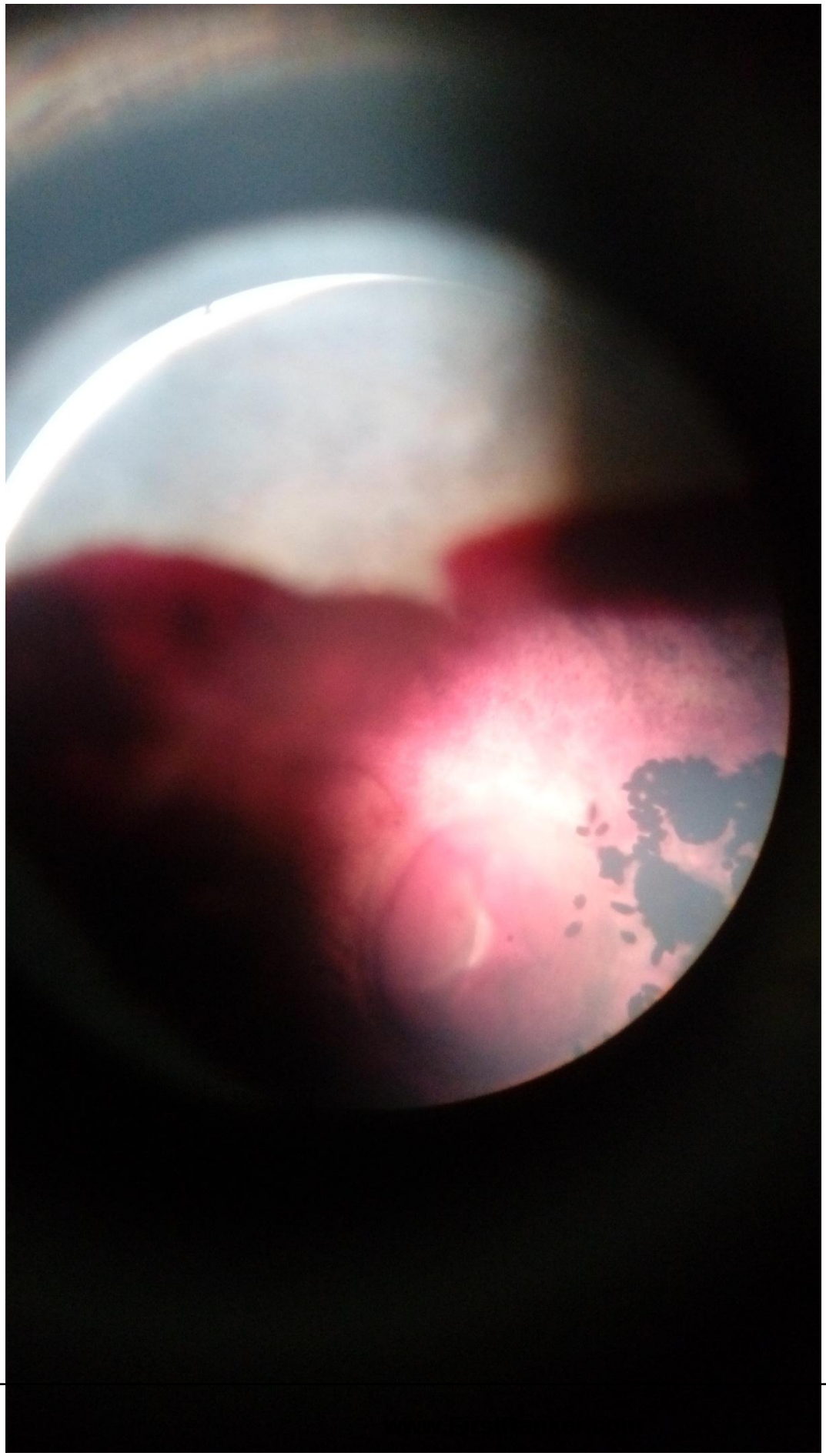
- It has hyaline septate hyphae.

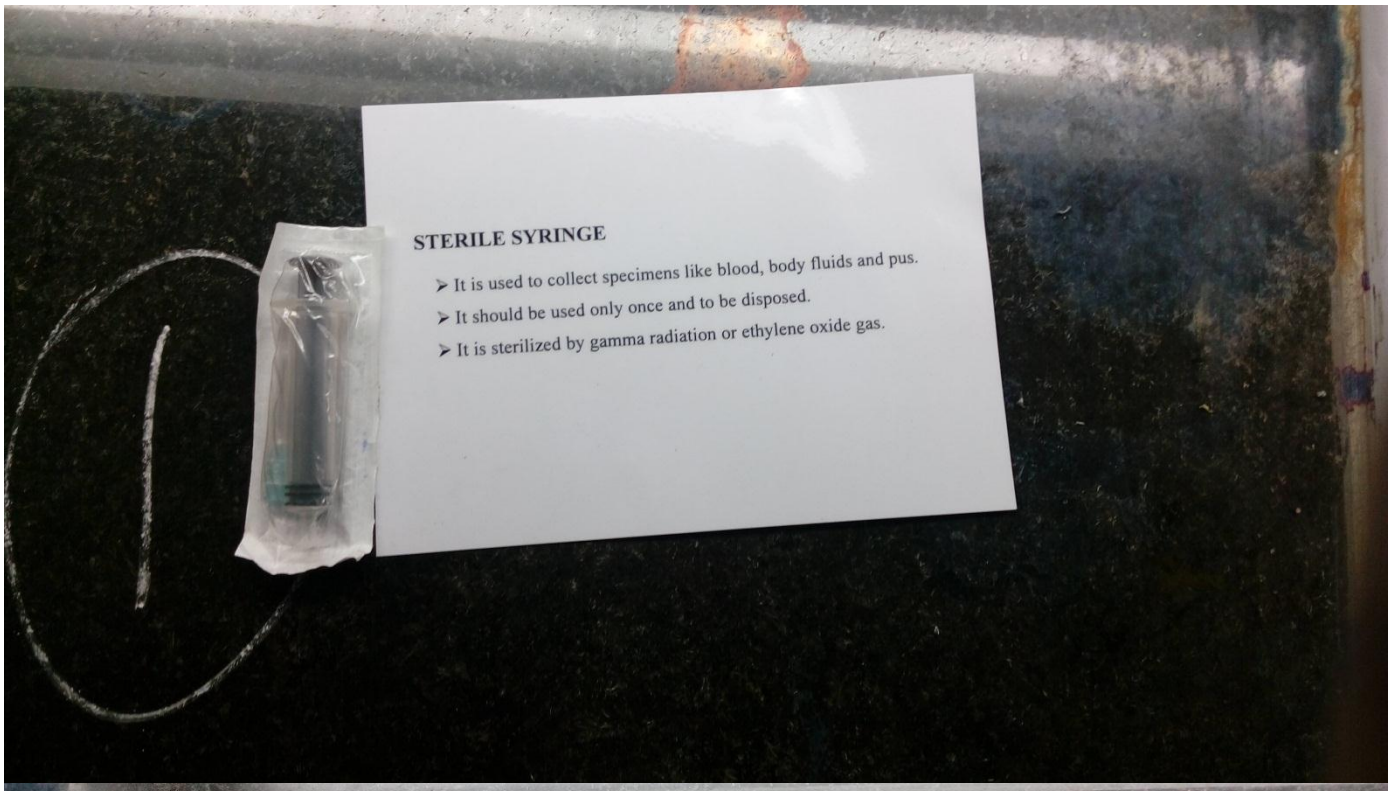
- Phialides are single(uniseriate), usually cover upper half of the vesicle, parallel to the axis of stalk.
- Conidiophores are smooth walled, they may be light green or brown.
- Vesicle is flask shaped.
- Conidia are green in colour.

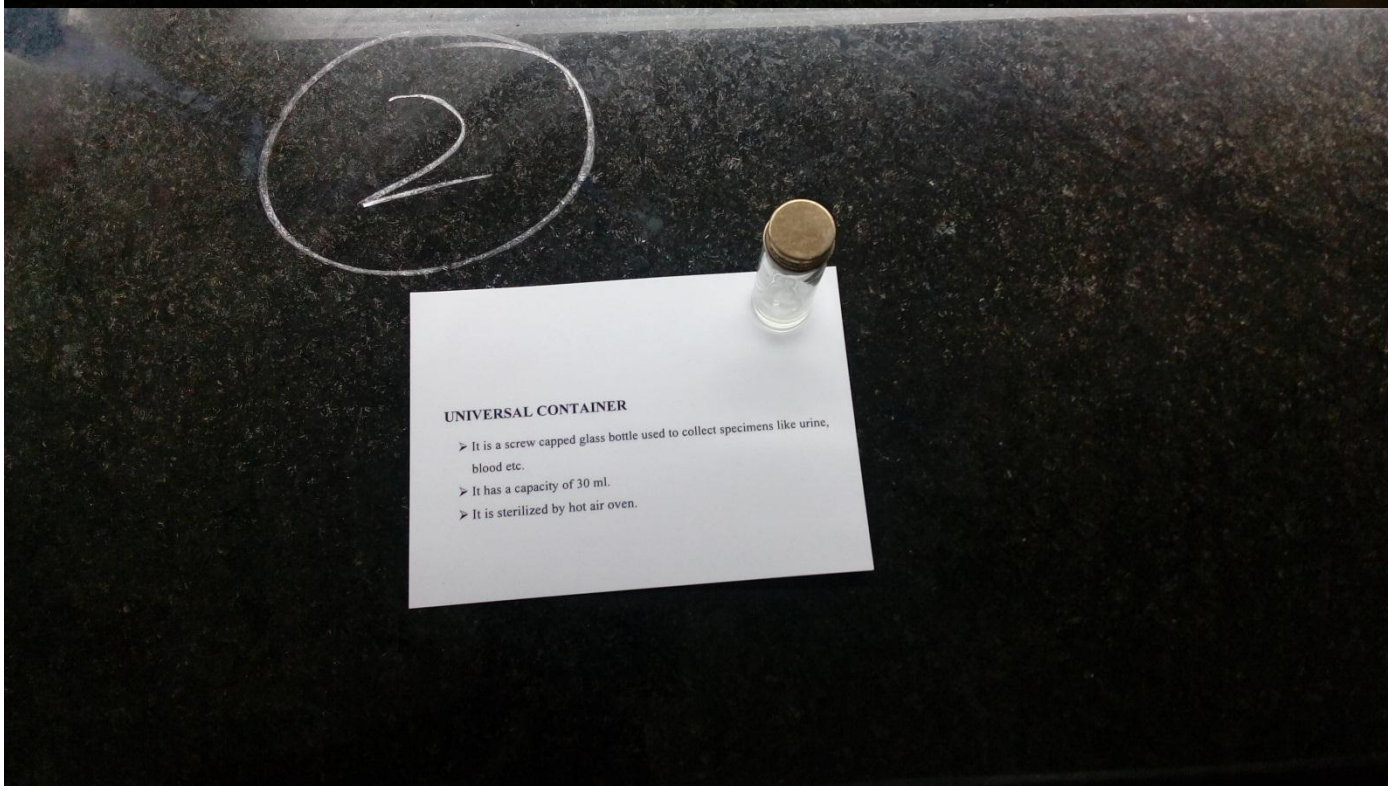
PENCILLIUM

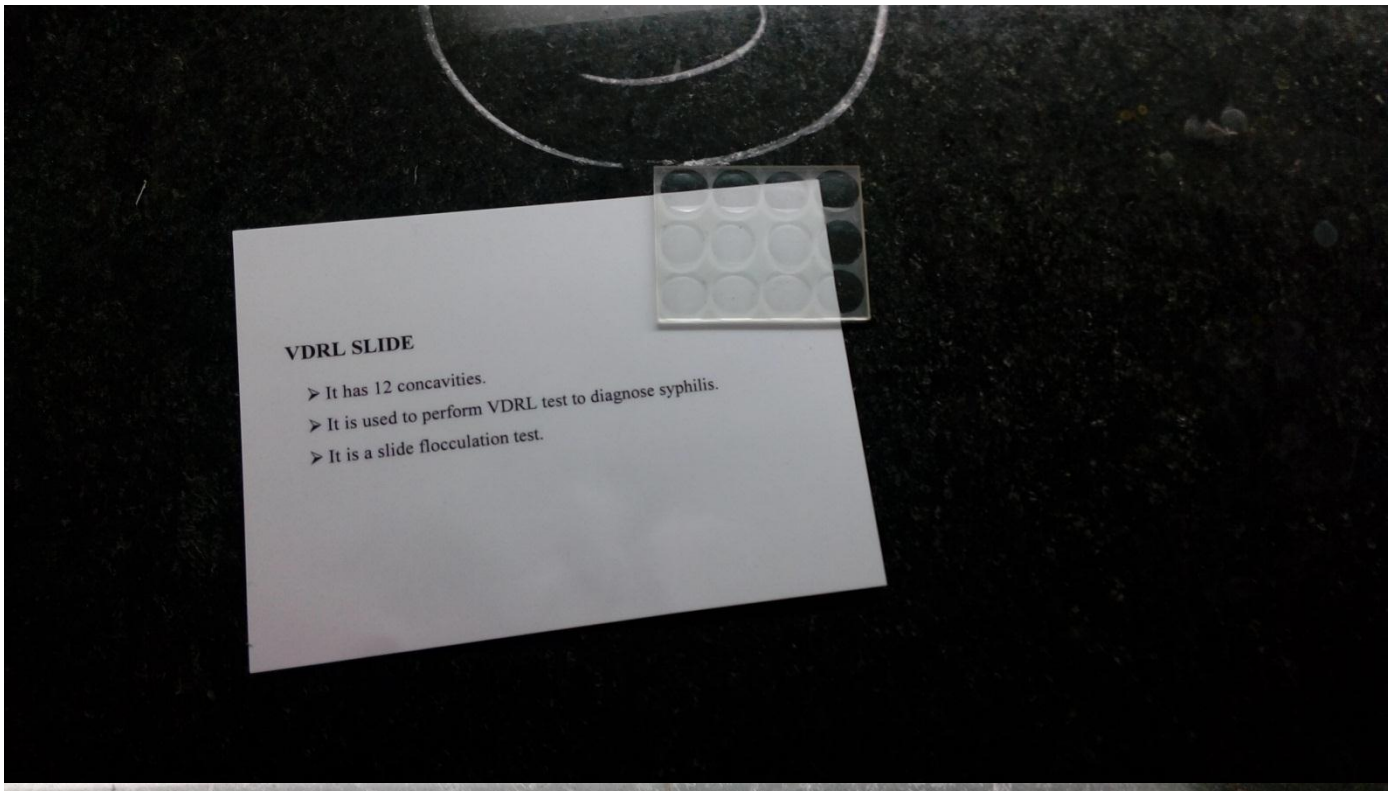
- It has hyaline septate hyphae.
- Conidiophores are branched with two rows of sterigmata bearing chains of spores, the appearance is like brush.

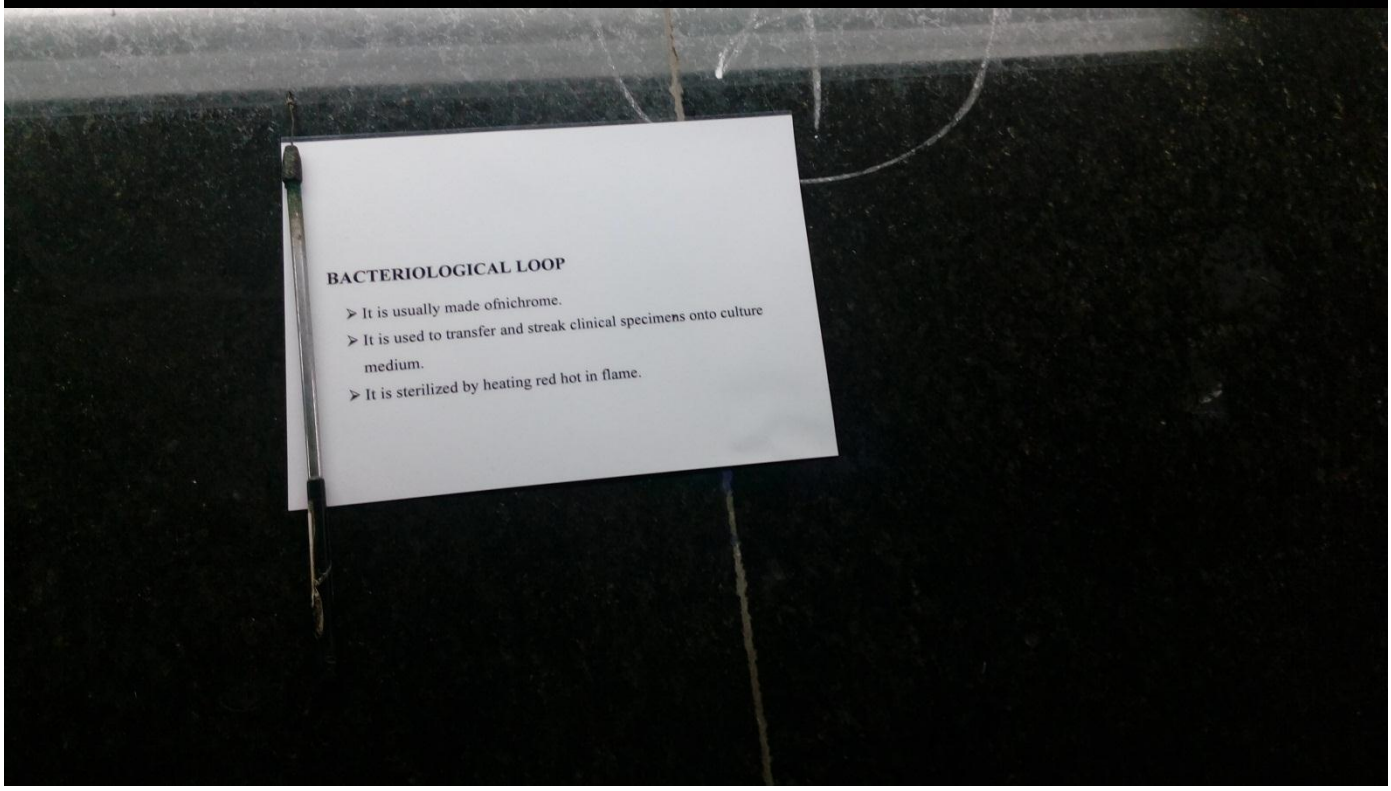


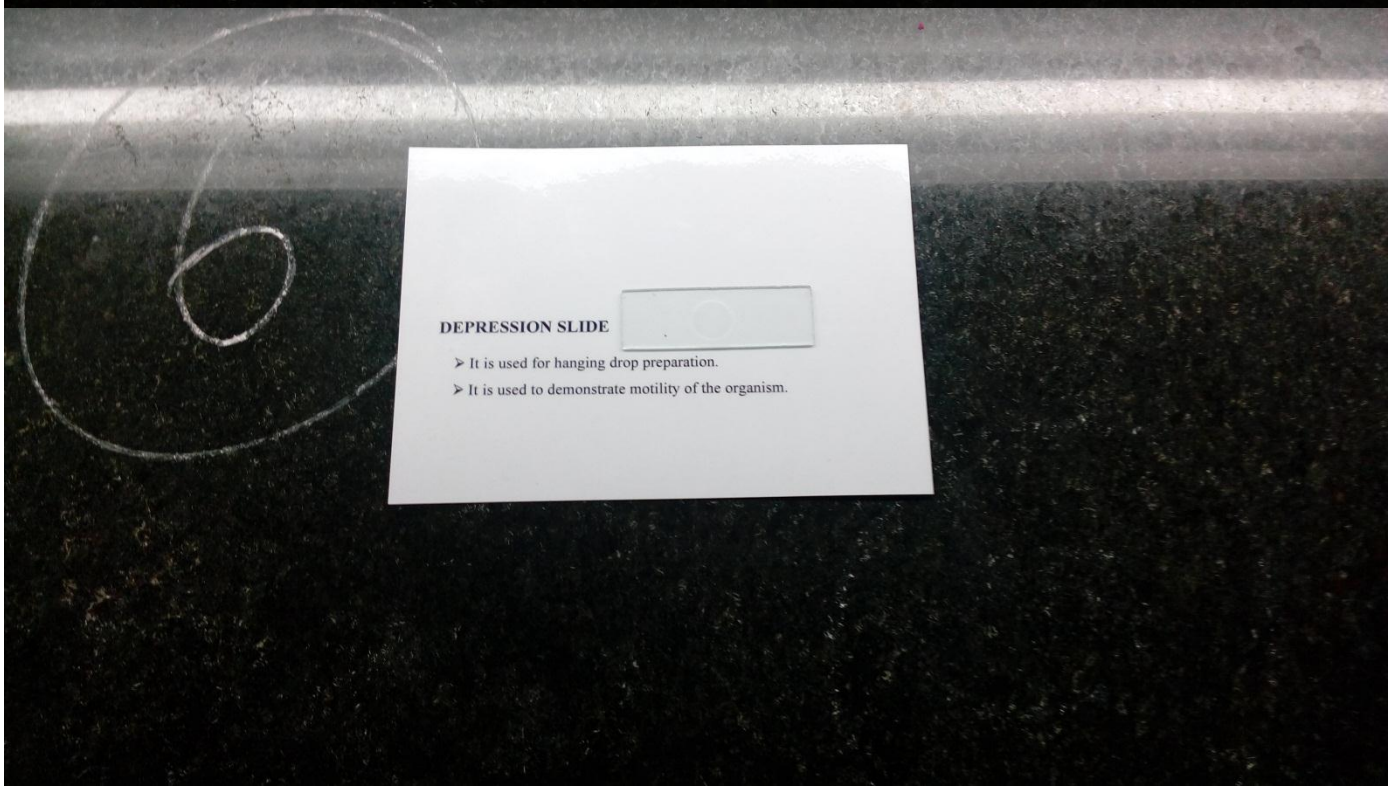


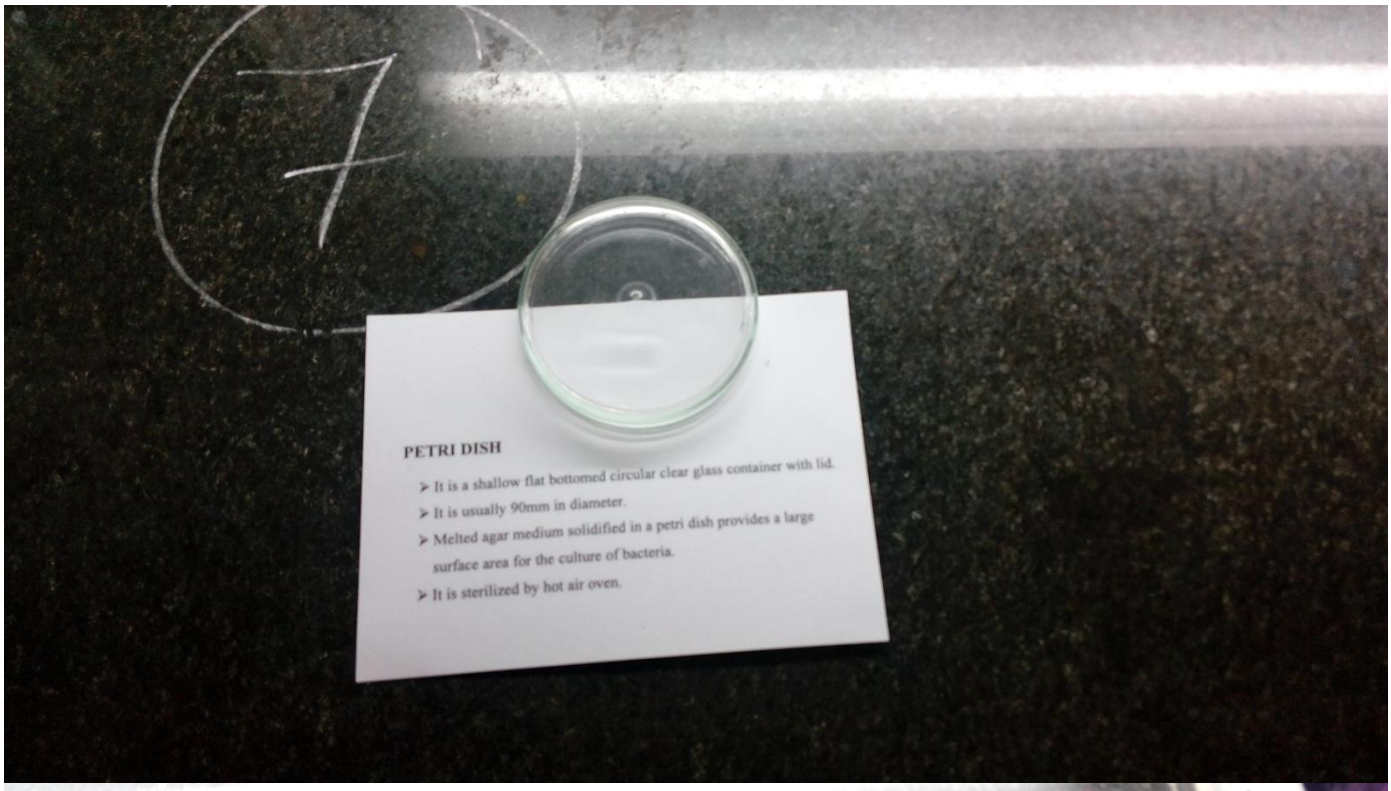


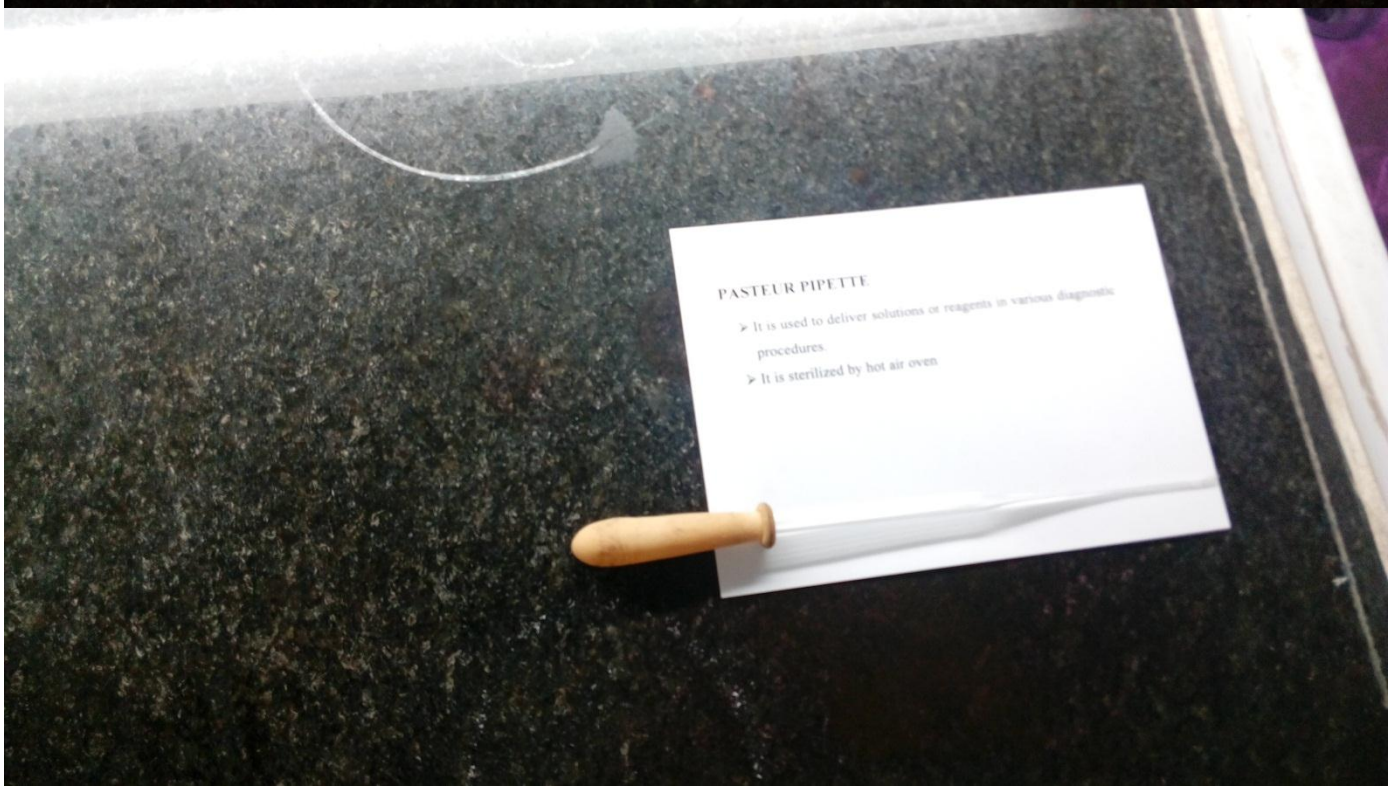


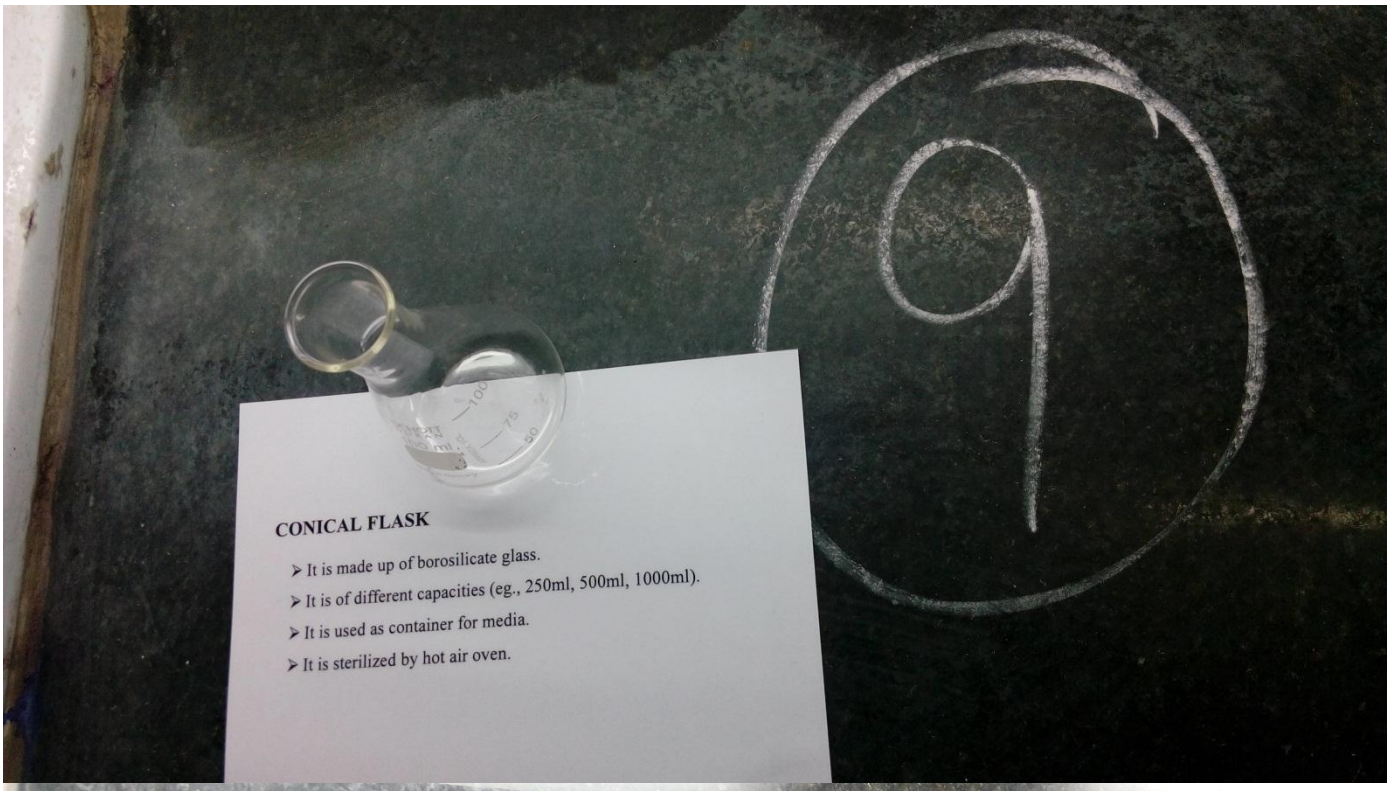


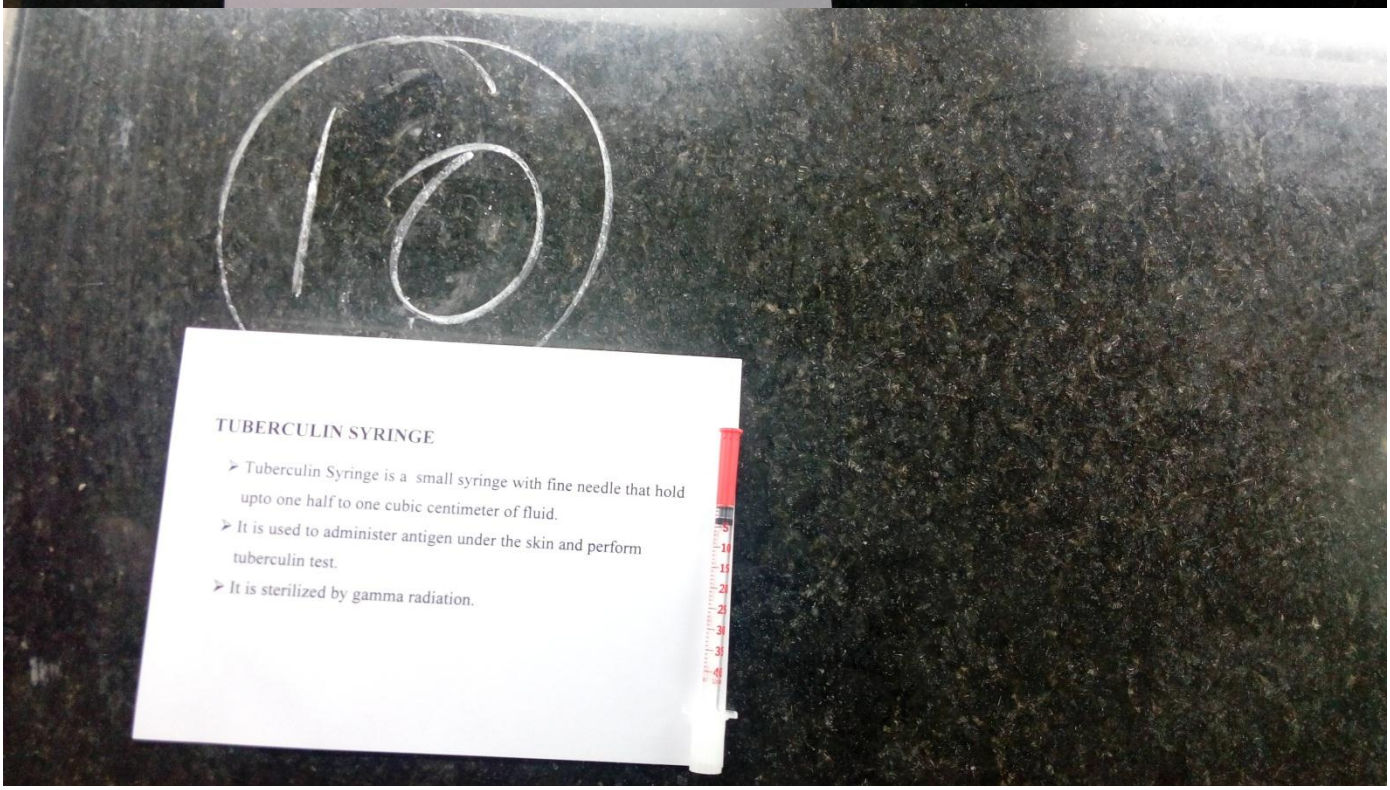


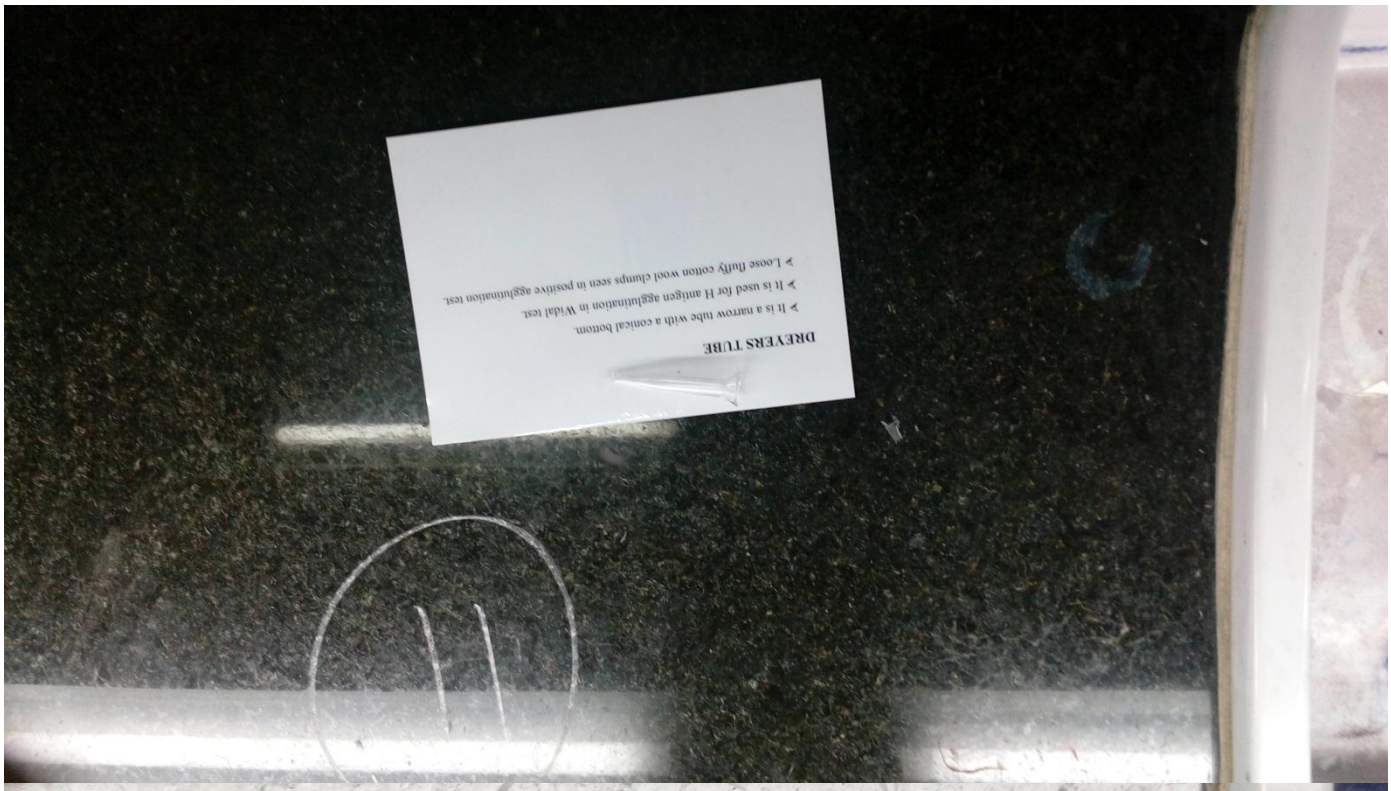


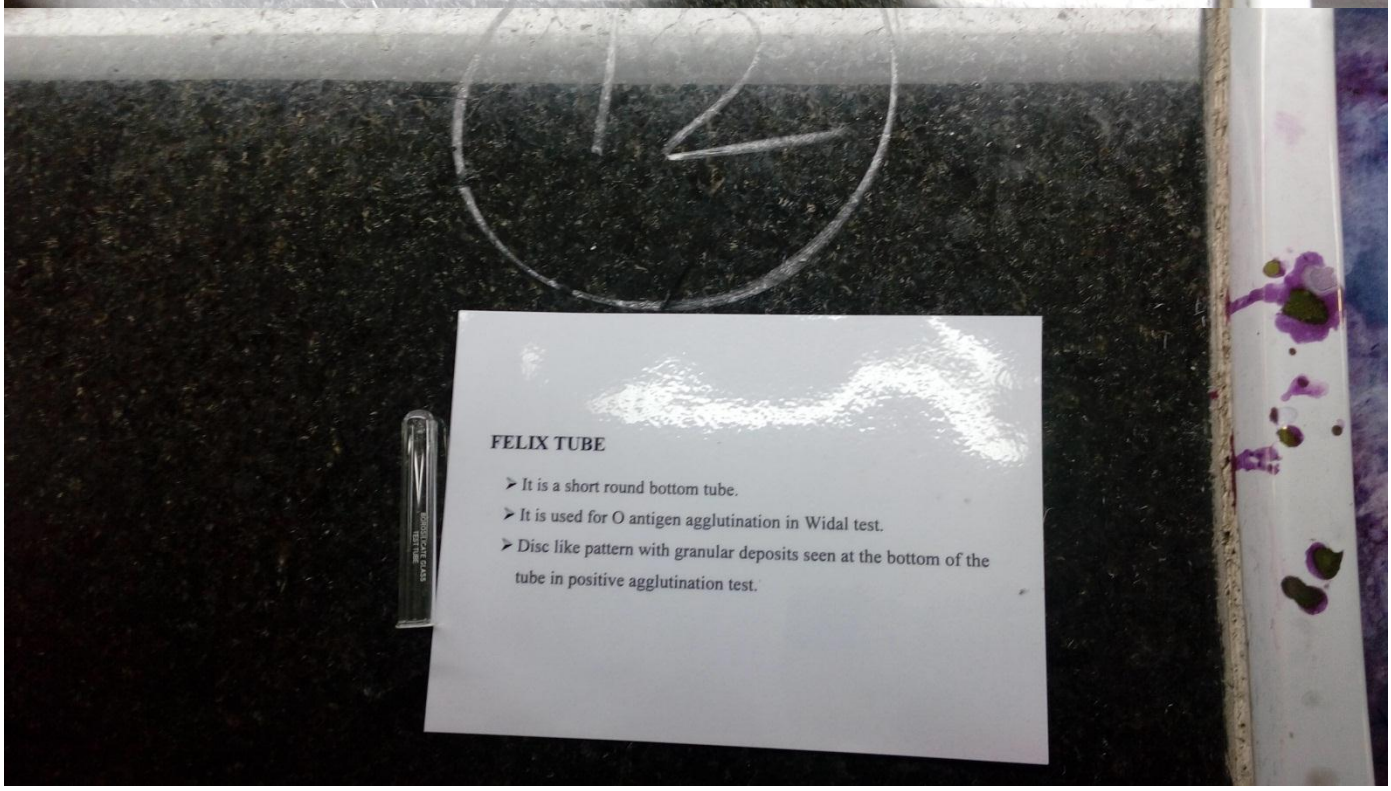


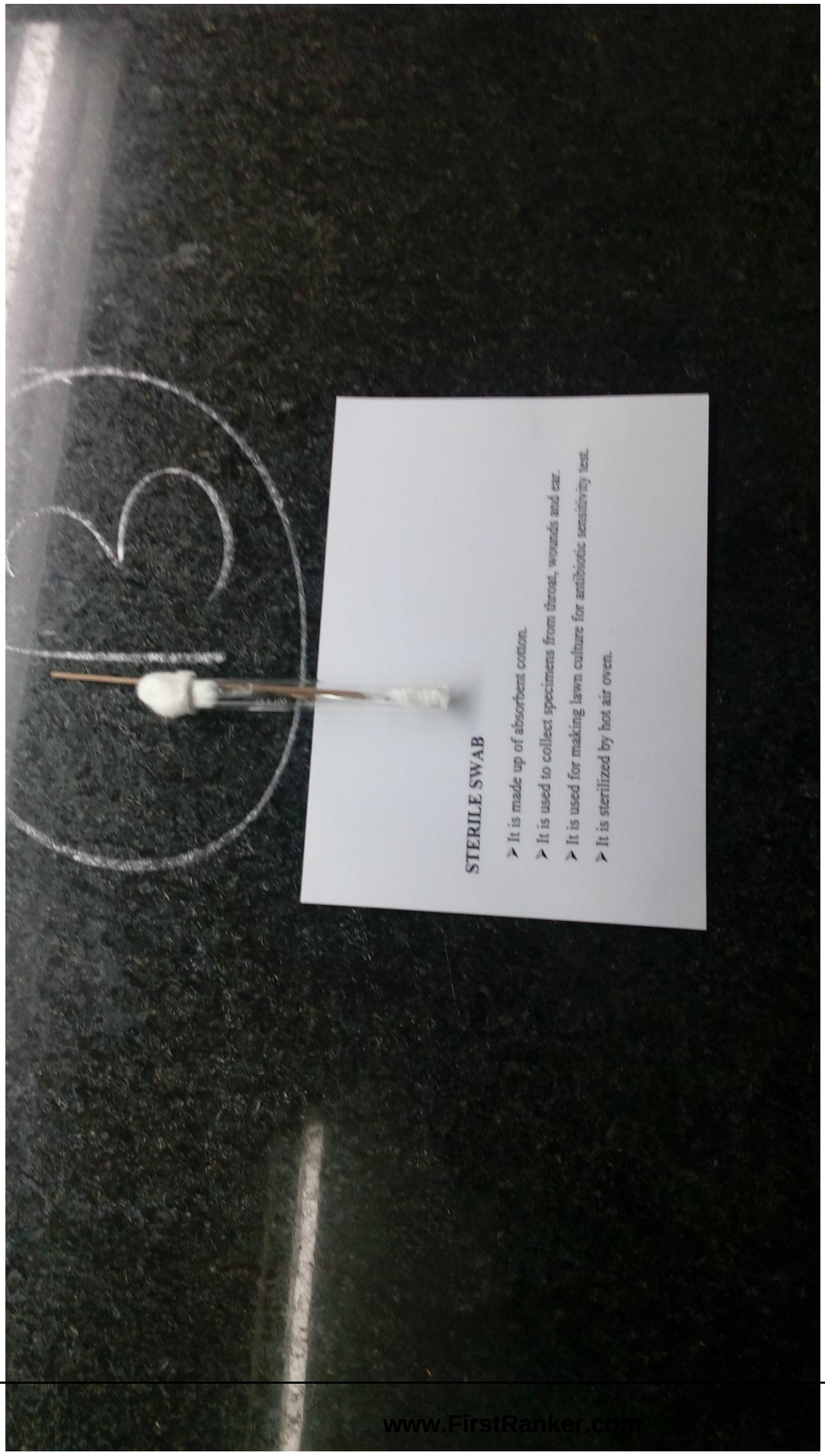


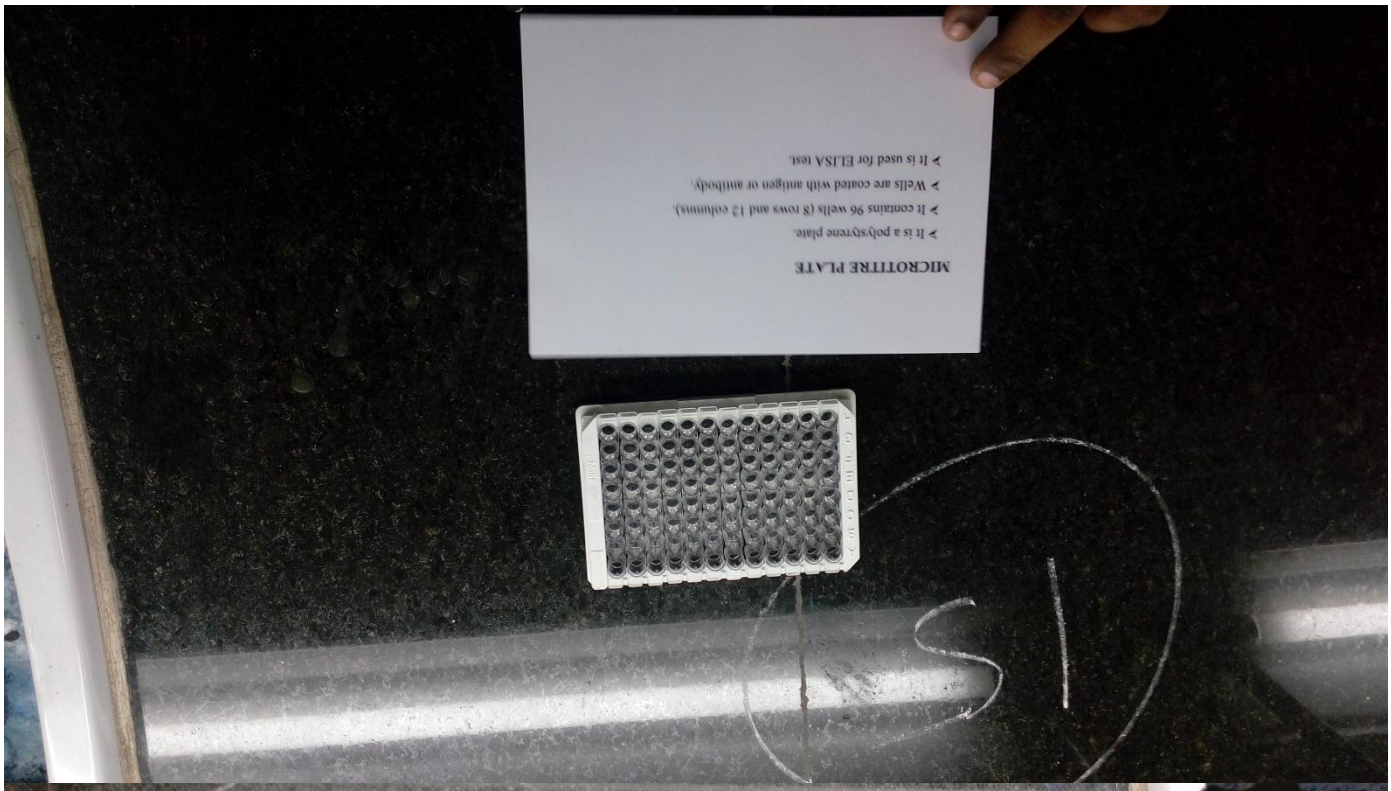


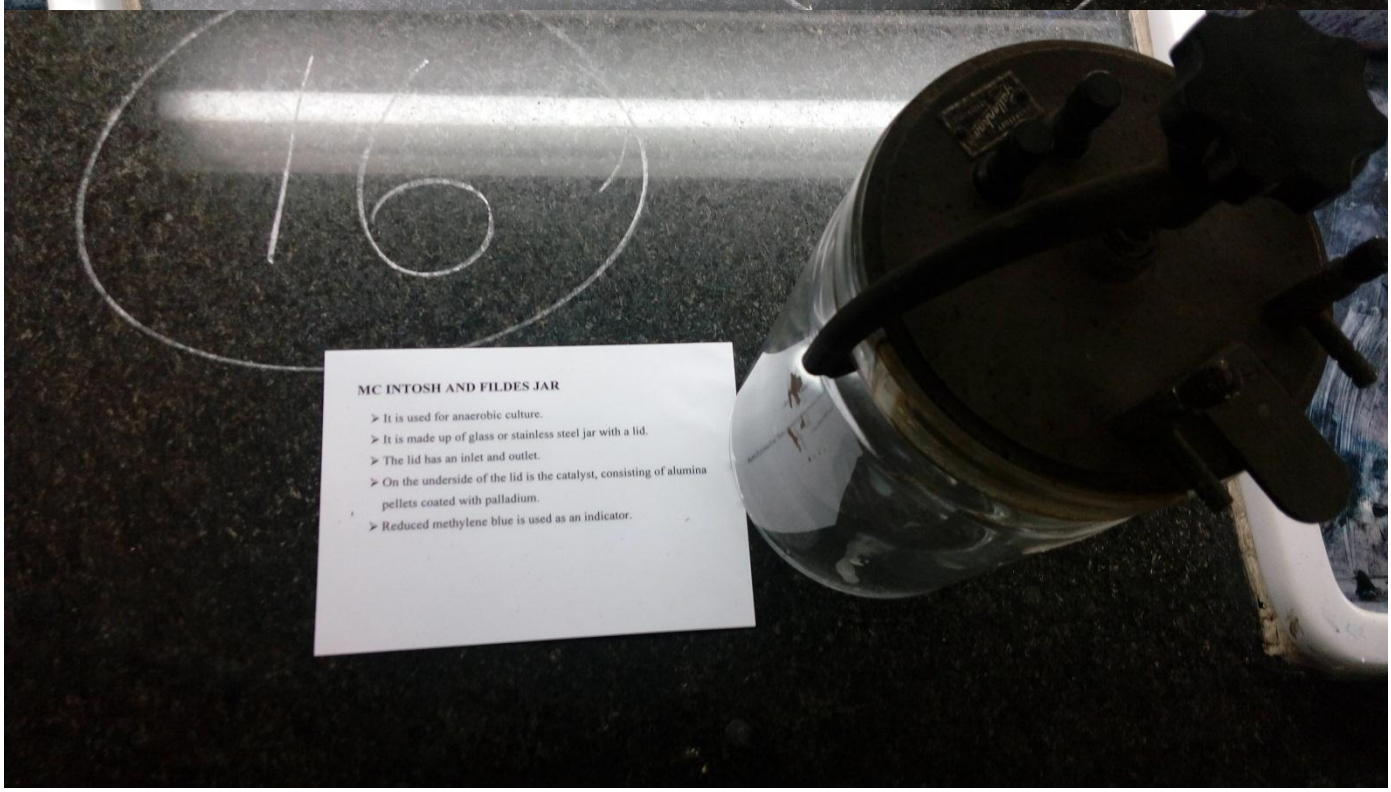


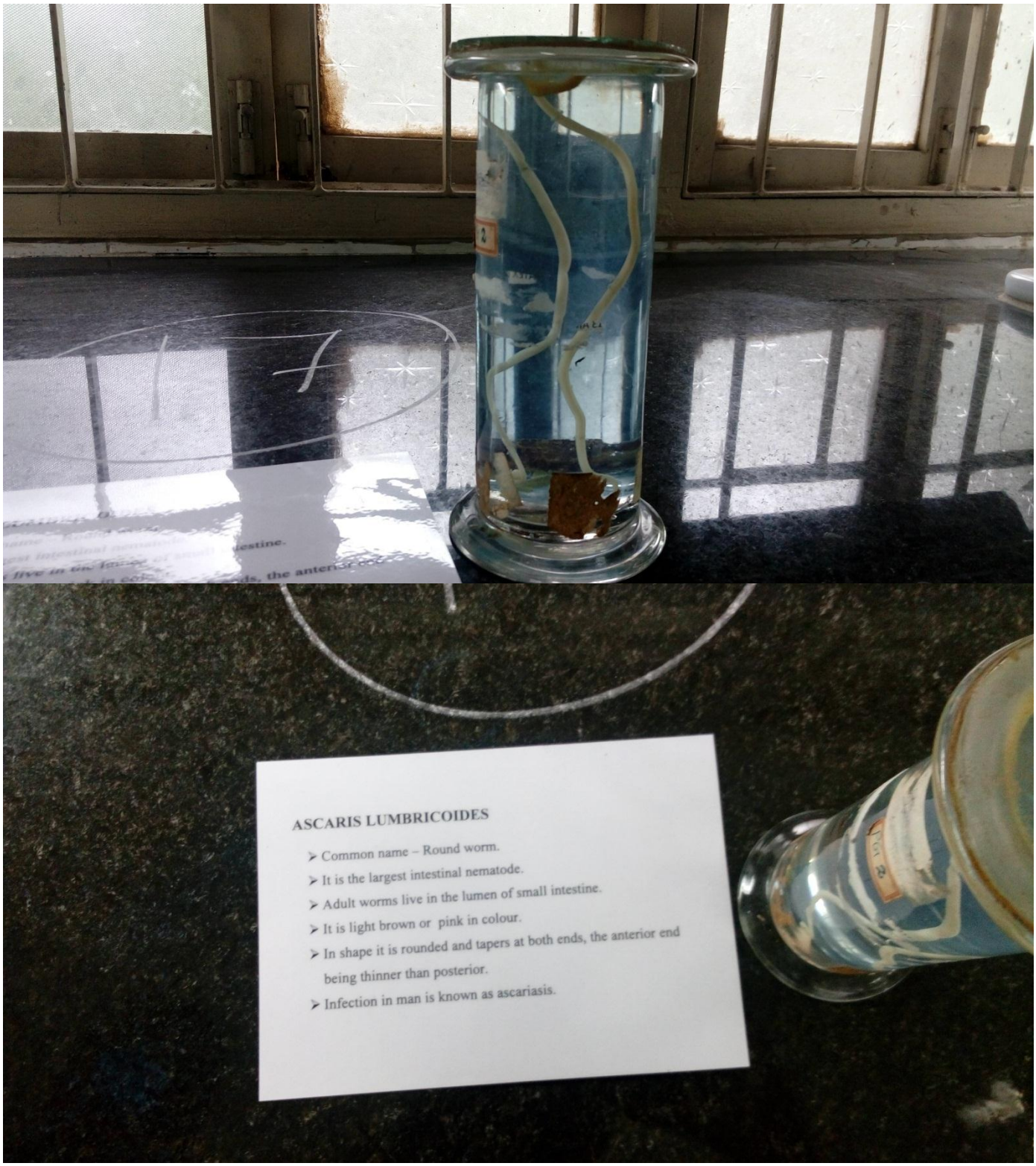






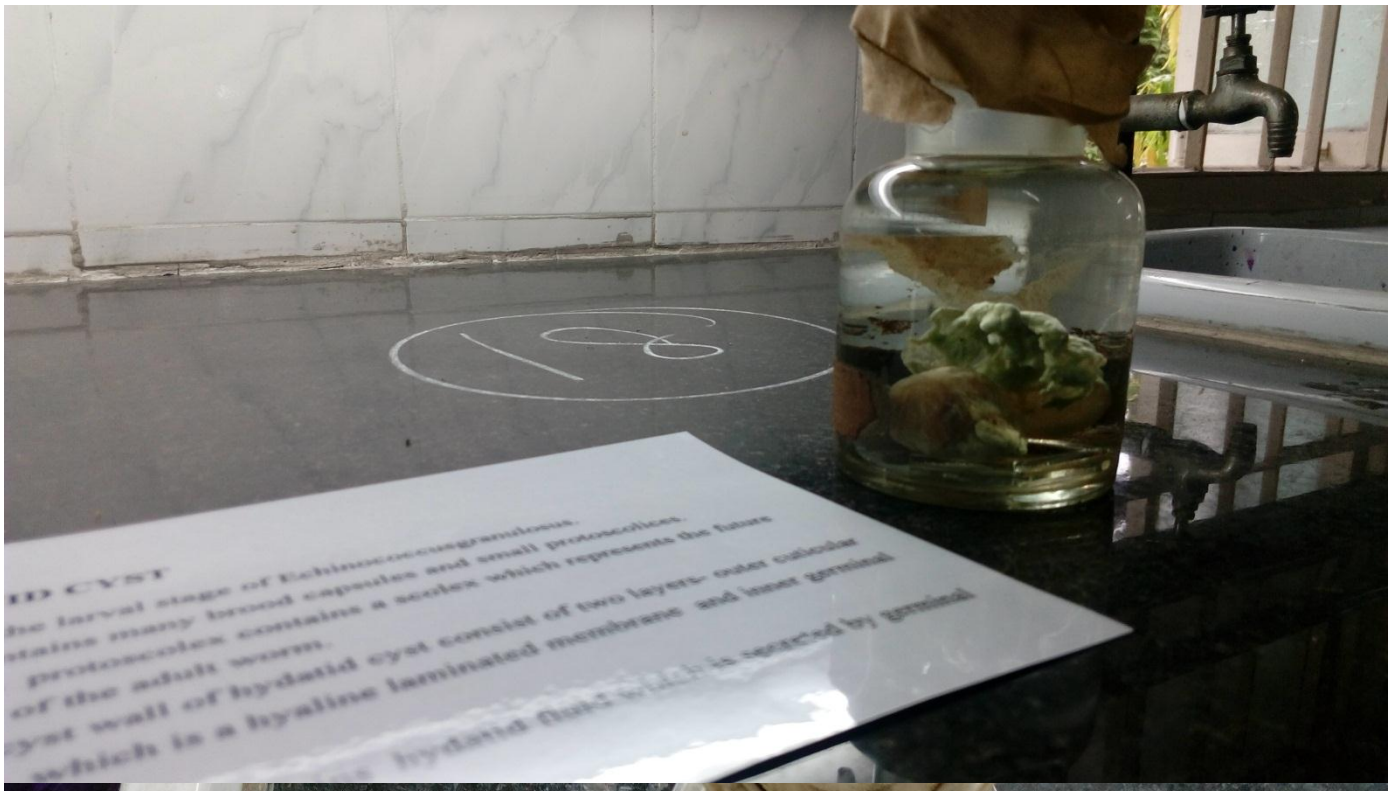


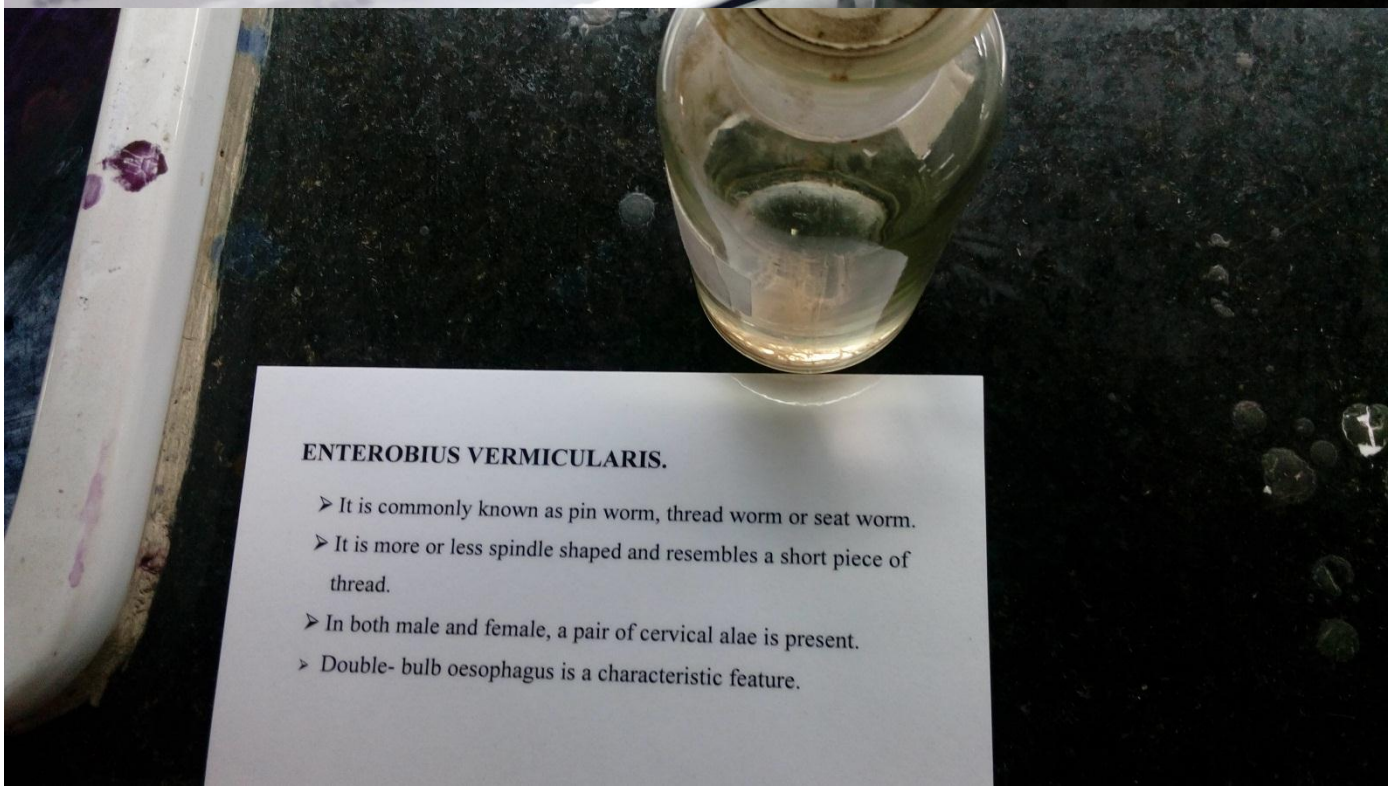




HYDATID CYST

- It is the larval stage of Echinococcusgranulosus.
- It contains many brood capsules and small protoscolices.
- Each protoscolex contains a scolex which represents the future head of the adult worm.
- The cyst wall of hydatid cyst consist of two layers- outer cuticular layer, which is a hyaline laminated membrane and inner germinal layer.
- Hydatid cyst contains hydatid fluid which is secreted by germinal layer.





TAPEWORM

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- Sexes are not separate, that is hermaphrodite (monoecious).
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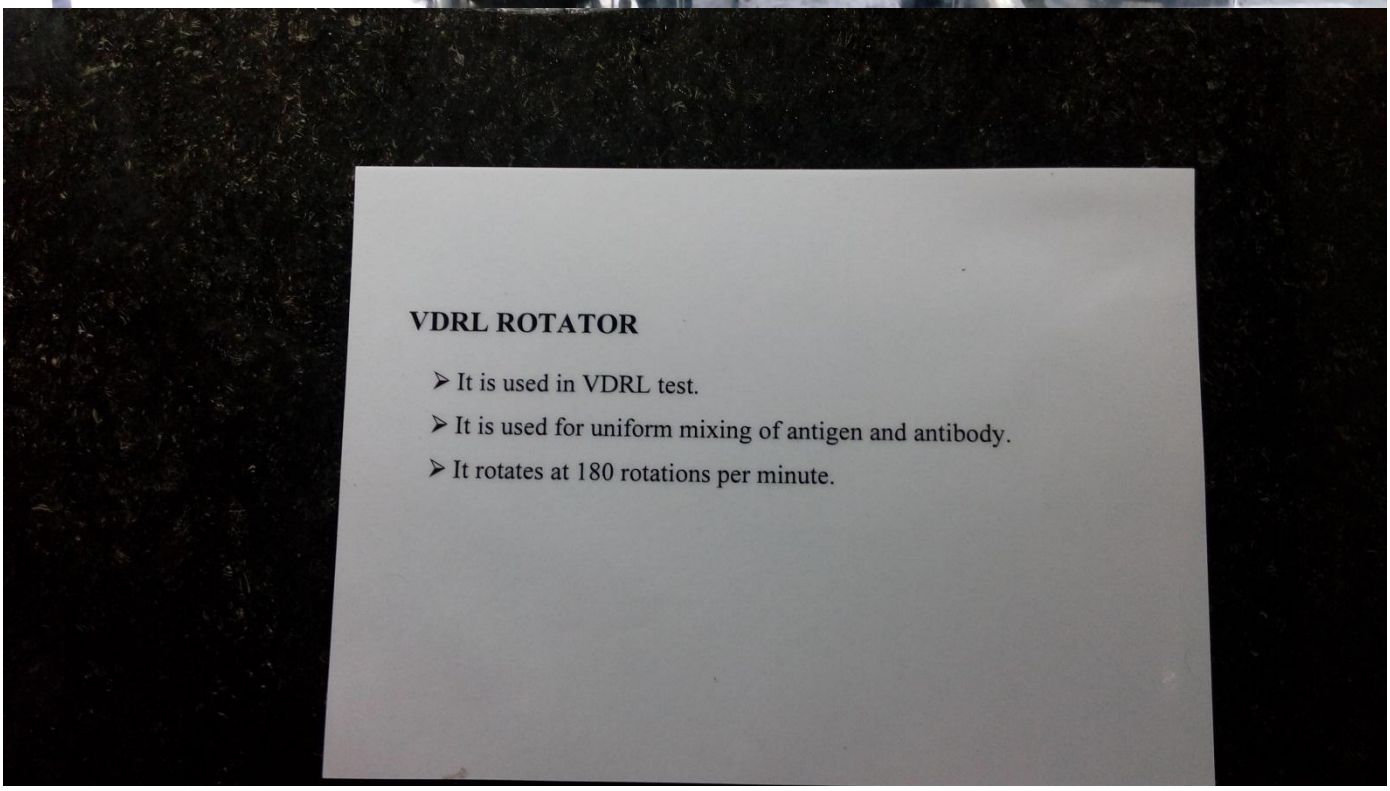
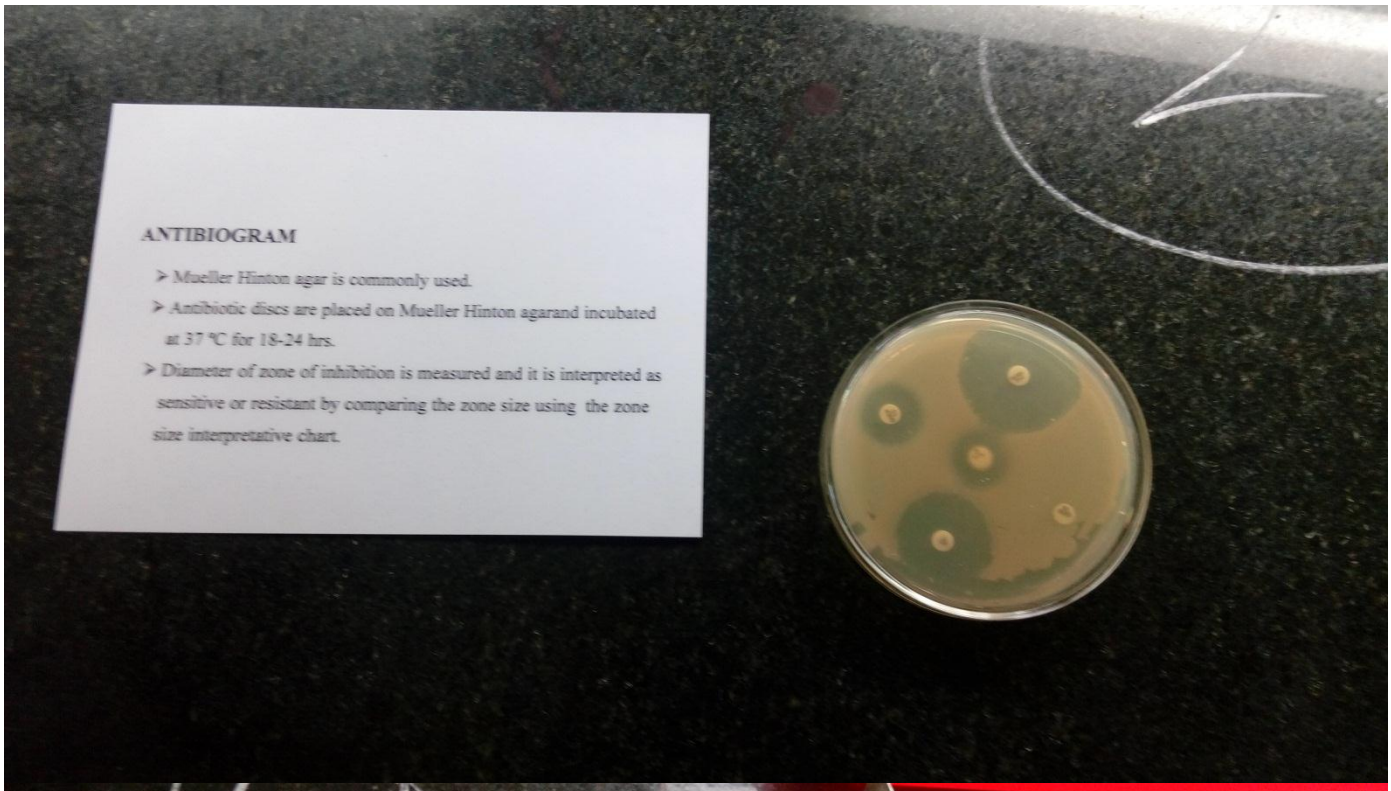
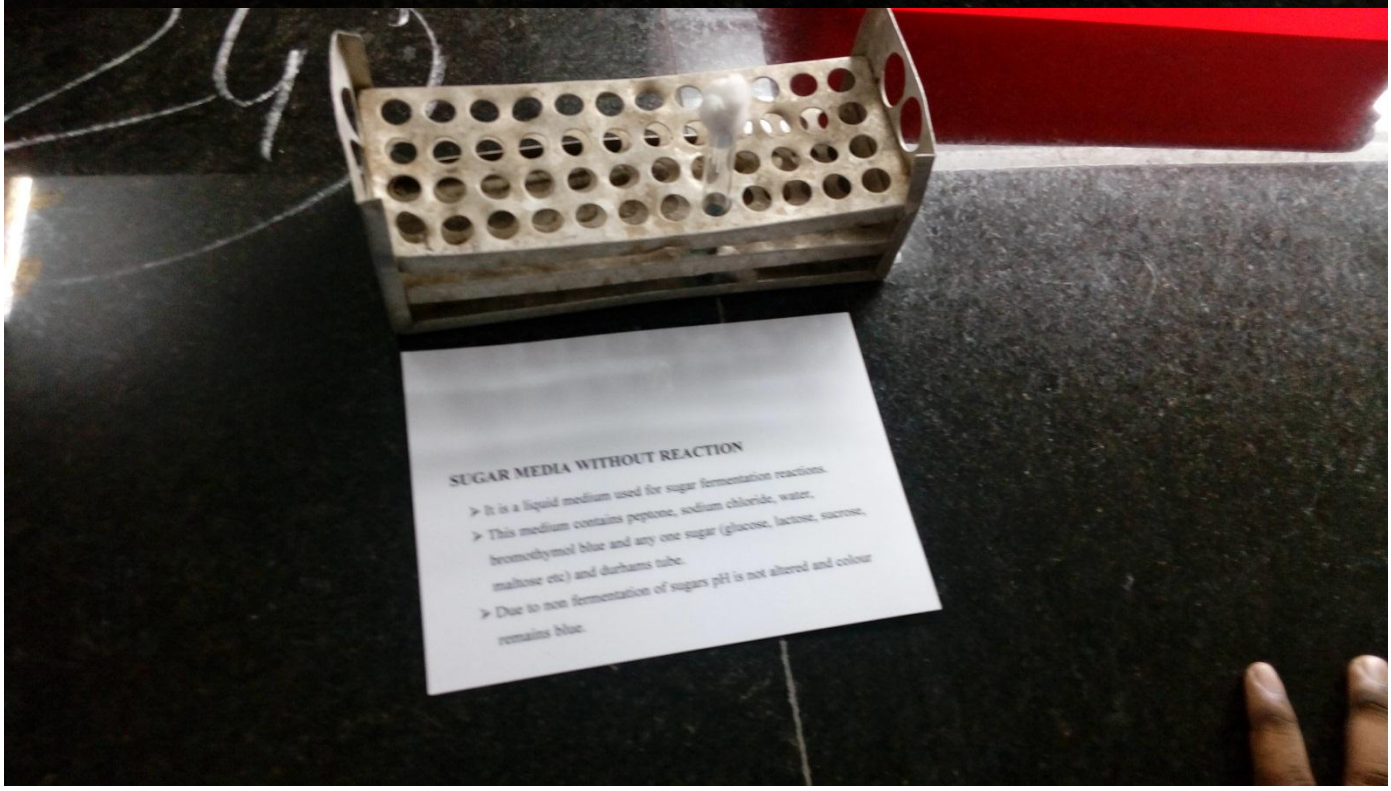
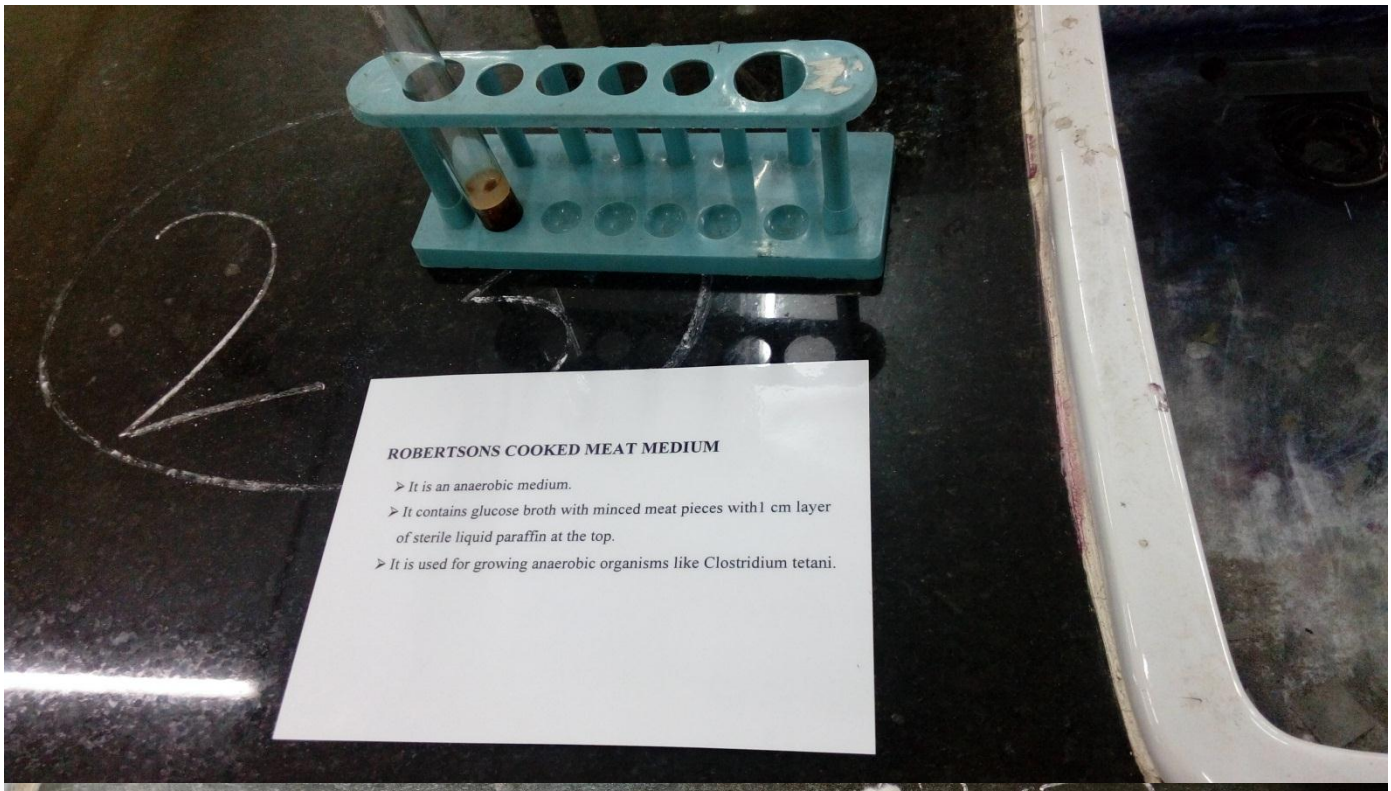
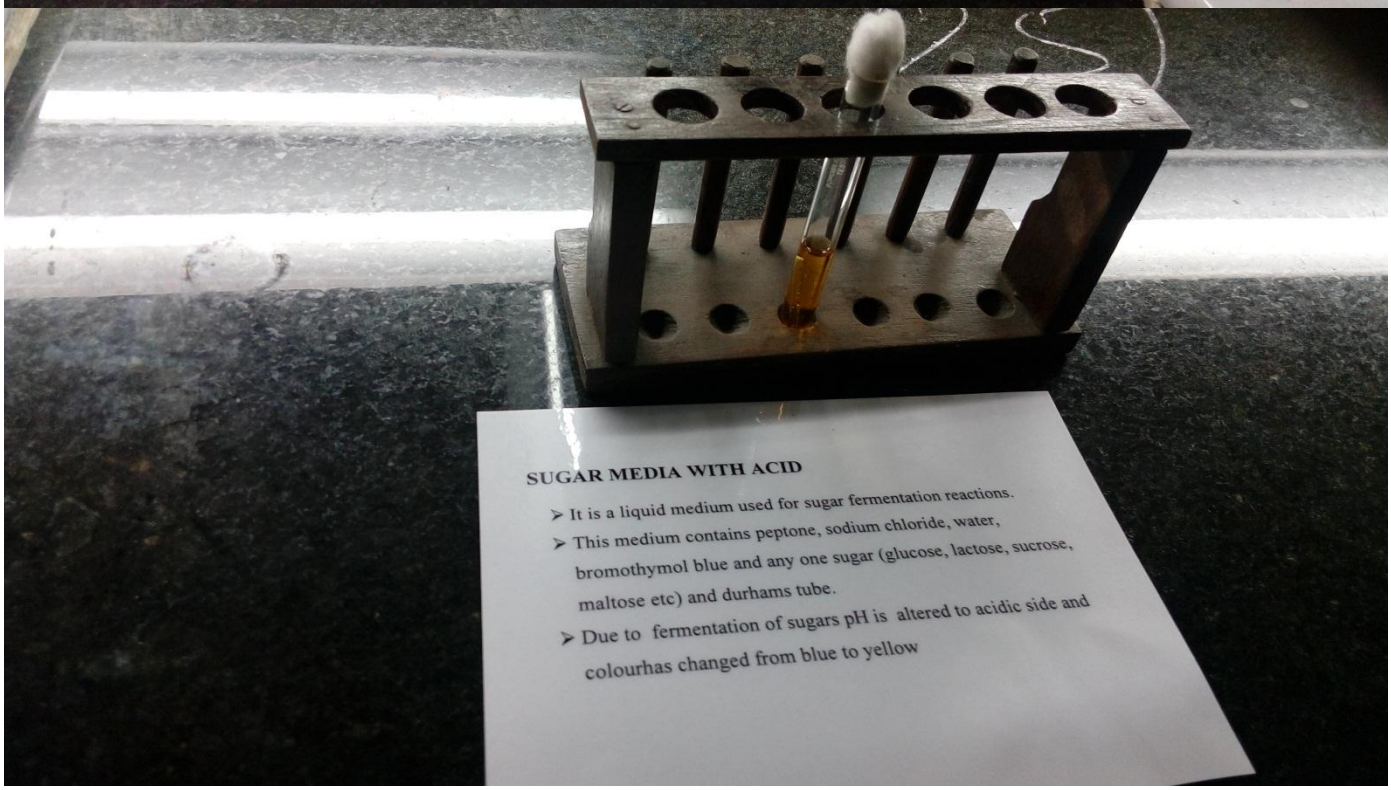




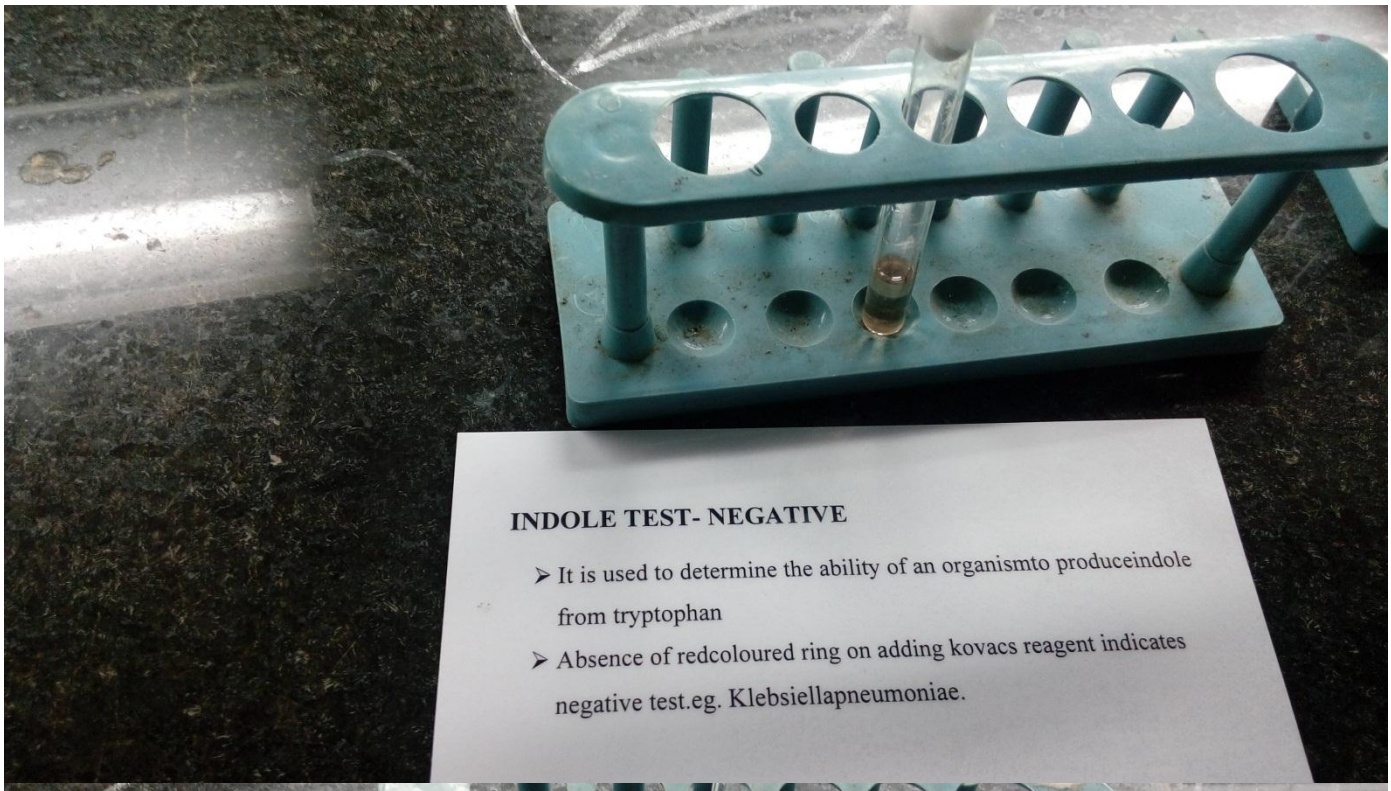


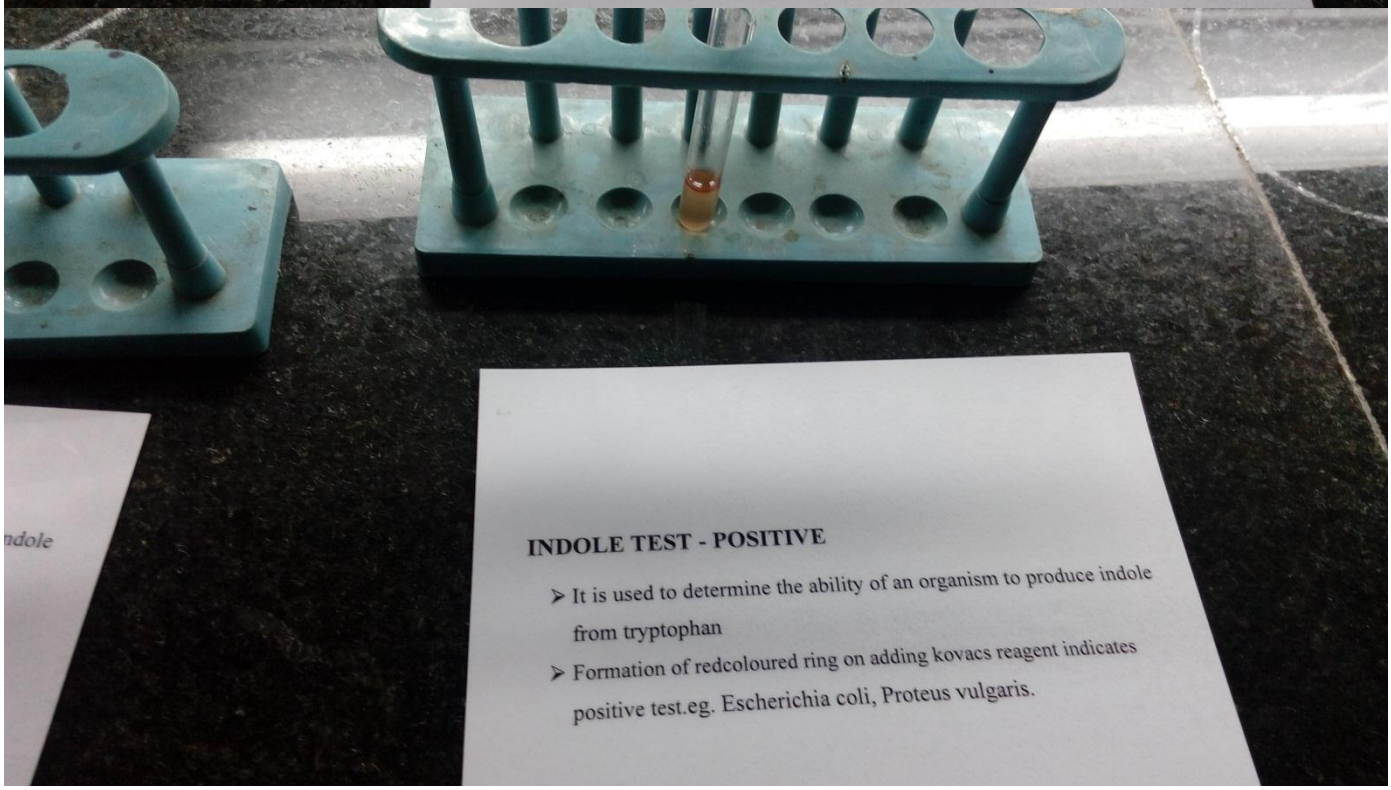




SUGAR MEDIA WITH ACID AND GAS

- It is a liquid biochemical medium used for sugar fermentation reactions.
- This medium contains peptone, sodium chloride, water, bromothymol blue and any one sugar (glucose, lactose, sucrose, maltose etc) and durhams tube.
- Due to fermentation of sugars pH is altered to acidic side and colour has changed from blue to yellow.
- Gas production is seen as air bubbles in durhams tube.



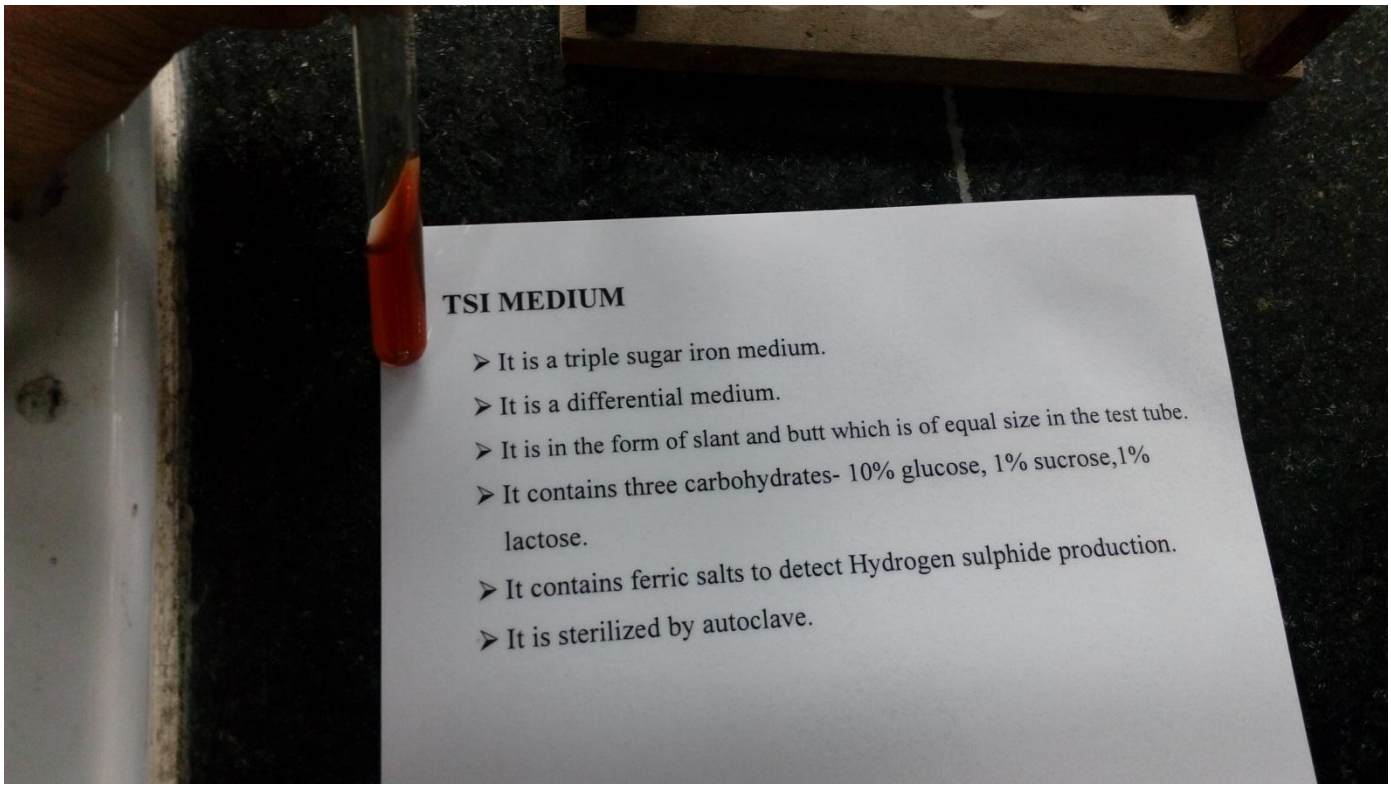


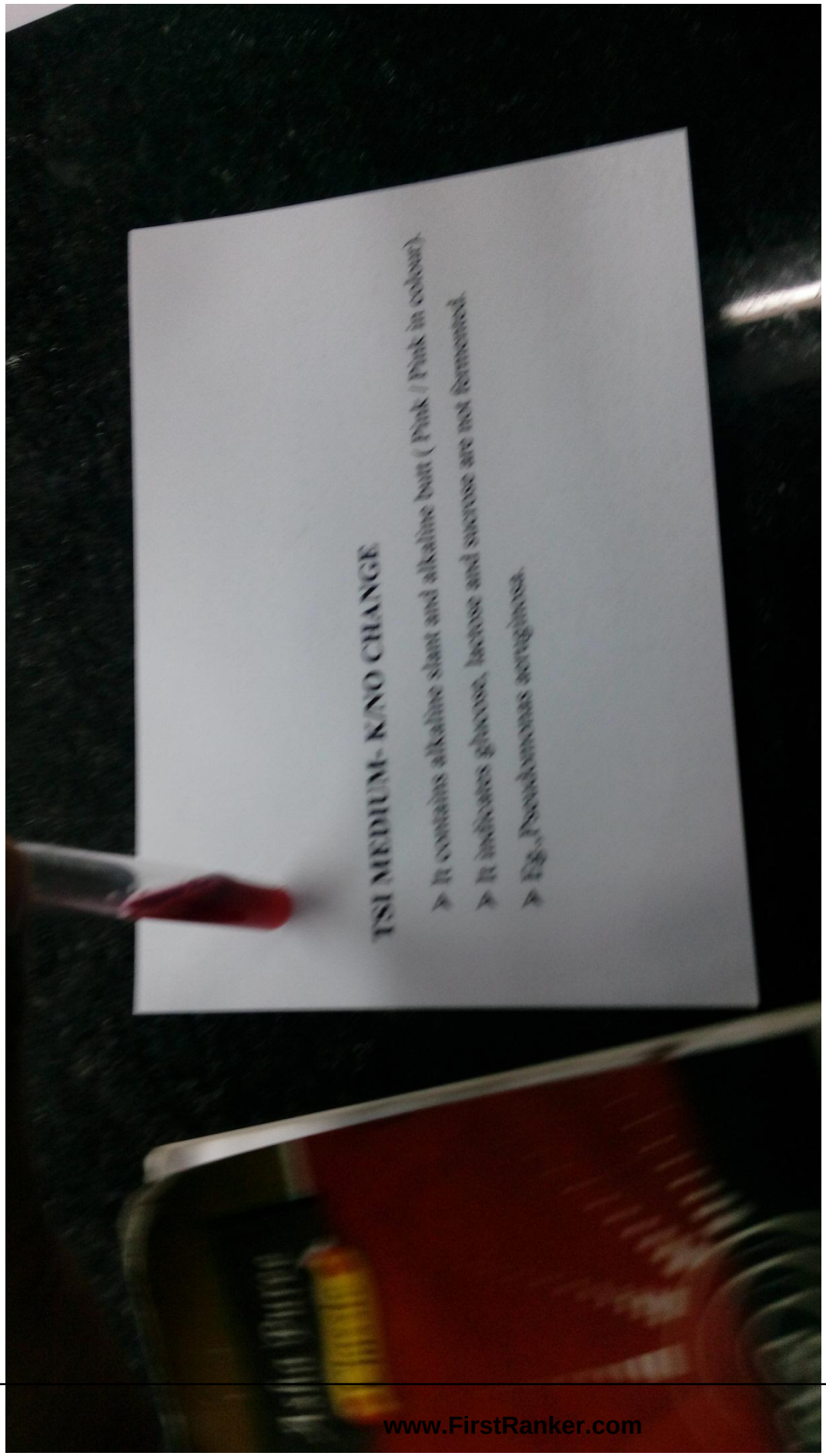
CITRATE MEDIUM WITHOUT REACTION

- Simmons citrate medium is used to determine the ability of an organism to utilise citrate as sole source of carbon for its growth.
- Bromothymol blue is used as an indicator.
- Green colour indicates negative test.eg. Escherichia coli.

CITRATE MEDIUM WITH REACTION

- Simmons citrate medium is used to determine the ability of an organism to utilisecitrate asthe sole source of carbon for its growth.
- Bromothymol blue is used as an indicator.
- Blue colour indicates positive test.eg.,Klebsiellapneumoniae, Citrobacter.





TSI MEDIUM- A/A

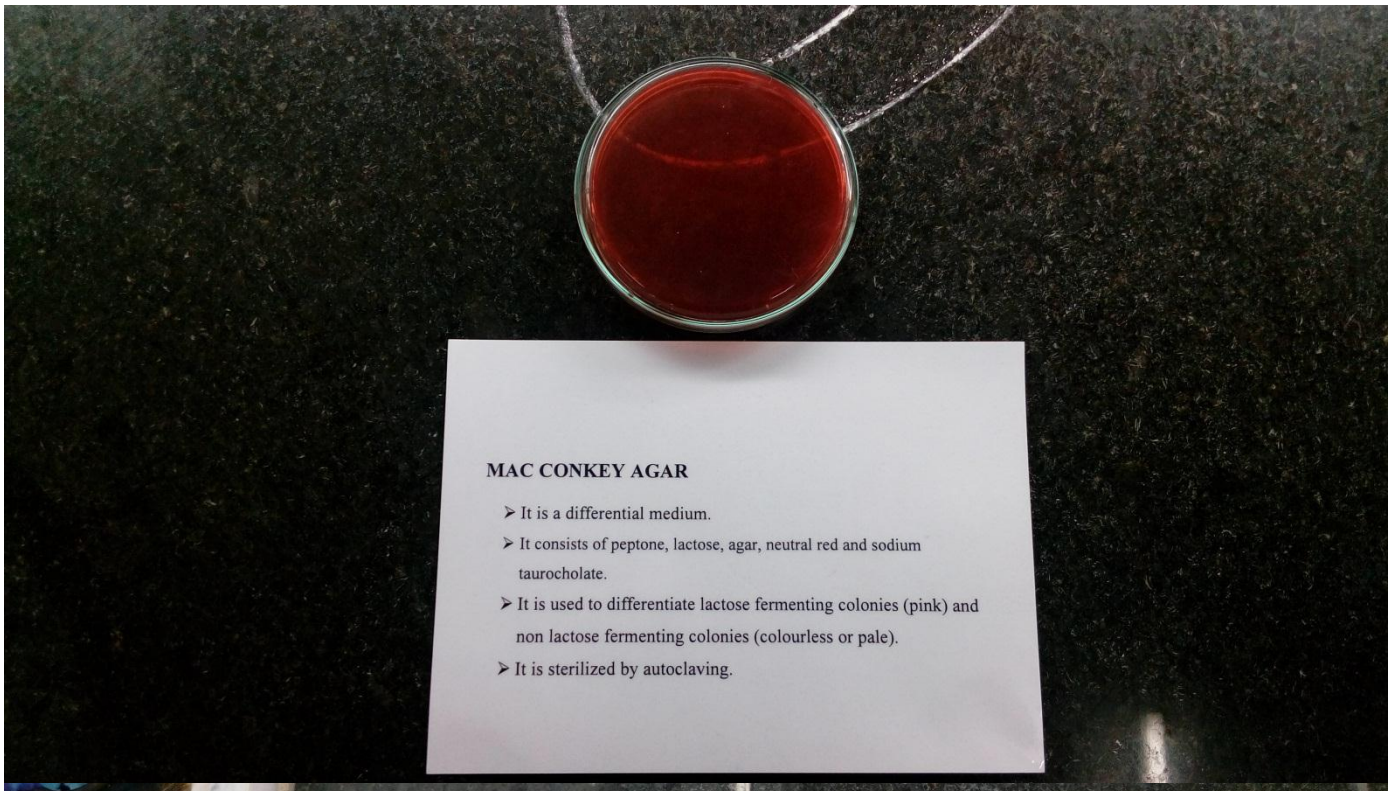
- It contains acid slant and acid butt (yellow / yellow in colour).
- It indicates fermentation of glucose, lactose and sucrose.
- Eg. Escherichia coli, Klebsiellapneumoniae.

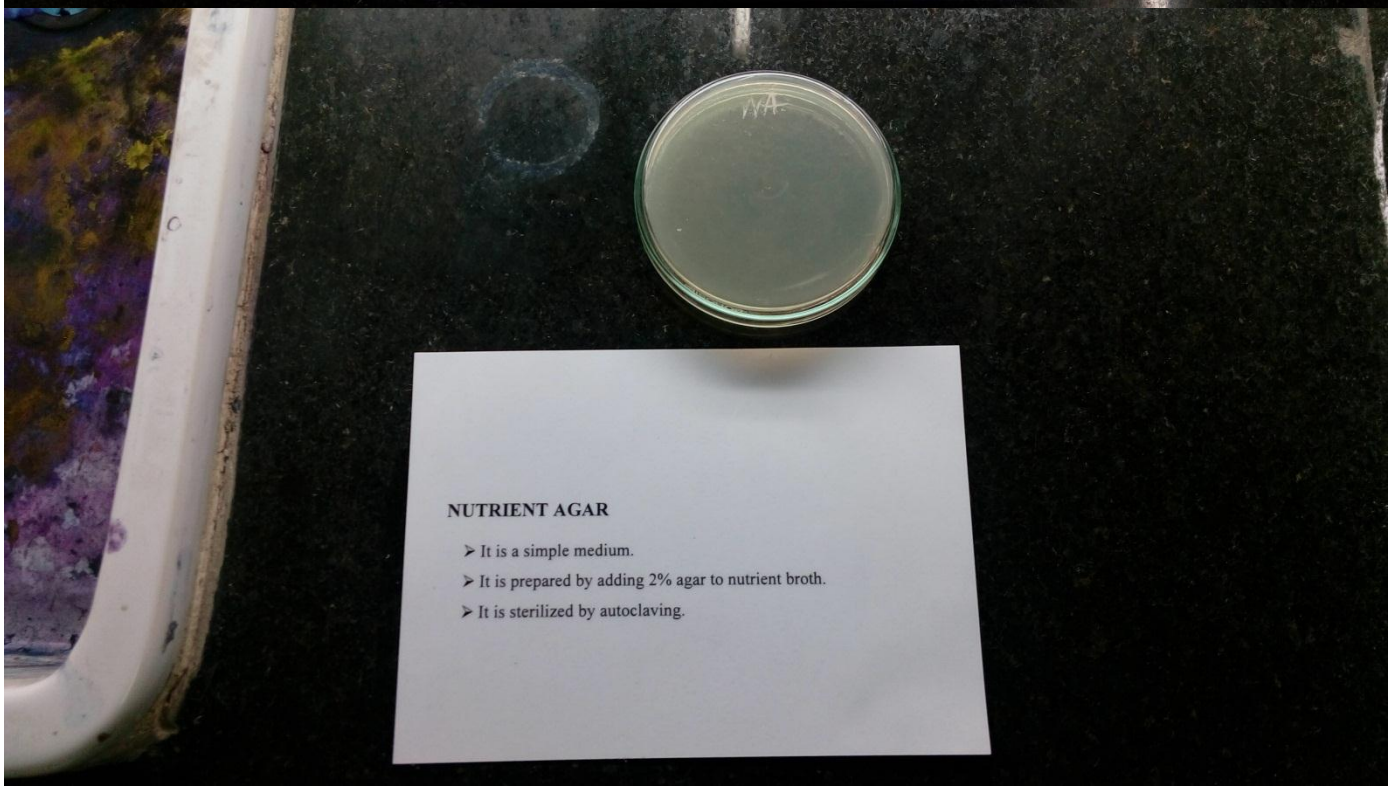
TSI MEDIUM- K/A WITH H2S

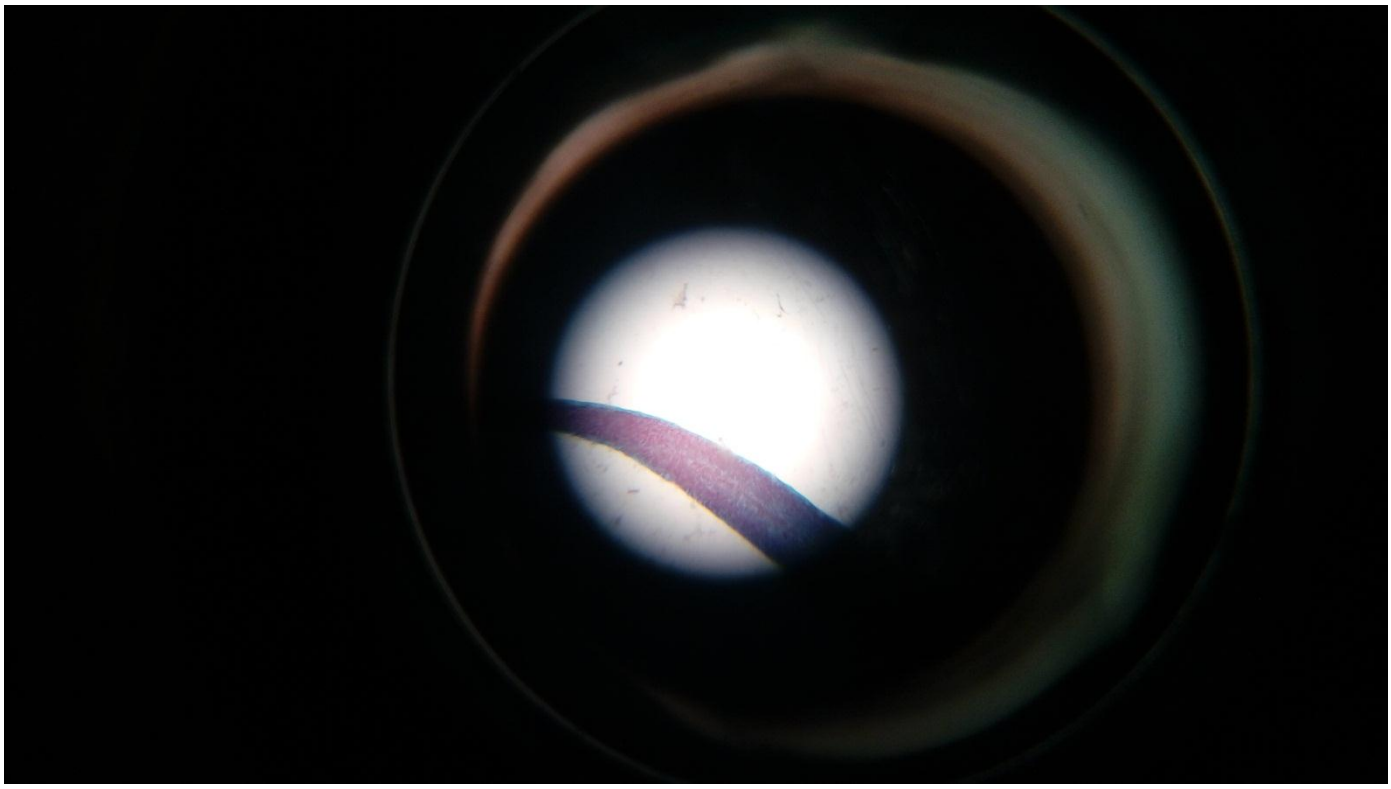
- It contains alkaline slant and acid butt (pink/yellow in colour) with abundant hydrogen sulphide production.
- Hydrogen sulphide production is detected by blackening of the medium.
- Eg. Proteus vulgaris, Salmonella typhipara B.

TSI MEDIUM –K/A

- It contains alkaline slant and acid butt(pink / yellow in colour).
- It indicates that glucose is only fermented.
- Eg. Shigella, Salmonella.







ENTEROBIUS VERMICULARIS

- It is commonly known as pinworm, thread worm, seat worm.
- Adult worms (gravid females) live in the caecum and vermiform appendix.
- It is small, more or less spindle-shaped and white in colour.
- In both male and female a pair of cervical alae is present at the anterior end.
- Double –bulb oesophagus is a characteristic feature of this nematode.

FASCIOLA HEPATICA.

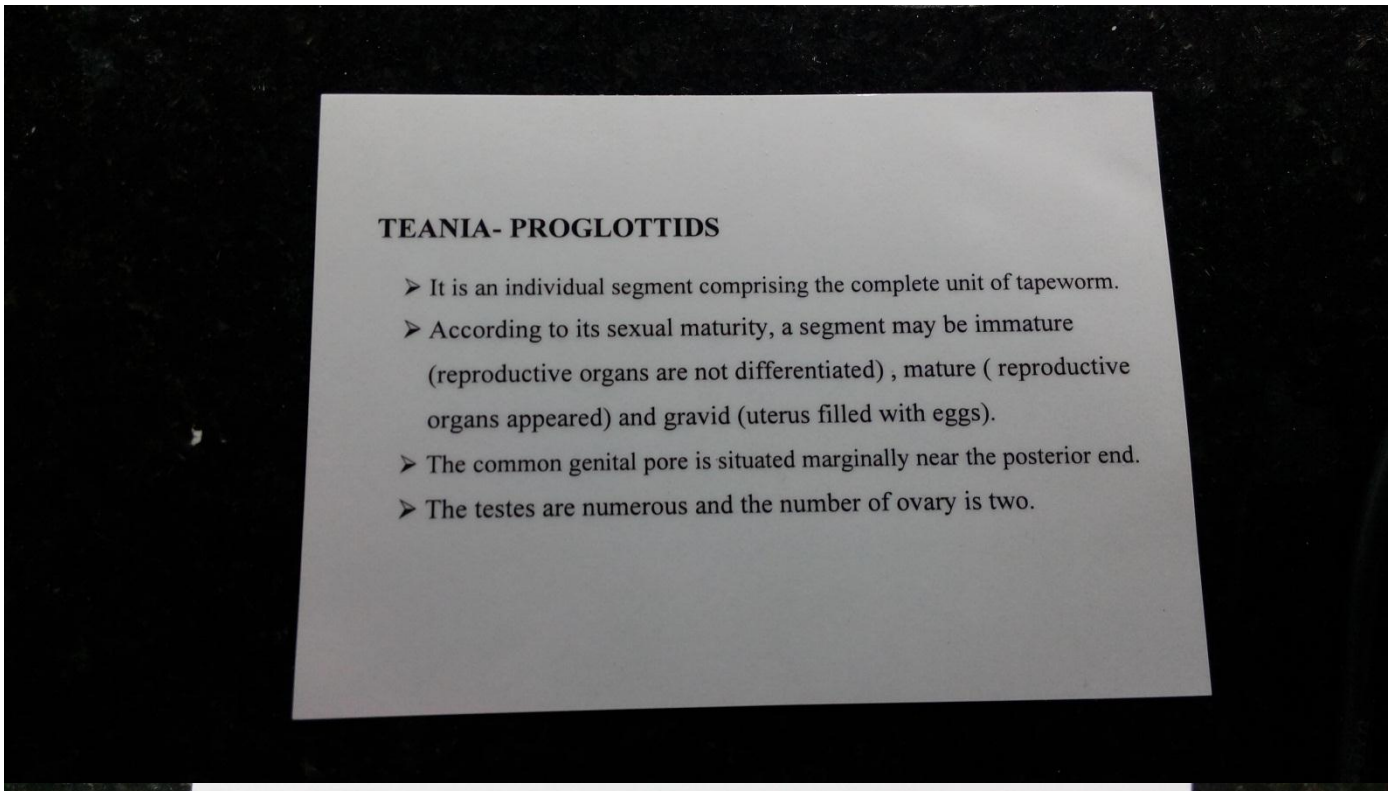
- It is commonly known a liver fluke.
- It is large leaf shaped fluke.
- It contains two suckers- oral and ventral.

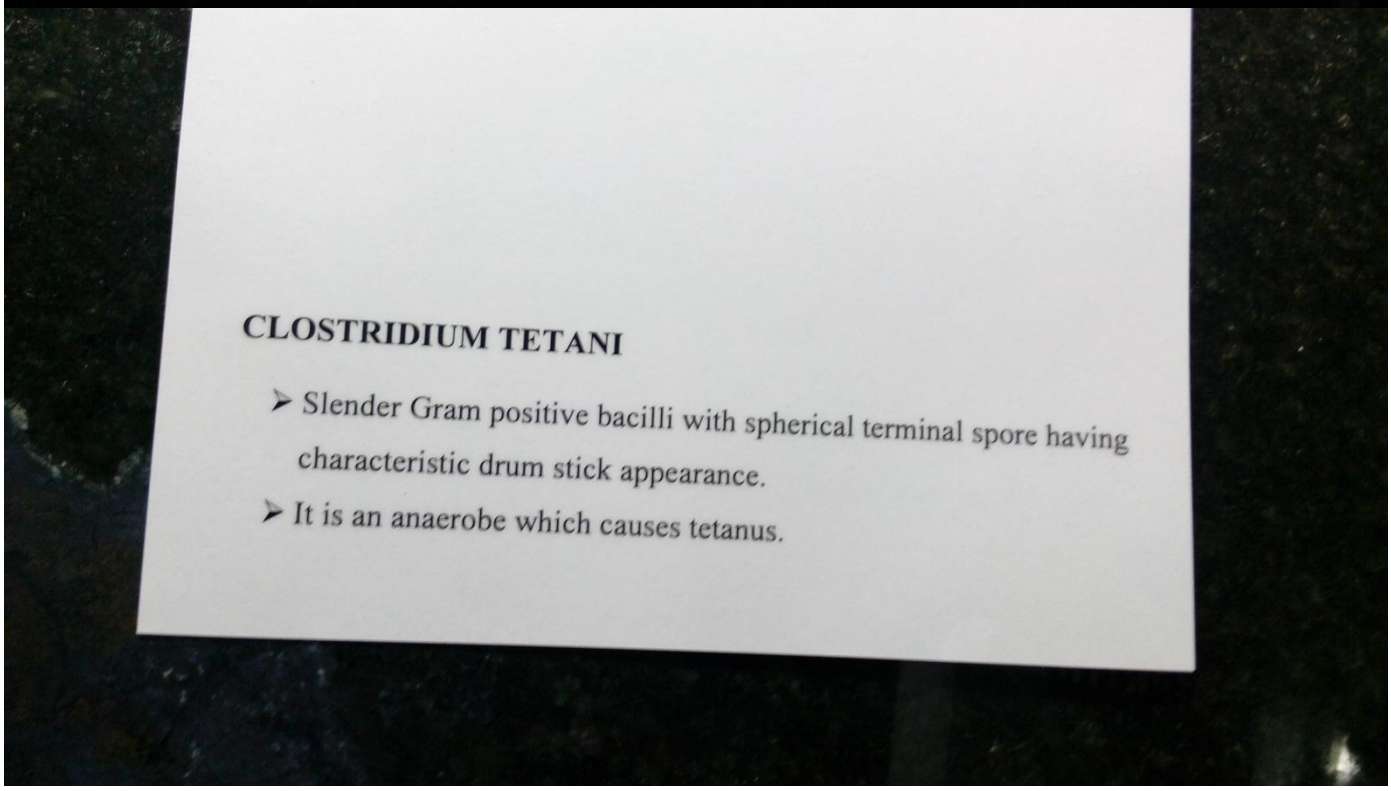
CERCARIA

- It is the final stage of larval development of trematodes in the mollusc. It possess a body and tail.
- According to the nature of tail different names are given. Eg Fork tailed (as in Schistosomes).

TEANIA- SCOLEX

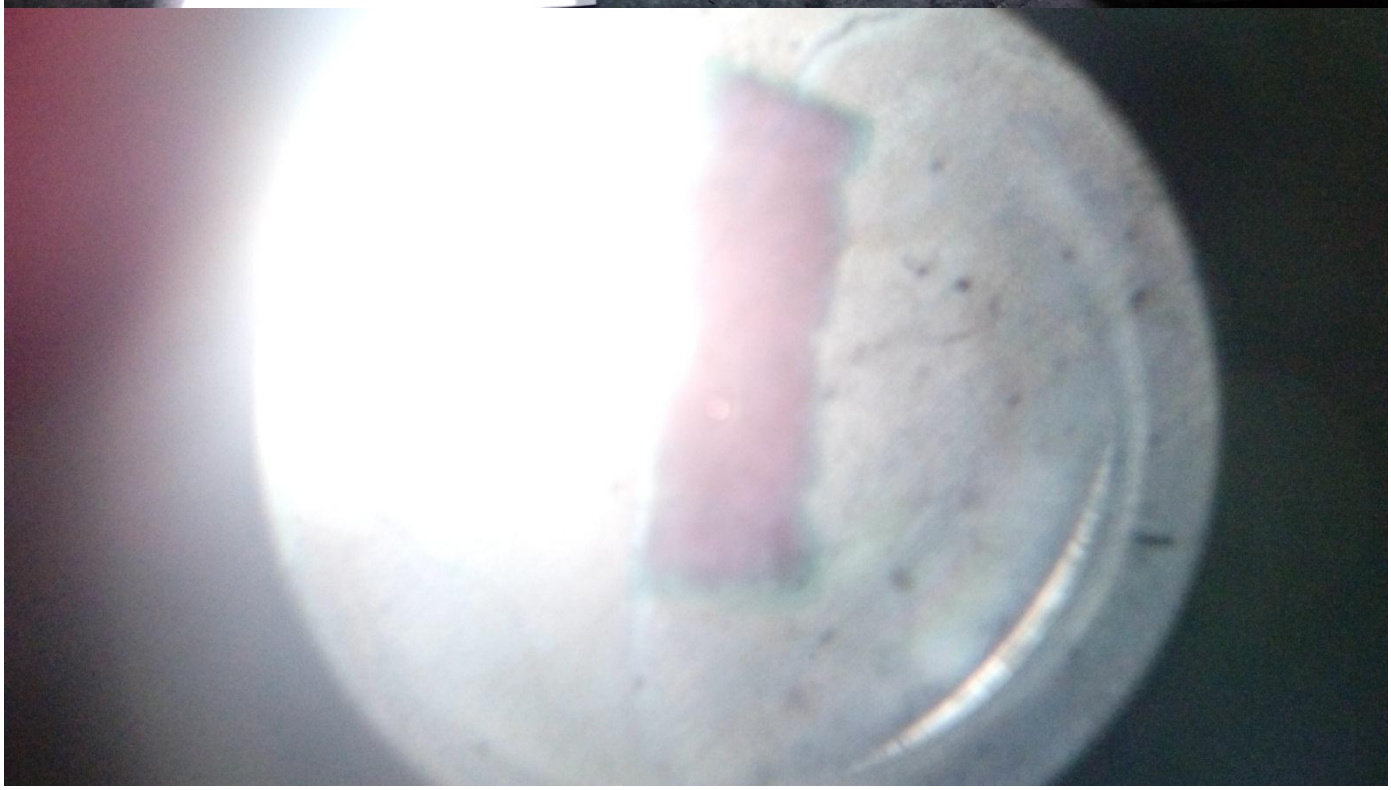
- Commonly called head.
- It is globular or quadrate in outline.
- It has four circular suckers.
- May or may not provided with rostellum and double row of hooklets.

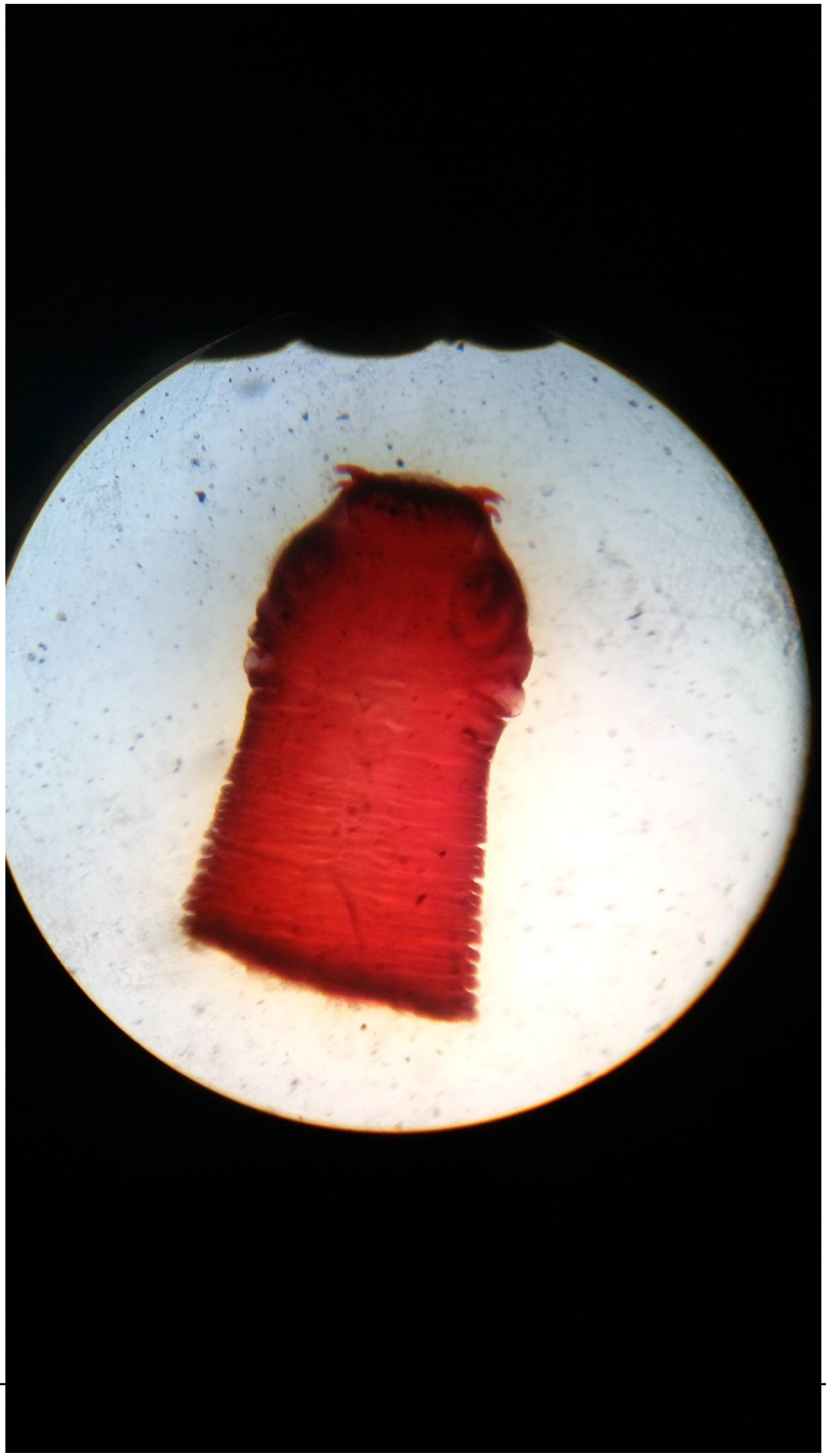


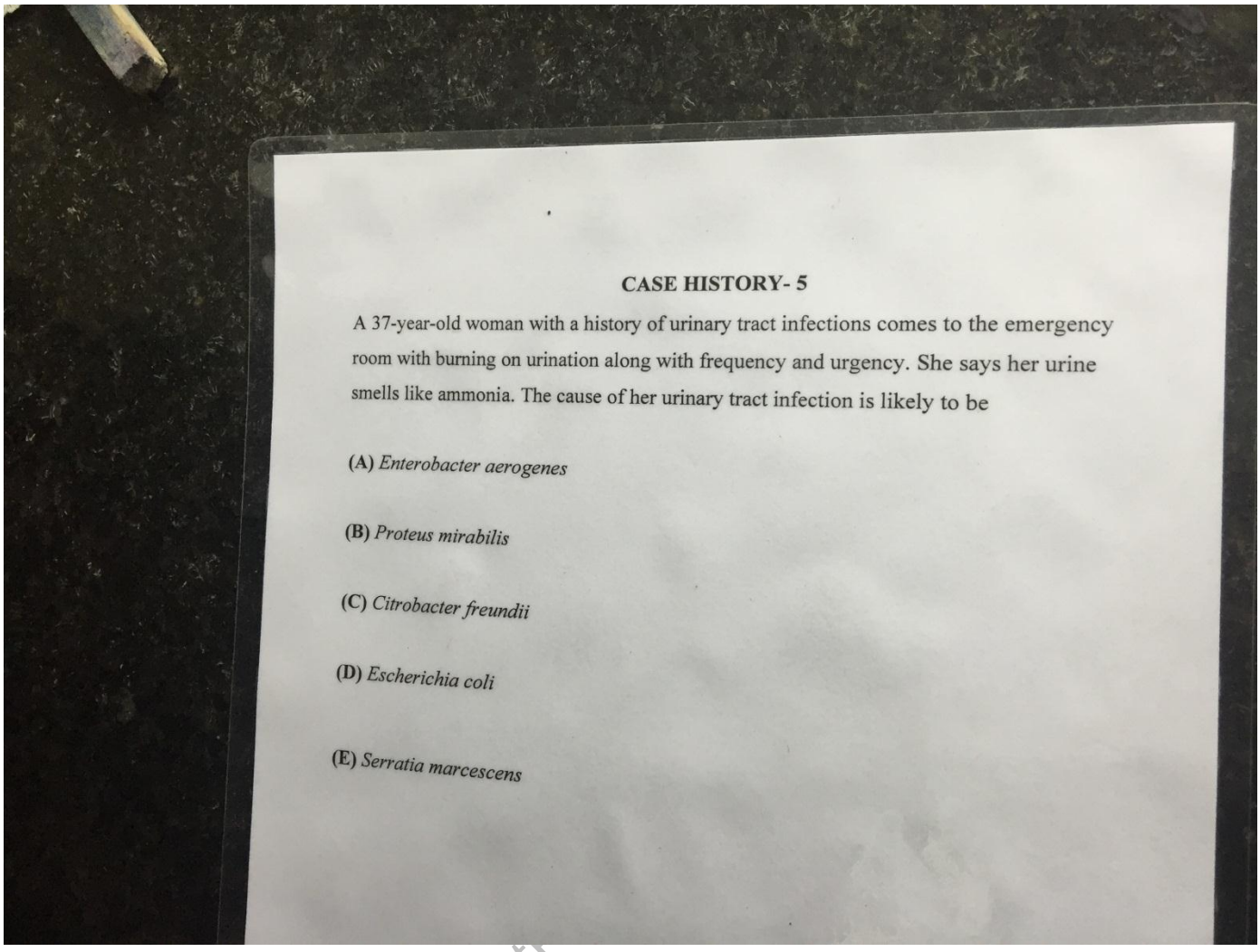


ACID FAST BACILLI

- Slender and pink coloured rod shaped bacilli seen against blue back ground.
- Eg. Mycobacterium tuberculosis.







CASE HISTORY- 5

A 37-year-old woman with a history of urinary tract infections comes to the emergency room with burning on urination along with frequency and urgency. She says her urine smells like ammonia. The cause of her urinary tract infection is likely to be

(A) *Enterobacter aerogenes*

(B) *Proteus mirabilis*

(C) *Citrobacter freundii*

(D) *Escherichia coli*

(E) *Serratia marcescens*

5. PROTEUS

Specimen: Urine of patient suffering from Urinary tract infection

Direct smear: Pus cells with gram negative bacilli

Culture: Plating done on

Culture media	Incubation	After 24 hr
Nutrient agar	Incubated at 37°c for 24 hr	Tiny colonies with swarming growth
Blood agar		
Mac conkey		Pale tiny non lactose fermenting colonies

Smear from colony : gram negative bacilli seen, exhibits Pleomorphism. Different morphological forms are seen in the same organisms.

Biochemical test:

	P.mirabilis	P.vulgaris
Indole	Negative	Positive
Urease	Positive	Positive
Citrate	Positive	Positive
TSI	Alk/acid	Alk/acid
	Gas+	Gas+
	H2S+	H2S+
Catalase	Positive	Positive
Oxidase	Negative	Negative

Hanging drop:motile gram negative bacilli seen

Sugar reactions:

Glu	Lactose	Sucrose	Maltose	Mannitol
Acid+	Not fermented	Not fermented	Acid+	Not fermented
Gas+			Gas+	

Antibiotic susceptibility pattern:

Amoxicillin Ciprofloxacin Cotrimoxazole Erythromycin Nalidicic acid Nitrofurantoin

Clinical significance: Urinary Calculi formation

CASEHISTORY - 4

A 37-year-old firefighter suffers smoke inhalation and is hospitalized for ventilatory support. He has a severe cough and begins to expectorate purulent sputum. Gram stain of his sputum specimen shows numerous polymorphonuclear cells and numerous gram-negative rods. Sputum culture grows numerous gram-negative rods that are oxidase-positive. They grow well at 42 °C. On clear agar medium they produce a blue-green color in the agar. The agar where the blue-green color is located fluoresces when exposed to ultraviolet light. The organism causing the patient's infection is

(A) *Burkholderia cepacia*

(B) *Klebsiella pneumoniae*

(C) *Escherichia coli*

(D) *Pseudomonas aeruginosa*

(E) *Burkholderia pseudomallei*

4. PSEUDOMONAS

Specimen: Wound swab from patient X

Direct smear: Gram staining shows plenty of pus cells with Gram negative bacilli seen.

Culture: After Incubation @ 37°c for 24 hr

Culture media	**Colony Characteristics**
Nutrient agar	Opaque irregular colonies with earthy smell. Pseudomonas pyogenes produce green pigment
Blood agar plate	Opaque irregular colonies surrounded by zone of hemolysis.
Macconkey agar	Non lactose fermenting colonies

Smear from colony: Gram negative bacilli.

Hanging drop: Motile rods seen

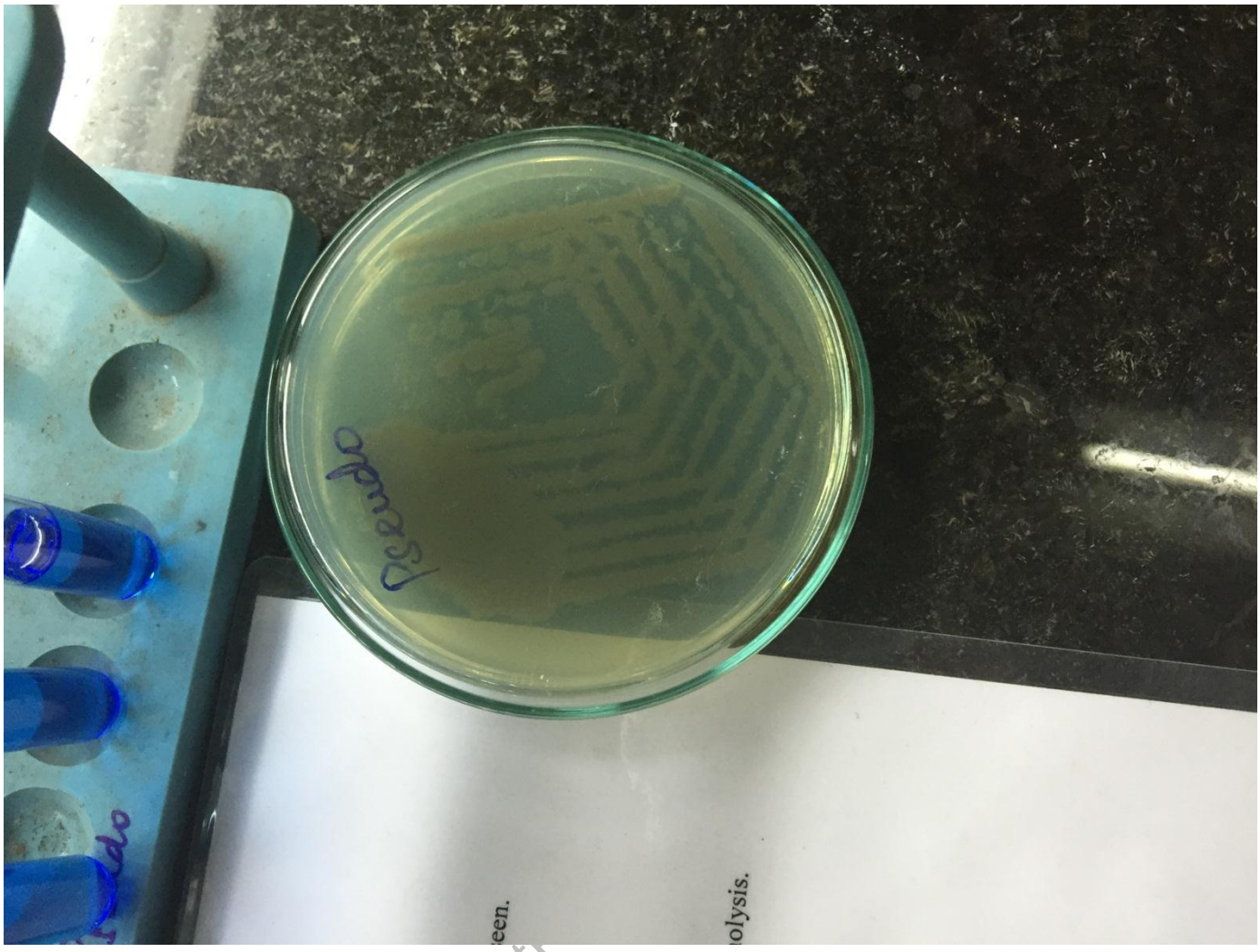
Biochemical Tests:

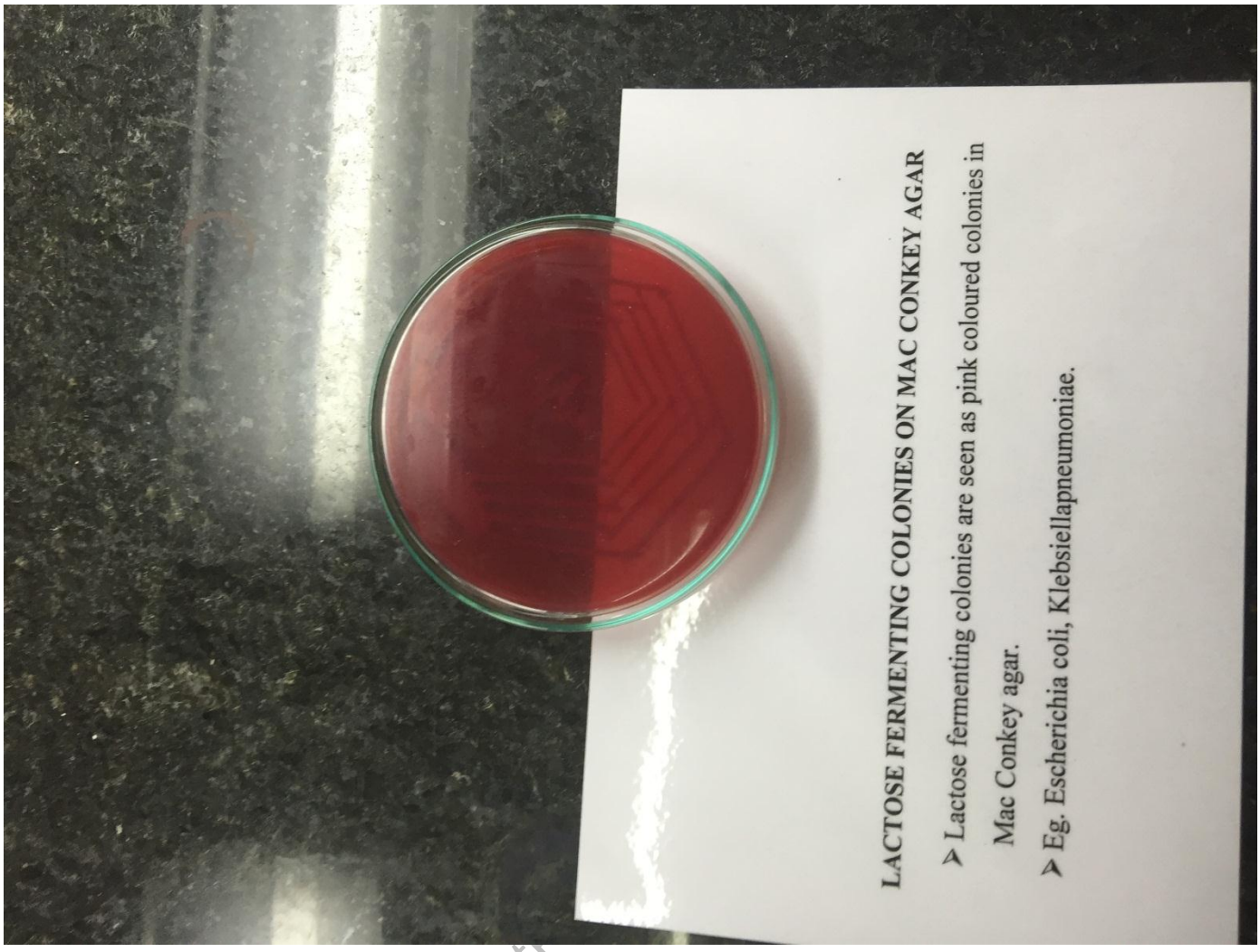
Indole	Negative
Urease	Negative
Citrate	Positive
TSI	k/no change No gas/no H2S
Oxidase	Positive
Catalase	Positive
Sugar reaction:	Glucose – is utilized oxidatively, form acid only; Lactose; sucrose; maltose; mannitol – not fermented

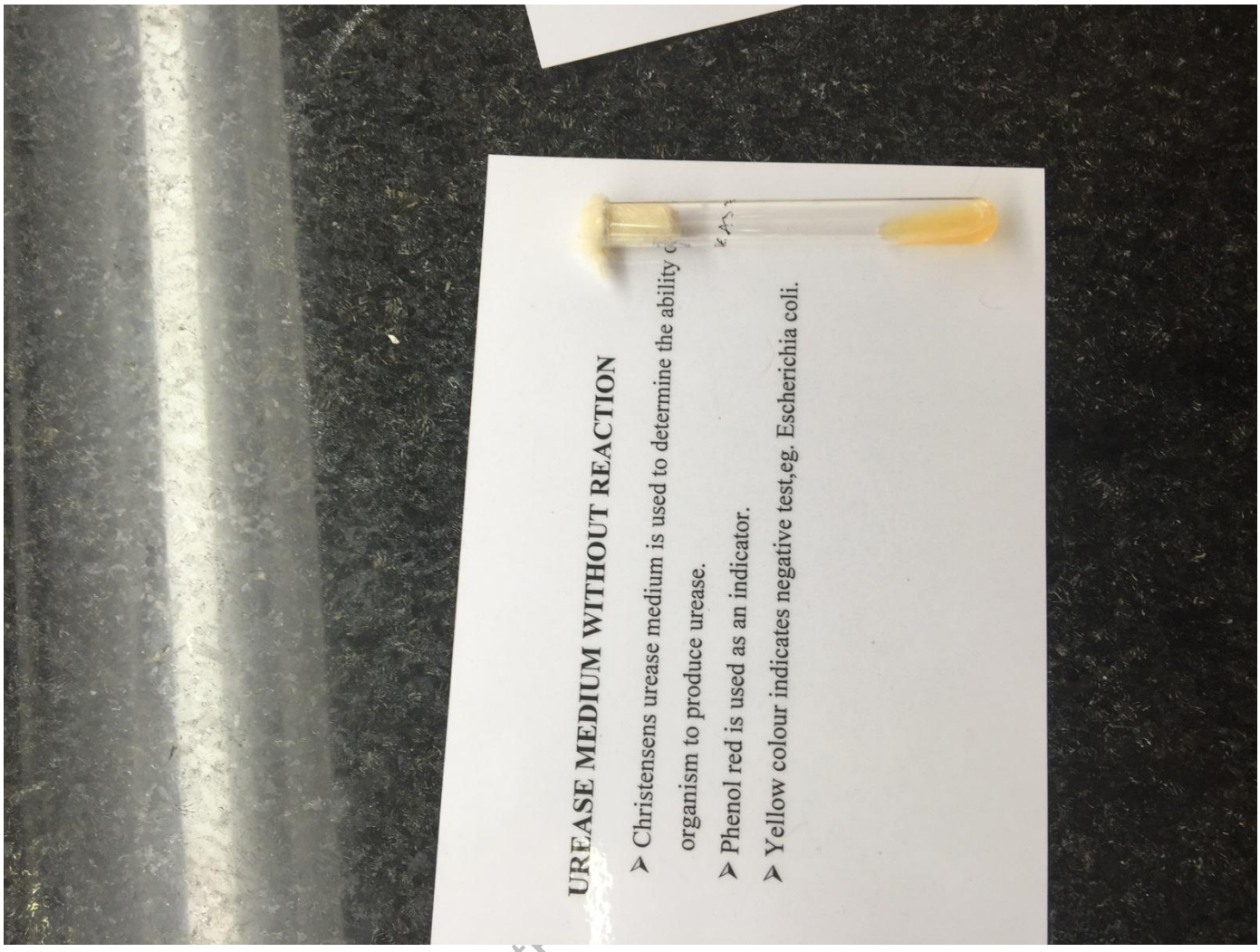
Pyocin typing and Antibiotic susceptibility testing.

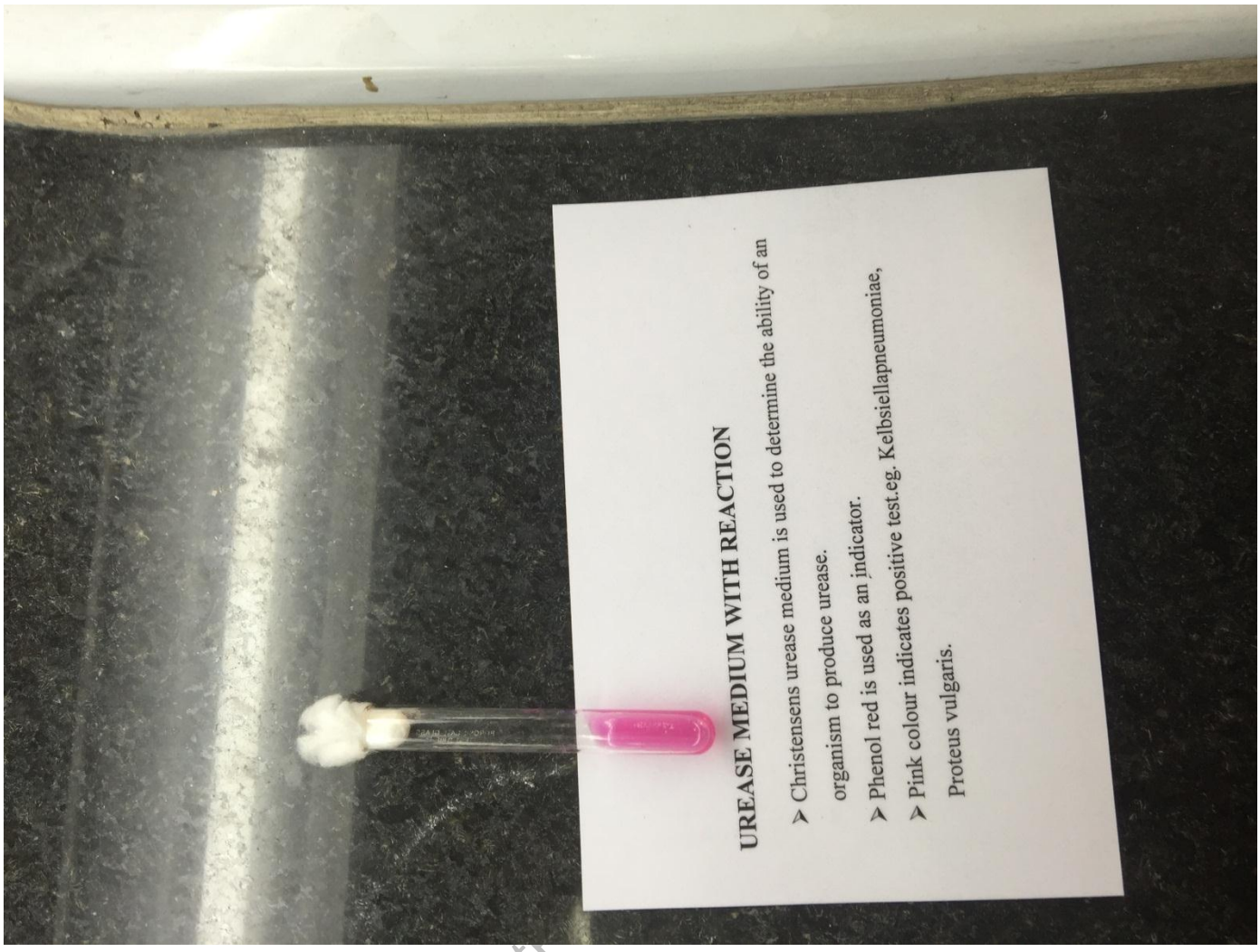
Clinical Significance: Pneumonia (Cystic fibrosis patient, Immunocompromised), Burns wound infection, bed sore infection, Skin and soft tissue infection, Urinary tract infection, Malignant otitis externa, Corneal ulcer for contact lens wearer or following trauma, Endocarditis for iv drug users, Septicemia.

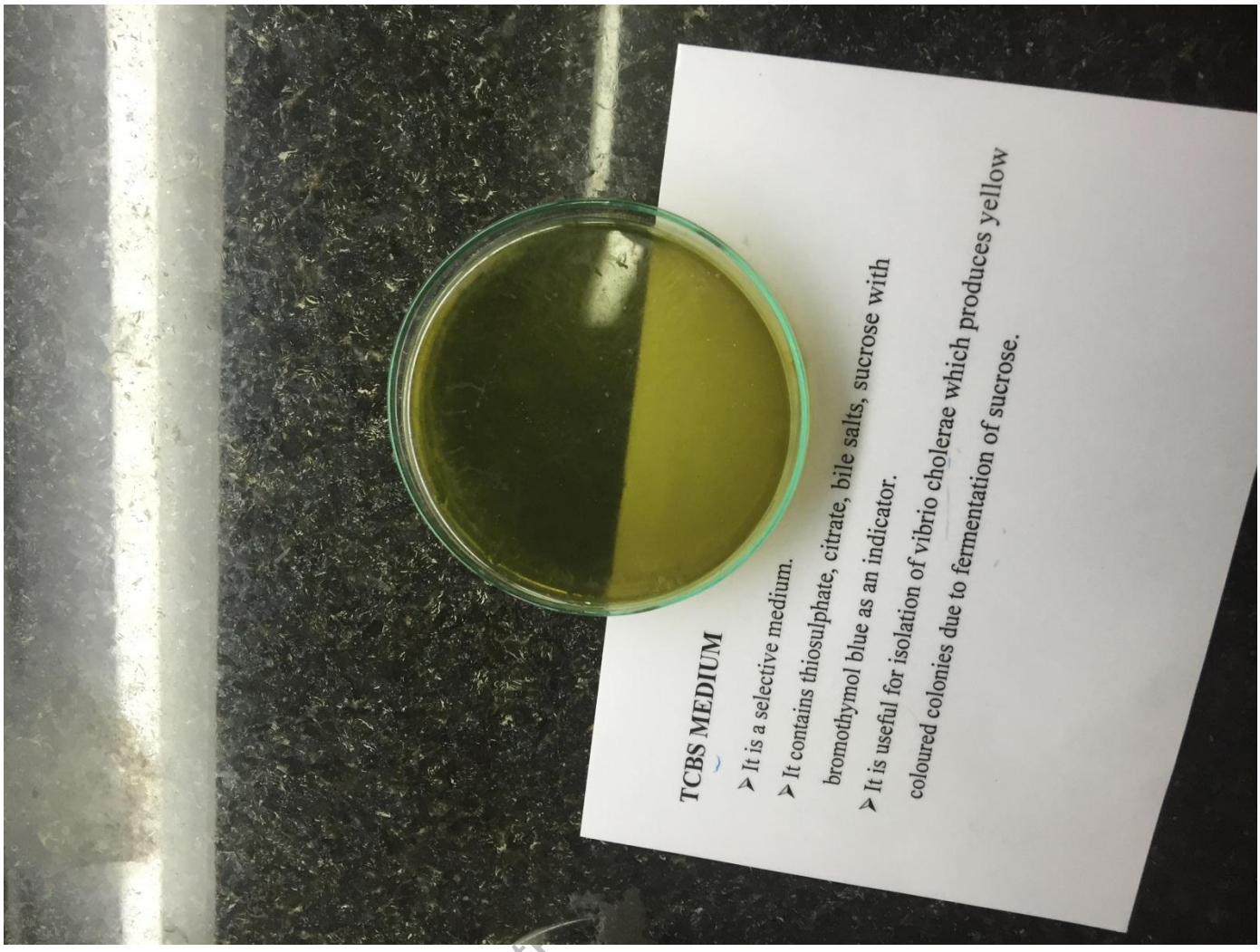
Most common cause for nosocomial infection

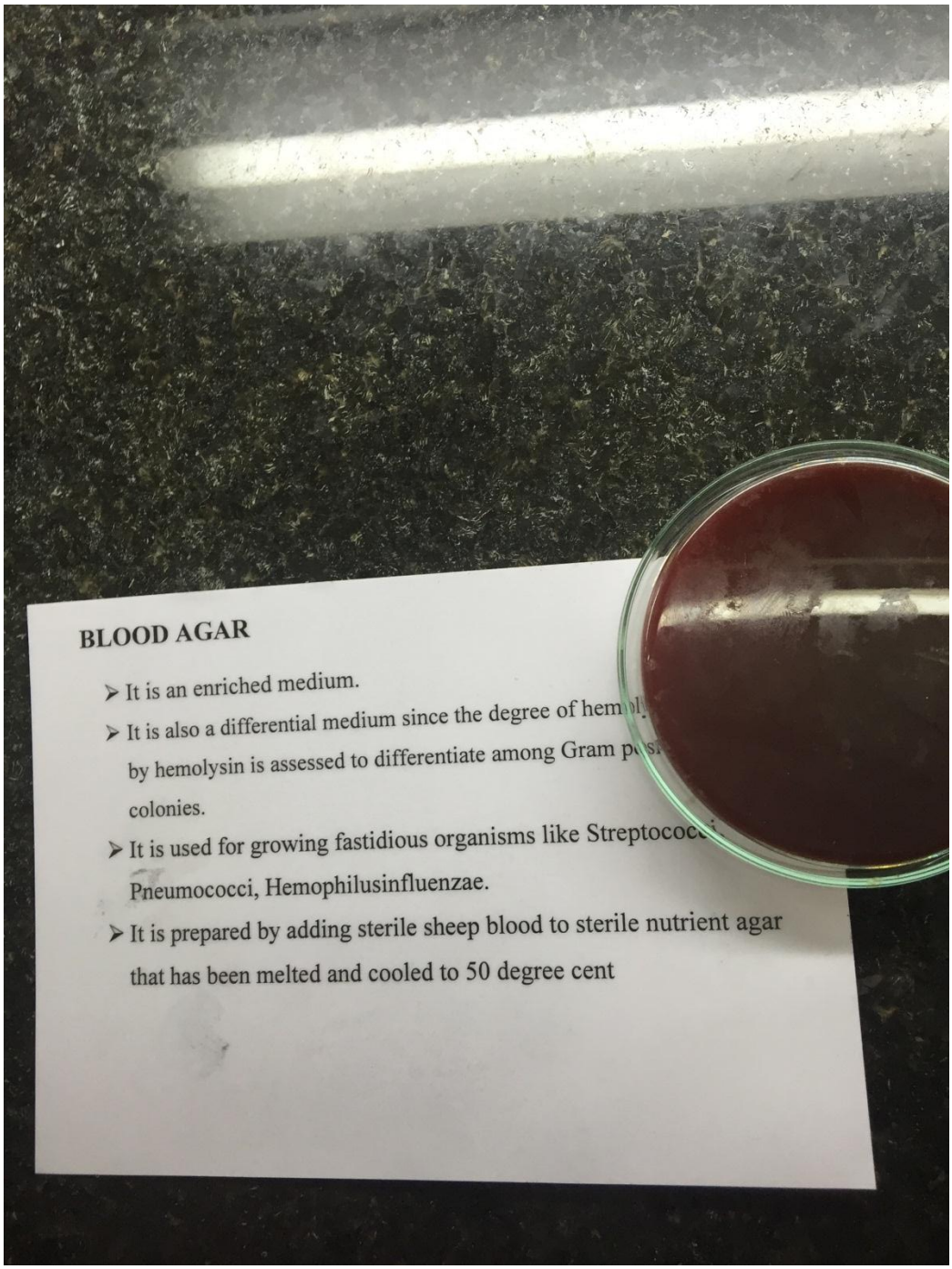


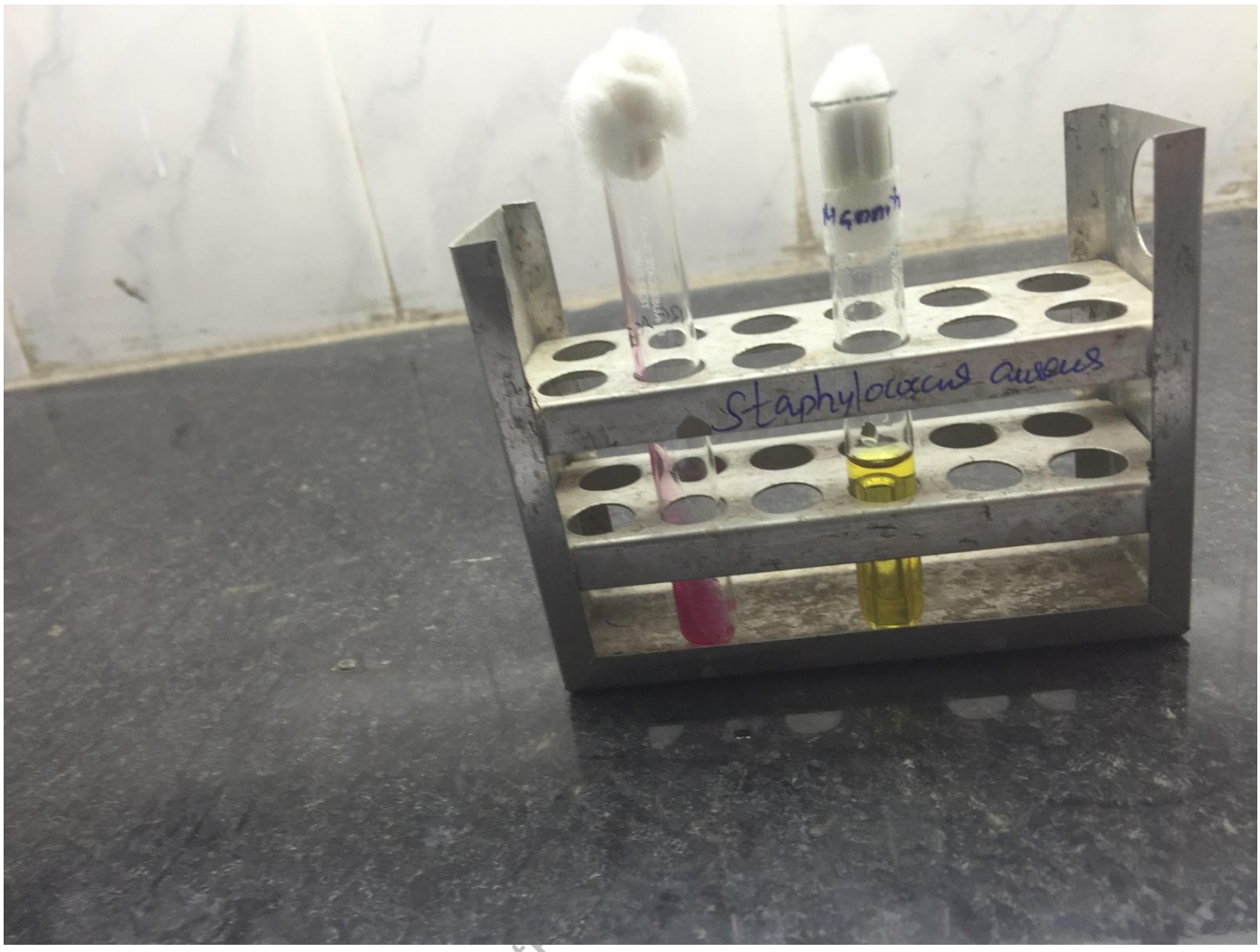


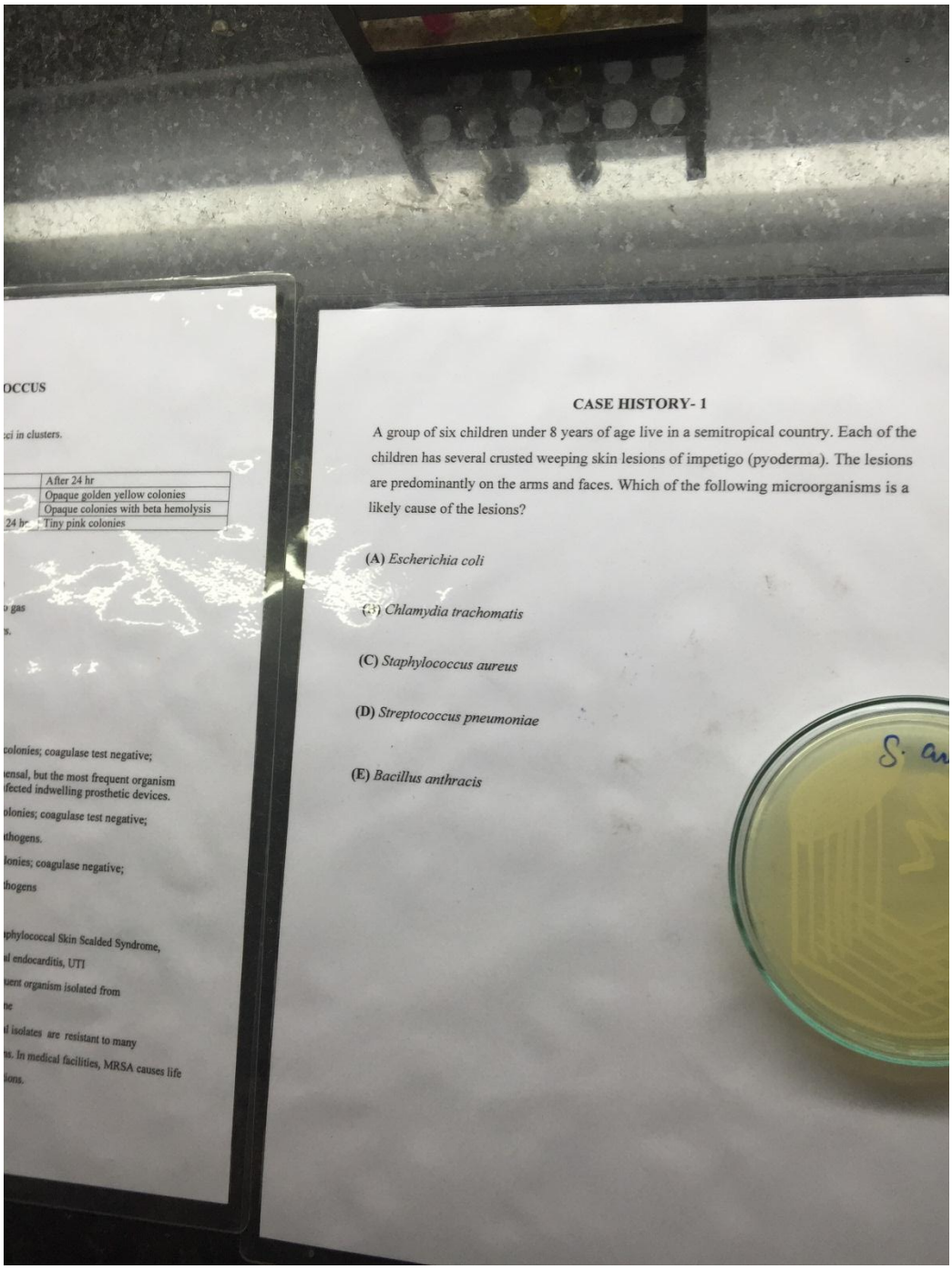












OCCUS

ci in clusters.

	After 24 hr
	Opaque golden yellow colonies
	Opaque colonies with beta hemolysis
24 hr	Tiny pink colonies

gas

colonies; coagulase test negative;
ensal, but the most frequent organism
fected indwelling prosthetic devices.
olonies; coagulase test negative;
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lonies; coagulase negative;
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phylococcal Skin Scalded Syndrome,
l endocarditis, UTI
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l isolates are resistant to many
s. In medical facilities, MRSA causes life
ions.

CASE HISTORY- 1

A group of six children under 8 years of age live in a semitropical country. Each of the children has several crusted weeping skin lesions of impetigo (pyoderma). The lesions are predominantly on the arms and faces. Which of the following microorganisms is a likely cause of the lesions?

(A) *Escherichia coli*

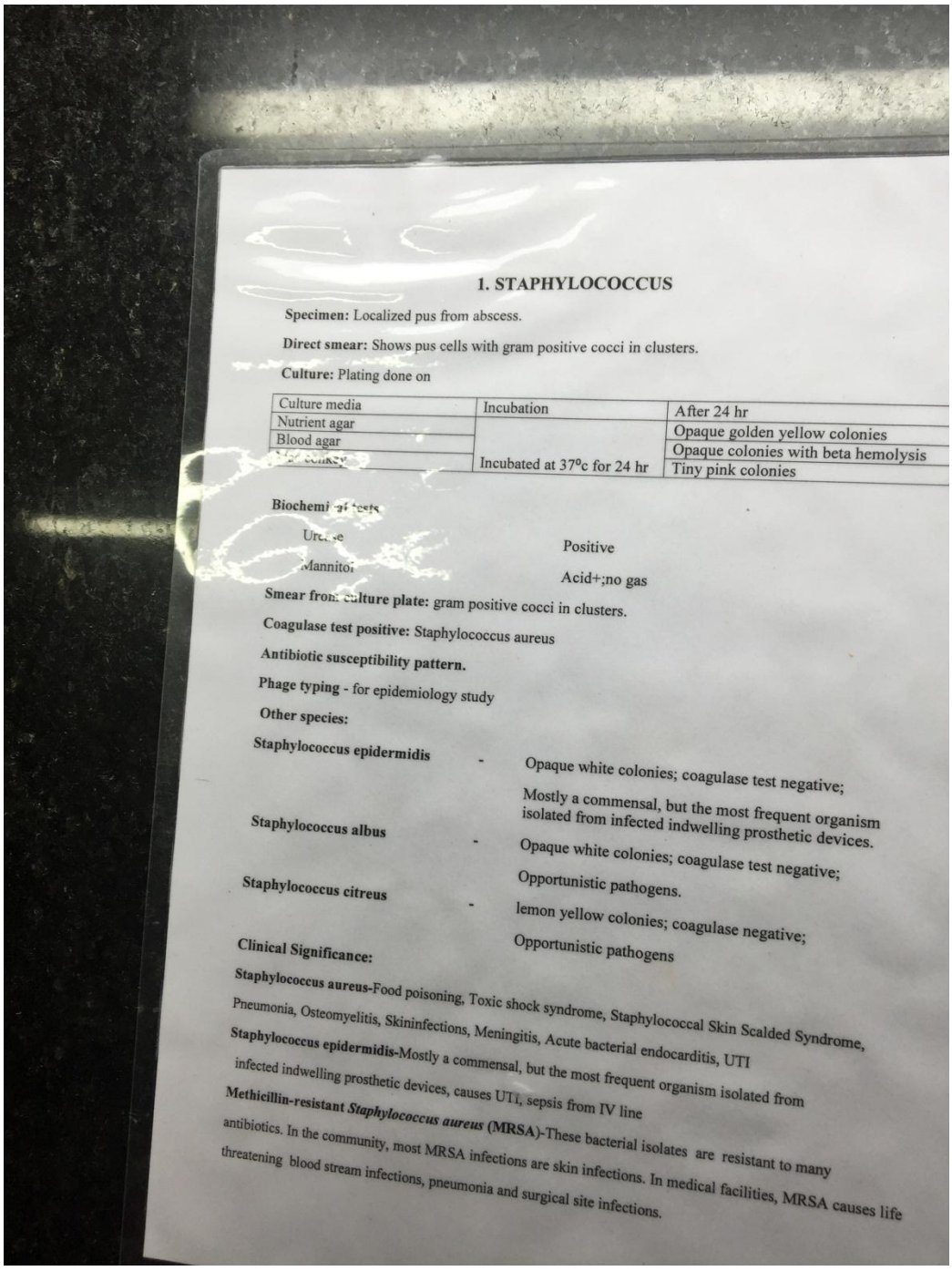
(B) *Chlamydia trachomatis*

(C) *Staphylococcus aureus*

(D) *Streptococcus pneumoniae*

(E) *Bacillus anthracis*

S. au



1. STAPHYLOCOCCUS

Specimen: Localized pus from abscess.

Direct smear: Shows pus cells with gram positive cocci in clusters.

Culture: Plating done on

Culture media	Incubation	After 24 hr
Nutrient agar	Incubated at 37°c for 24 hr	Opaque golden yellow colonies
Blood agar		Opaque colonies with beta hemolysis
Mac conkey		Tiny pink colonies

Biochemical tests

Urease — Positive

Mannitol — Acid+;no gas

Smear from culture plate: gram positive cocci in clusters.

Coagulase test positive: Staphylococcus aureus

Antibiotic susceptibility pattern.

Phage typing - for epidemiology study

Other species:

Staphylococcus epidermidis - Opaque white colonies; coagulase test negative; Mostly a commensal, but the most frequent organism isolated from infected indwelling prosthetic devices.

Staphylococcus albus - Opaque white colonies; coagulase test negative; Opportunistic pathogens.

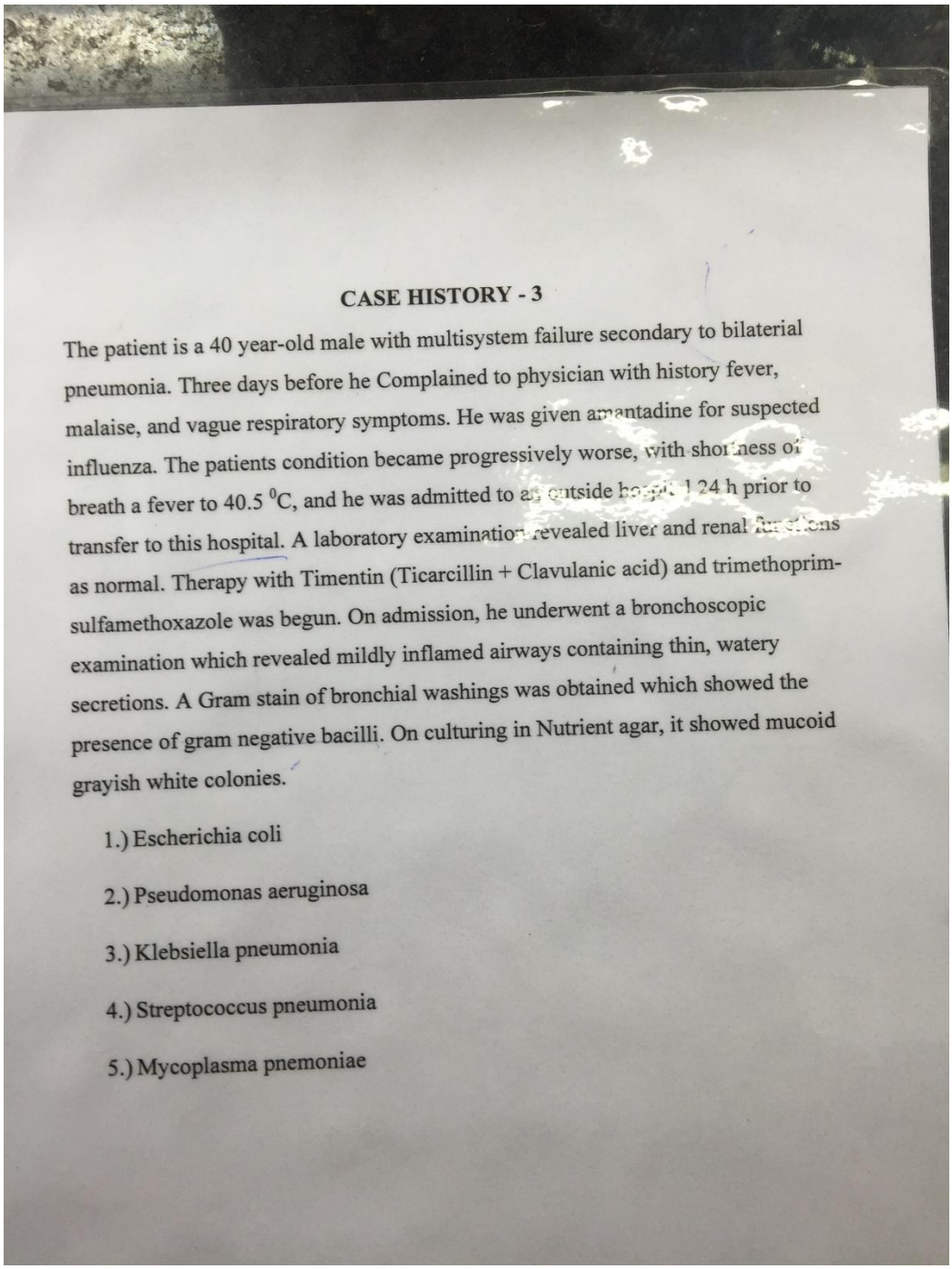
Staphylococcus citreus - lemon yellow colonies; coagulase negative; Opportunistic pathogens

Clinical Significance:

Staphylococcus aureus-Food poisoning, Toxic shock syndrome, Staphylococcal Skin Scalded Syndrome, Pneumonia, Osteomyelitis, Skininfections, Meningitis, Acute bacterial endocarditis, UTI

Staphylococcus epidermidis-Mostly a commensal, but the most frequent organism isolated from infected indwelling prosthetic devices, causes UTI, sepsis from IV line

Methicillin-resistant *Staphylococcus aureus* (MRSA)-These bacterial isolates are resistant to many antibiotics. In the community, most MRSA infections are skin infections. In medical facilities, MRSA causes life threatening blood stream infections, pneumonia and surgical site infections.



CASE HISTORY - 3

The patient is a 40 year-old male with multisystem failure secondary to bilaterial pneumonia. Three days before he Complained to physician with history fever, malaise, and vague respiratory symptoms. He was given amantadine for suspected influenza. The patients condition became progressively worse, with shortness of breath a fever to 40.5 ^{0}C, and he was admitted to an outside hospital 24 h prior to transfer to this hospital. A laboratory examination revealed liver and renal functions as normal. Therapy with Timentin (Ticarcillin + Clavulanic acid) and trimethoprim-sulfamethoxazole was begun. On admission, he underwent a bronchoscopic examination which revealed mildly inflamed airways containing thin, watery secretions. A Gram stain of bronchial washings was obtained which showed the presence of gram negative bacilli. On culturing in Nutrient agar, it showed mucoid grayish white colonies.

1.) Escherichia coli

2.) Pseudomonas aeruginosa

3.) Klebsiella pneumonia

4.) Streptococcus pneumonia

5.) Mycoplasma pnemoniae

3. KLEBSIELLA

Specimen: Urine from patient X who complaints of fever, Lower abdominal pain, increased frequency of micturition.

Direct smear: Grams smear shows pus cells with gram negative rods.

Wet Mount : Centrifuged, Plenty of pus cells seen

Culture : After Incubation at 37°c for 24 hr

Culture media	**Cultural Charecteristics**
Blood agar	Greyish white Colonies
Mac conkey	Pink, mucoid Lactose fermenting colonies

Smear from plate: Thick Gram negative bacilli seen, with some bacilli showing halo around it.

Biochemical tests:

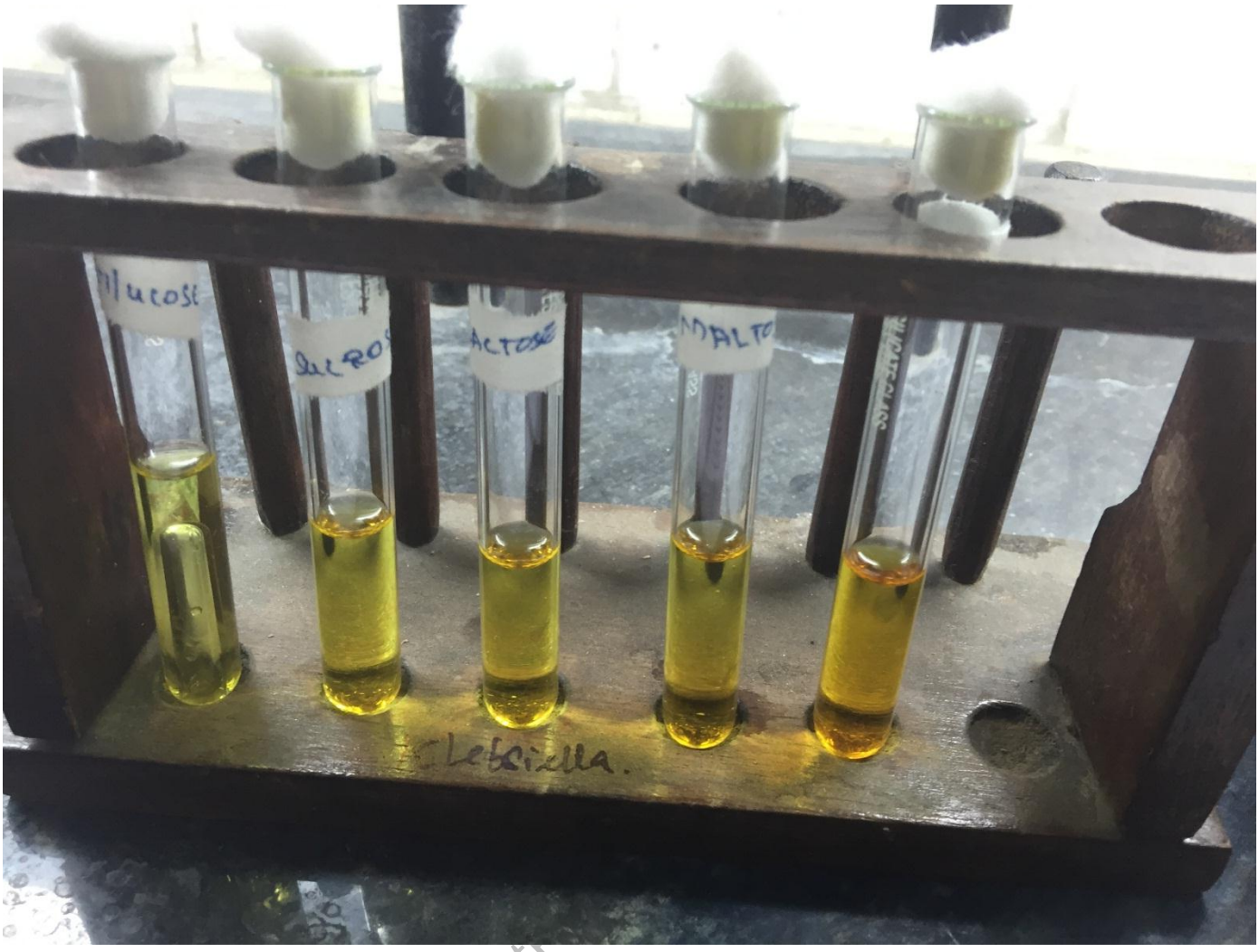
Indole	Negative
Citrate	Positive
Urease	Positive
TSI	A/A;gas+;no H2S
Oxidase	Negative
Catalase	Positive
MR	Negative
VP	Positive

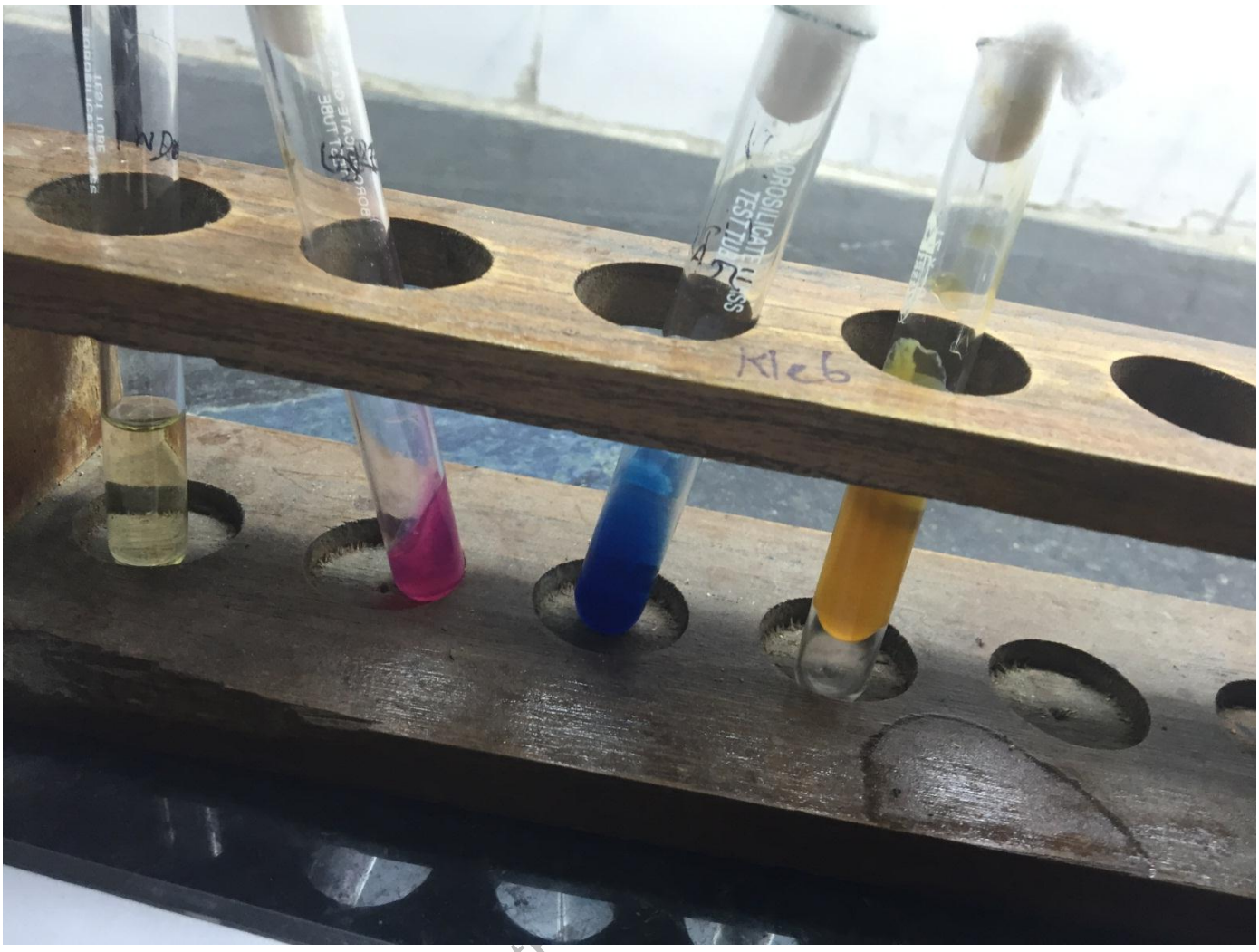
Sugar reactions: Glucose, lactose, sucrose, maltose, mannitol, starch are fermented with acid and gas.

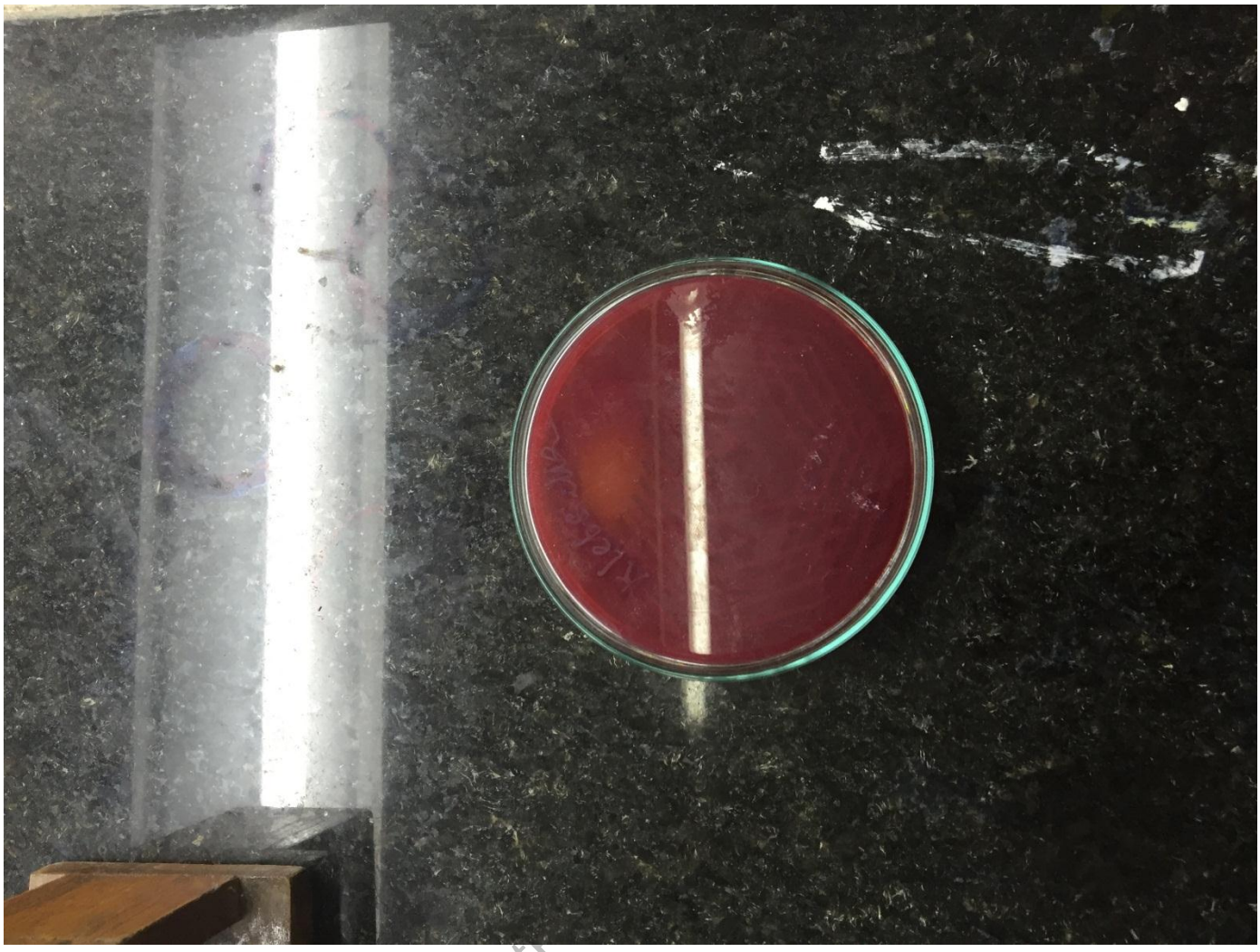
Animal Pathogenicity Test Done – Mice intraperitoneal inoculation done & organisms were demonstrated in the peritoneal fluid

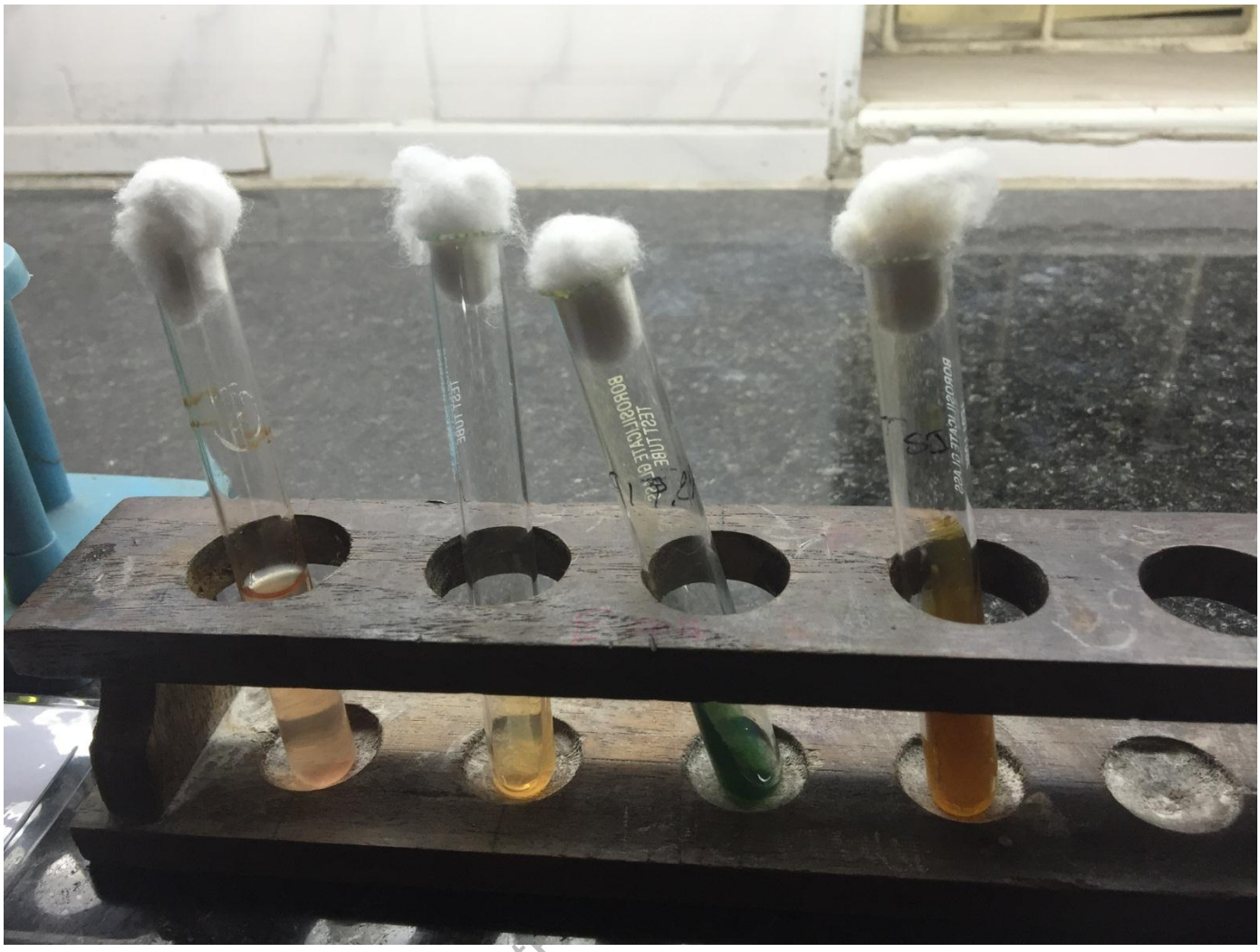
Antibiotic Susceptibility Testing And Klebocin Typing

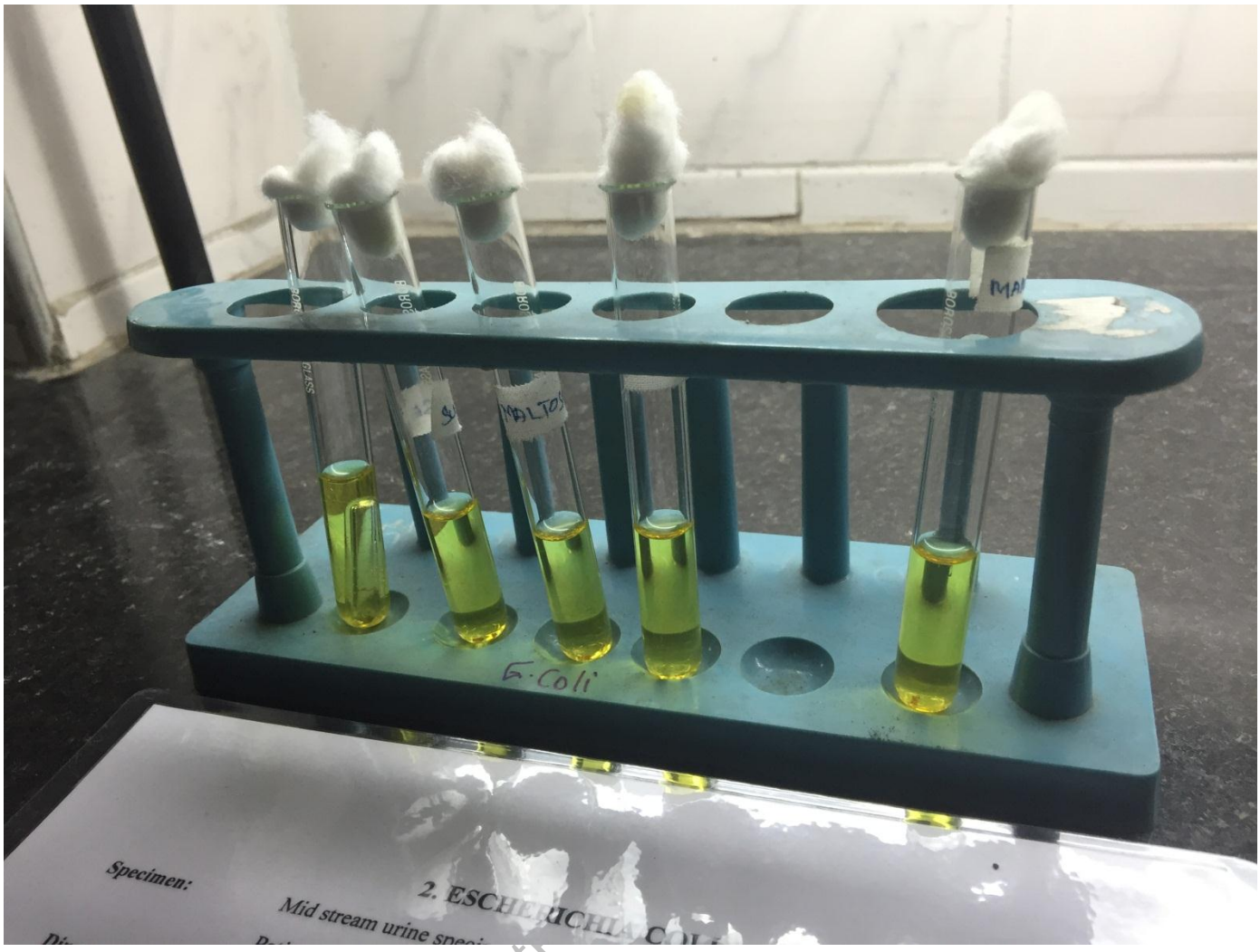
Clinical Significance: Pneumonia caused by Klebsiella species frequently involves the necrotic destruction of alveolar spaces, formation of cavities, and the production of blood-tinged sputum. These bacteria also cause wound & soft-tissue infection, and UTIs. Extended Spectrum Beta Lactamses (ESBL) producing isolates are resistance to 3rd generation cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, Cefpodoxime) and Monobactams (Aztreonam). ESBL can pose a intimidating challenge with limited therapeutic options.

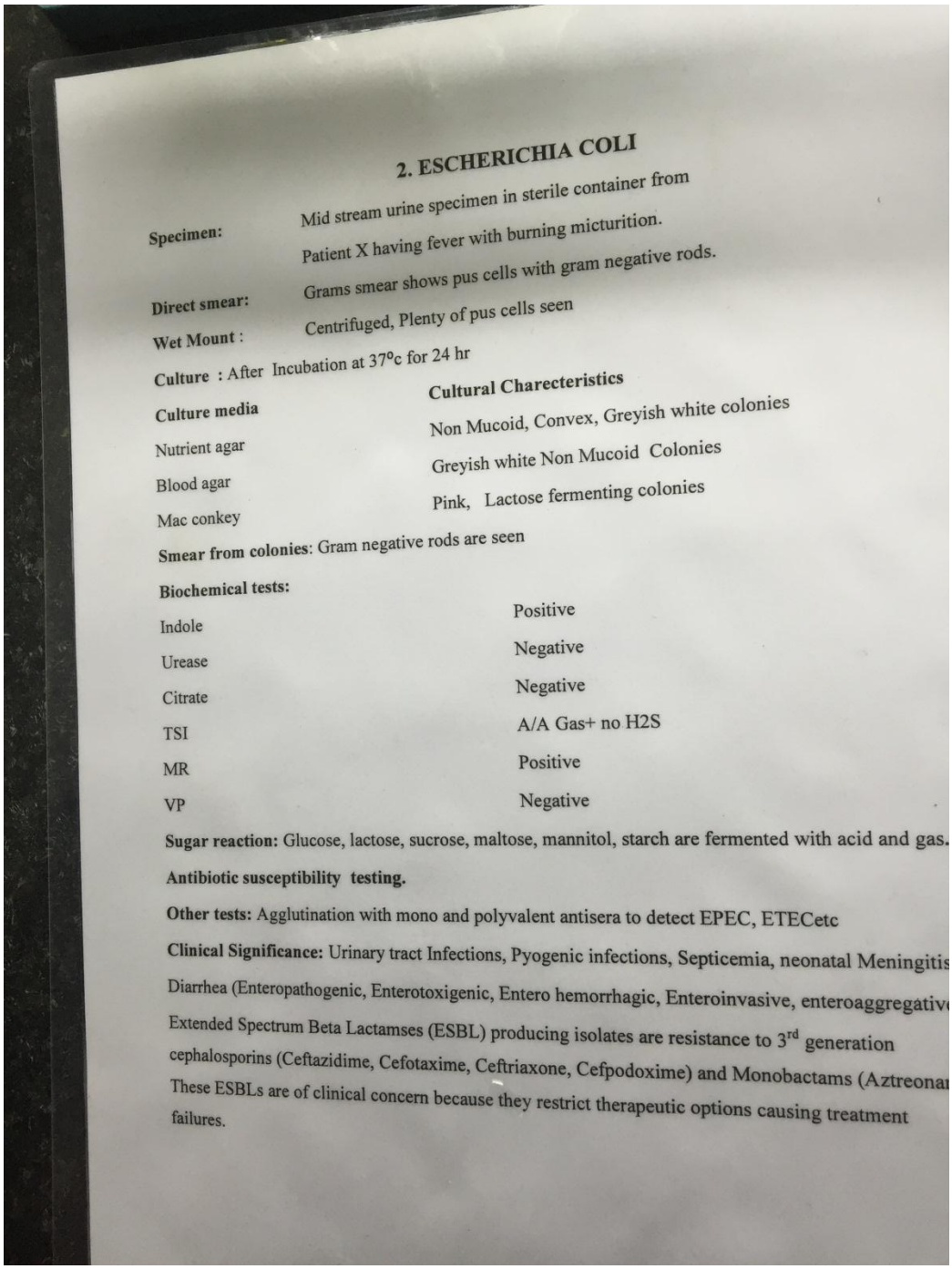












2. ESCHERICHIA COLI

Specimen: Mid stream urine specimen in sterile container from Patient X having fever with burning micturition.

Direct smear: Grams smear shows pus cells with gram negative rods.

Wet Mount : Centrifuged, Plenty of pus cells seen

Culture : After Incubation at 37°c for 24 hr

Culture media	**Cultural Charecteristics**
Nutrient agar	Non Mucoid, Convex, Greyish white colonies
Blood agar	Greyish white Non Mucoid Colonies
Mac conkey	Pink, Lactose fermenting colonies

Smear from colonies: Gram negative rods are seen

Biochemical tests:

Indole	Positive
Urease	Negative
Citrate	Negative
TSI	A/A Gas+ no H2S
MR	Positive
VP	Negative

Sugar reaction: Glucose, lactose, sucrose, maltose, mannitol, starch are fermented with acid and gas.

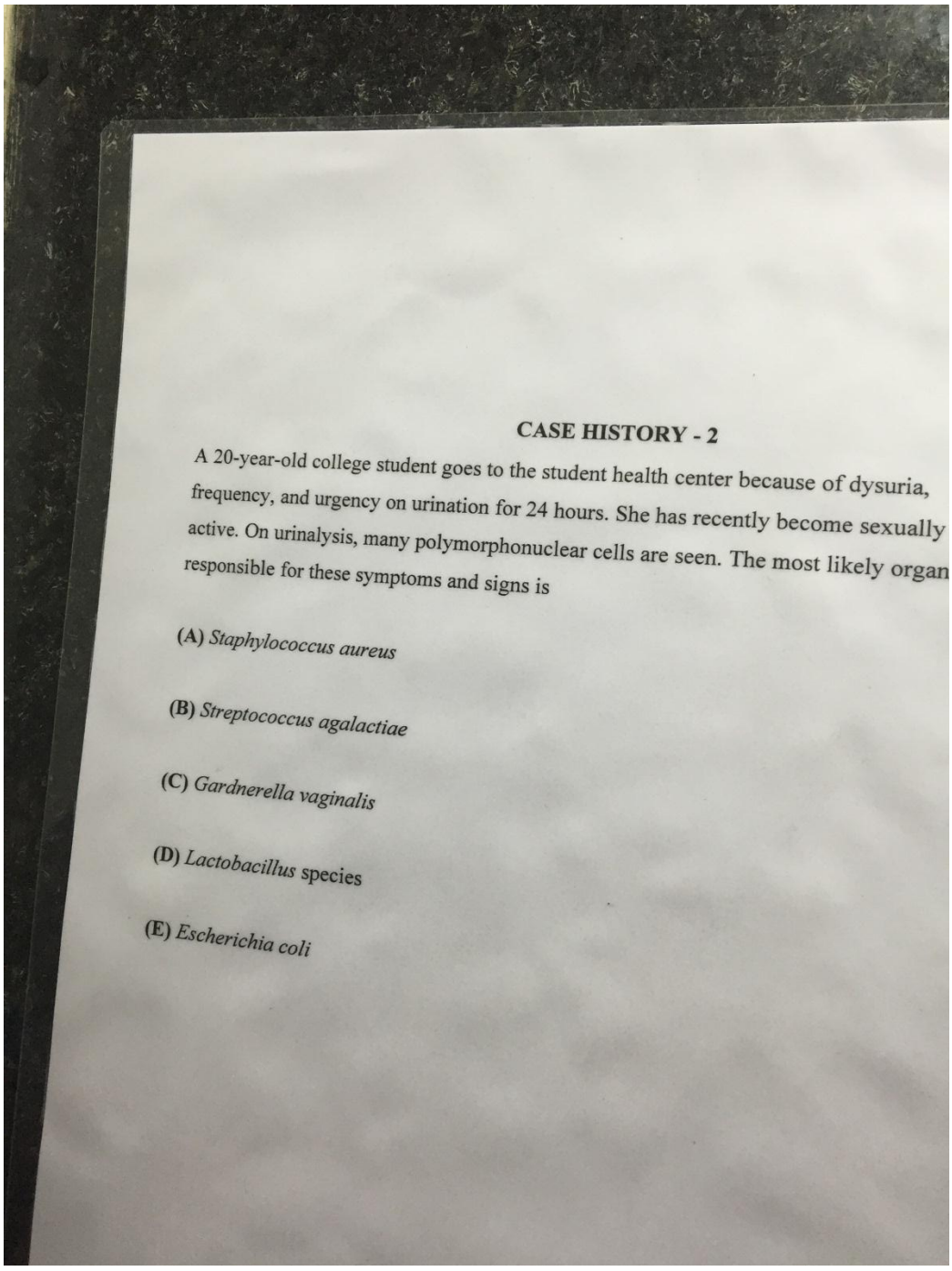
Antibiotic susceptibility testing.

Other tests: Agglutination with mono and polyvalent antisera to detect EPEC, ETECetc

Clinical Significance: Urinary tract Infections, Pyogenic infections, Septicemia, neonatal Meningitis

Diarrhea (Enteropathogenic, Enterotoxigenic, Entero hemorrhagic, Enteroinvasive, enteroaggregativ

Extended Spectrum Beta Lactamses (ESBL) producing isolates are resistance to 3rd generation cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, Cefpodoxime) and Monobactams (Aztreonar These ESBLs are of clinical concern because they restrict therapeutic options causing treatment failures.



CASE HISTORY - 2

A 20-year-old college student goes to the student health center because of dysuria, frequency, and urgency on urination for 24 hours. She has recently become sexually active. On urinalysis, many polymorphonuclear cells are seen. The most likely organ responsible for these symptoms and signs is

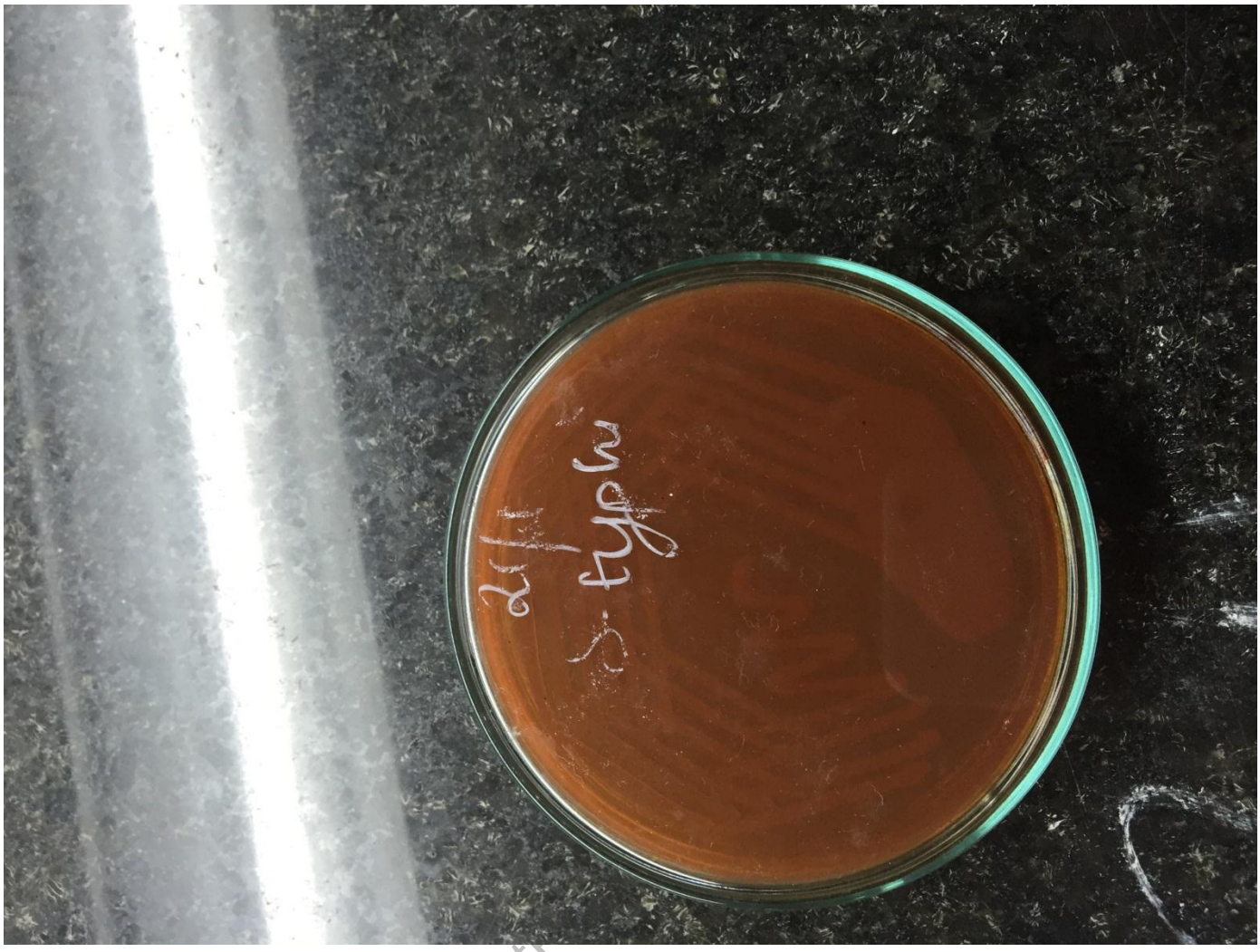
(A) *Staphylococcus aureus*

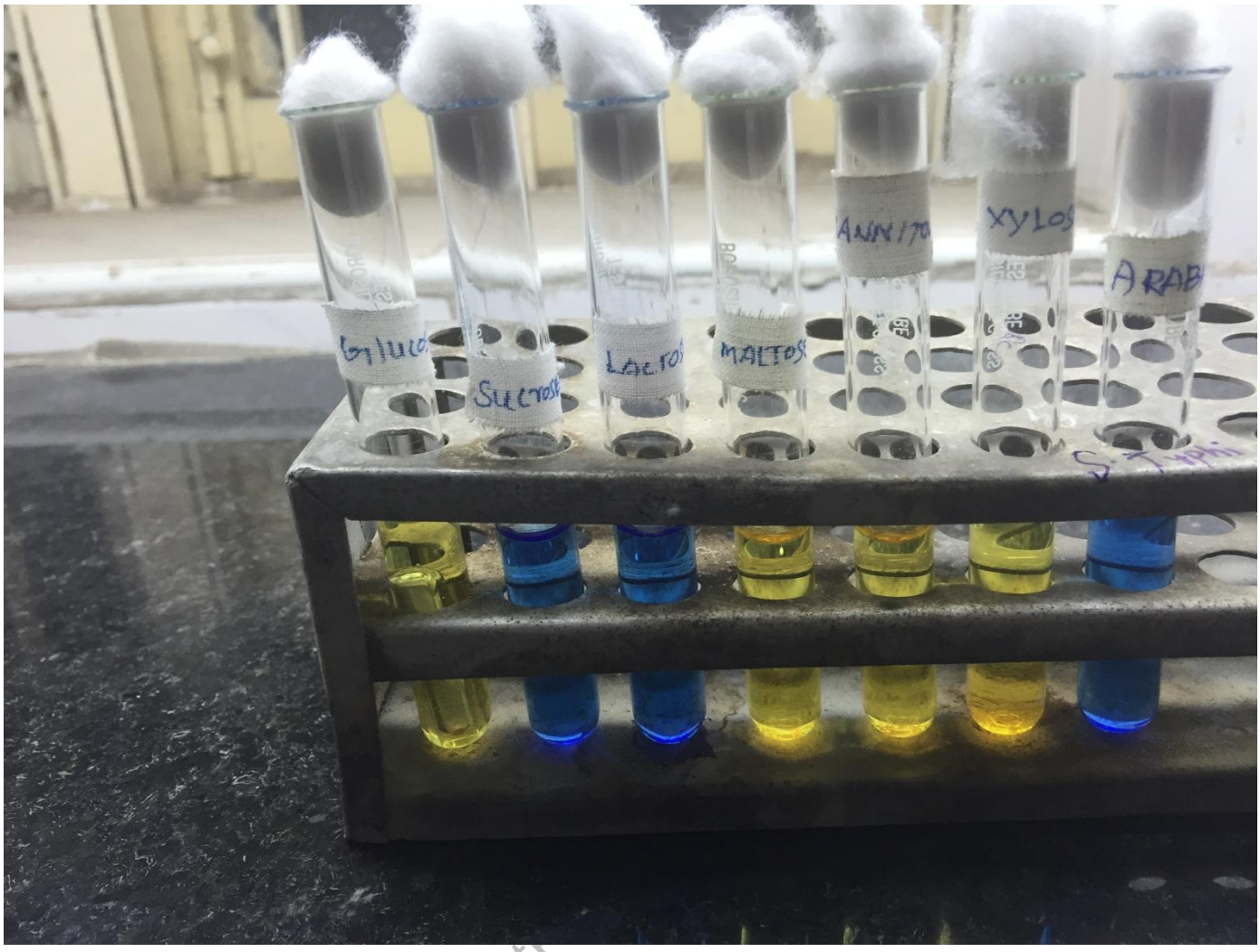
(B) *Streptococcus agalactiae*

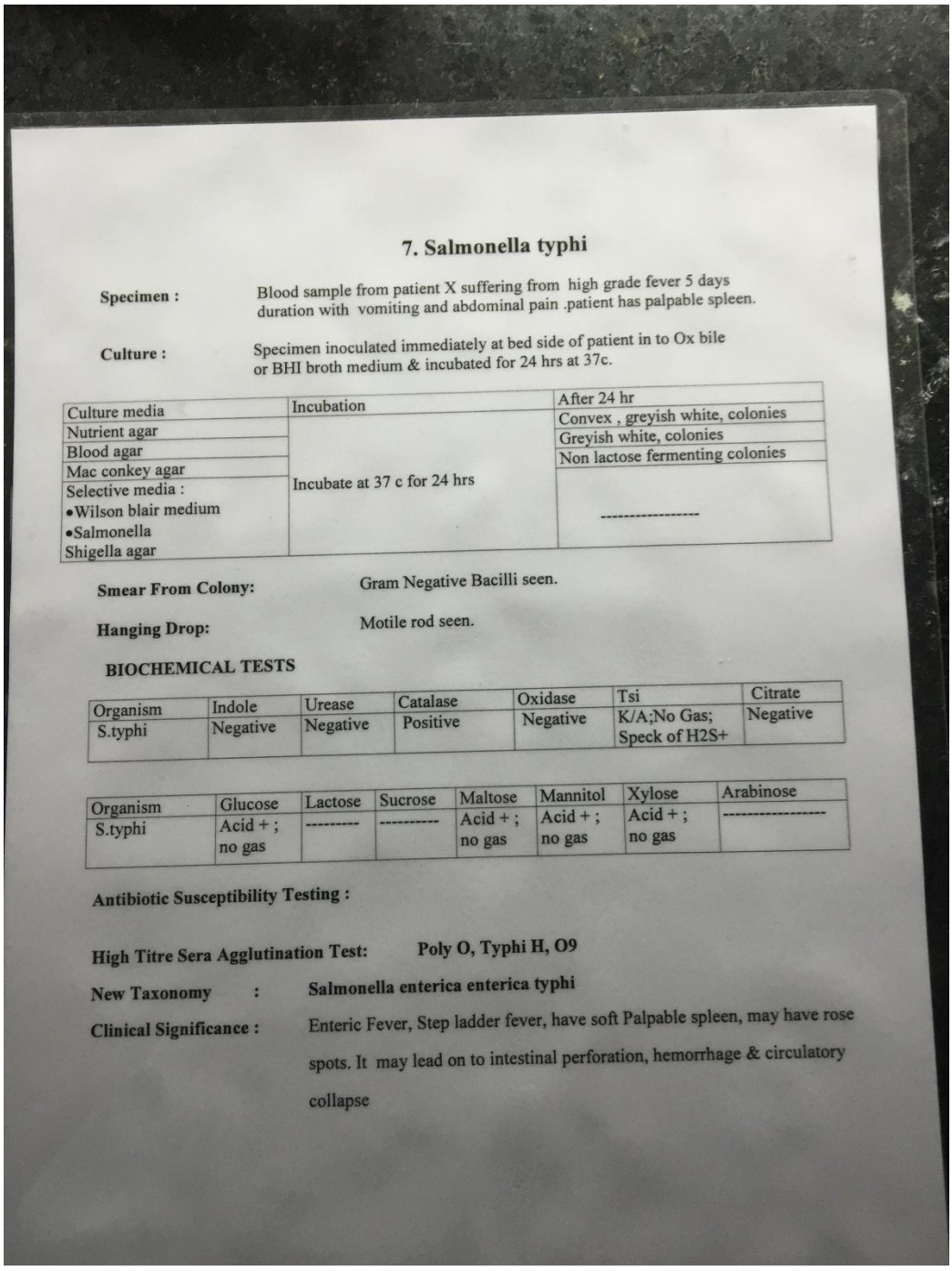
(C) *Gardnerella vaginalis*

(D) *Lactobacillus* species

(E) *Escherichia coli*







7. Salmonella typhi

Specimen : Blood sample from patient X suffering from high grade fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : Specimen inoculated immediately at bed side of patient in to Ox bile or BHI broth medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli seen.

Hanging Drop: Motile rod seen.

BIOCHEMICAL TESTS

Organism	Indole	Urease	Catalase	Oxidase	Tsi	Citrate
S.typhi	Negative	Negative	Positive	Negative	K/A;No Gas; Speck of H2S+	Negative

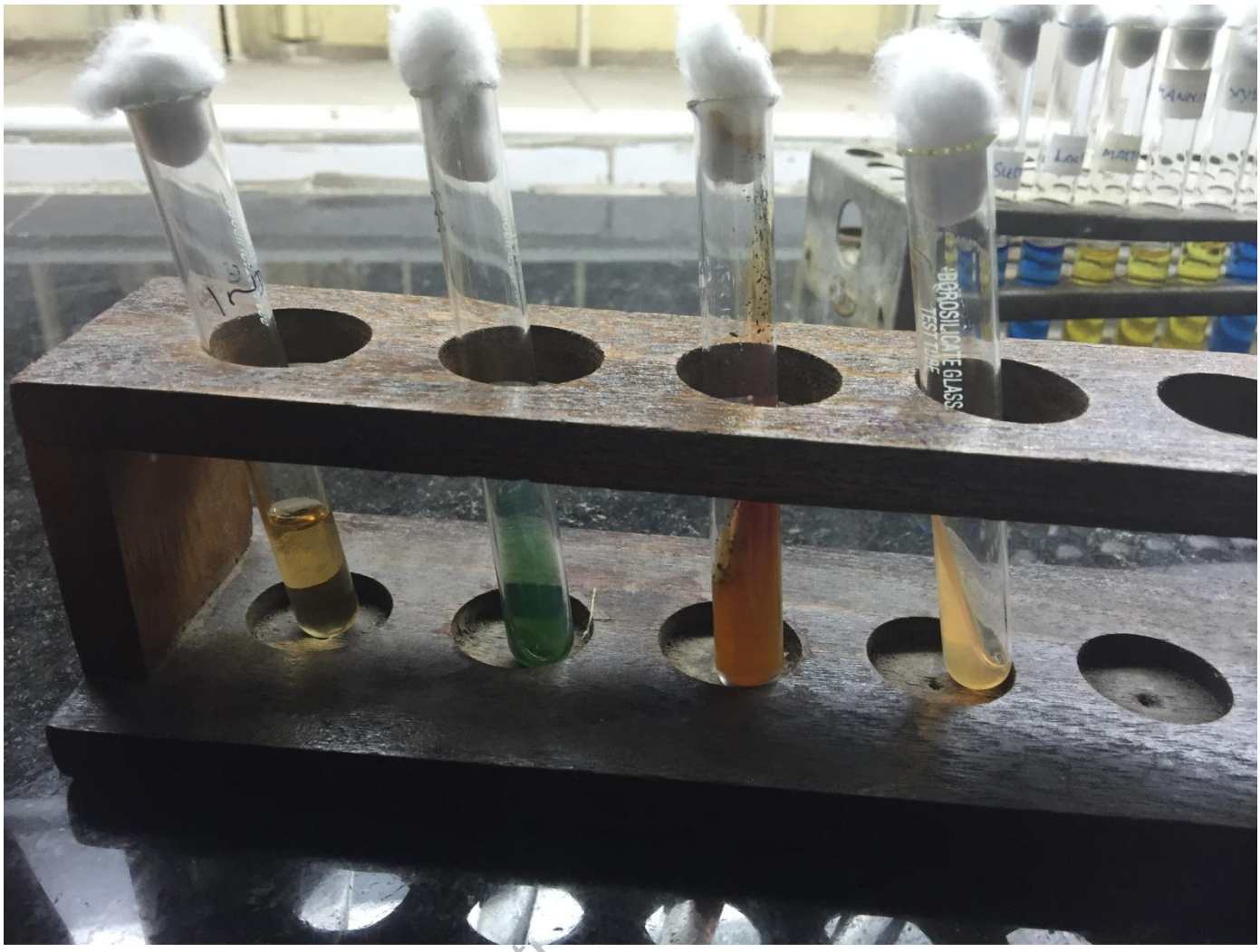
Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.typhi	Acid + ; no gas	---------	----------	Acid + ; no gas	Acid + ; no gas	Acid + ; no gas	----------

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: **Poly O, Typhi H, O9**

New Taxonomy : **Salmonella enterica enterica typhi**

Clinical Significance : Enteric Fever, Step ladder fever, have soft Palpable spleen, may have rose spots. It may lead on to intestinal perforation, hemorrhage & circulatory collapse



CASE HISTORY - 7

A 27-year-old woman is admitted to the hospital because of fever, with increasing anorexia, headache, weakness, and altered mental status of 2 days' duration. She works for an airline as a cabin attendant, flying between the Indian subcontinent and other places in Southeast Asia and the West Coast of the United States. Ten days prior to admission she had a diarrheal illness that lasted for about 36 hours. She has been constipated for the last 3 days. Her temperature is 39 °C, heart rate 68/min, blood pressure 120/80 mm Hg, and respirations 18/ min. She knows who she is and where she is but does not know the date. She is picking at the bedclothes. Rose spots are seen on the trunk. The remainder of the physical examination is normal. Blood cultures are done and an intravenous line is placed. The most likely cause of her illness is

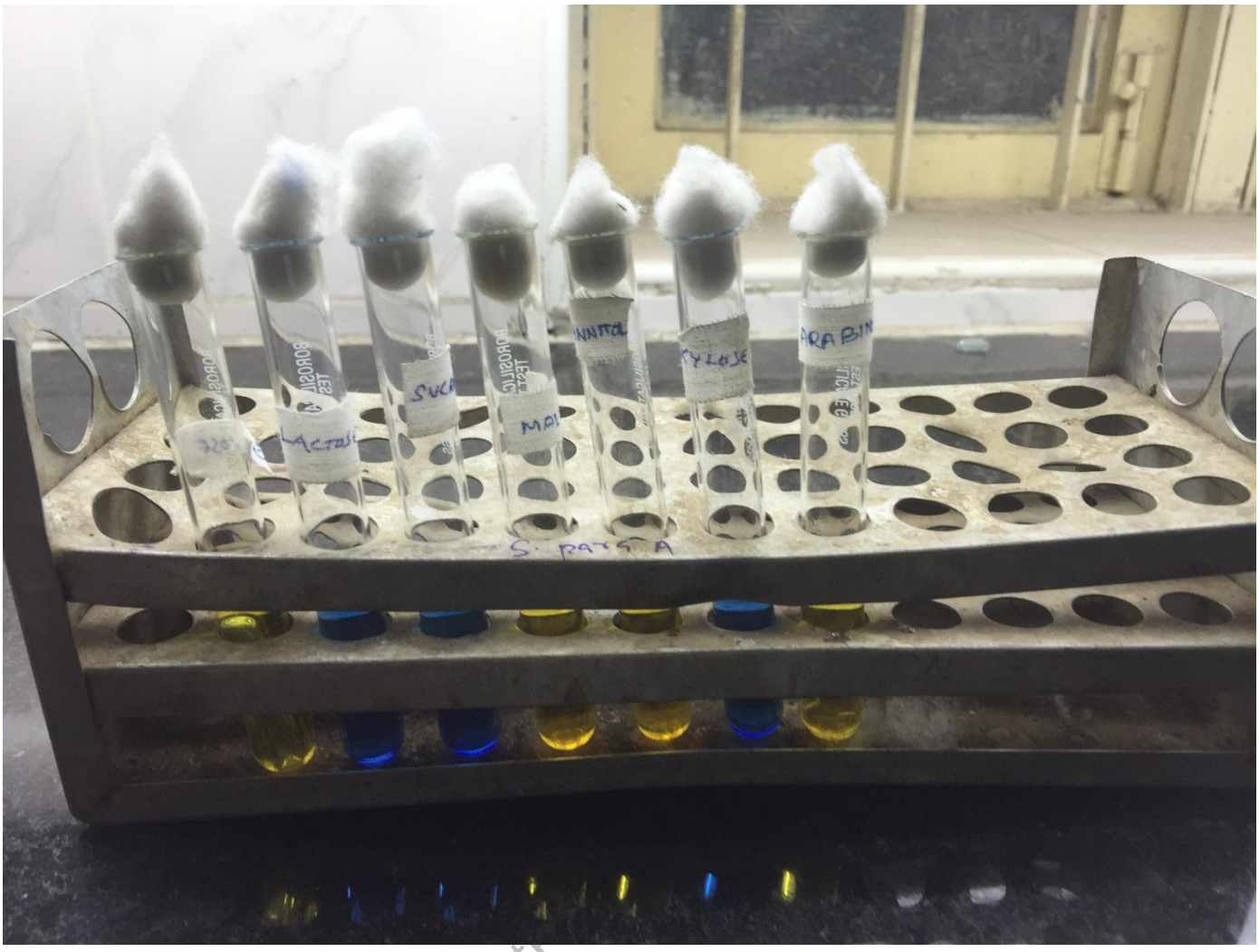
(A) Enterotoxigenic *Escherichia coli (ETEC)*

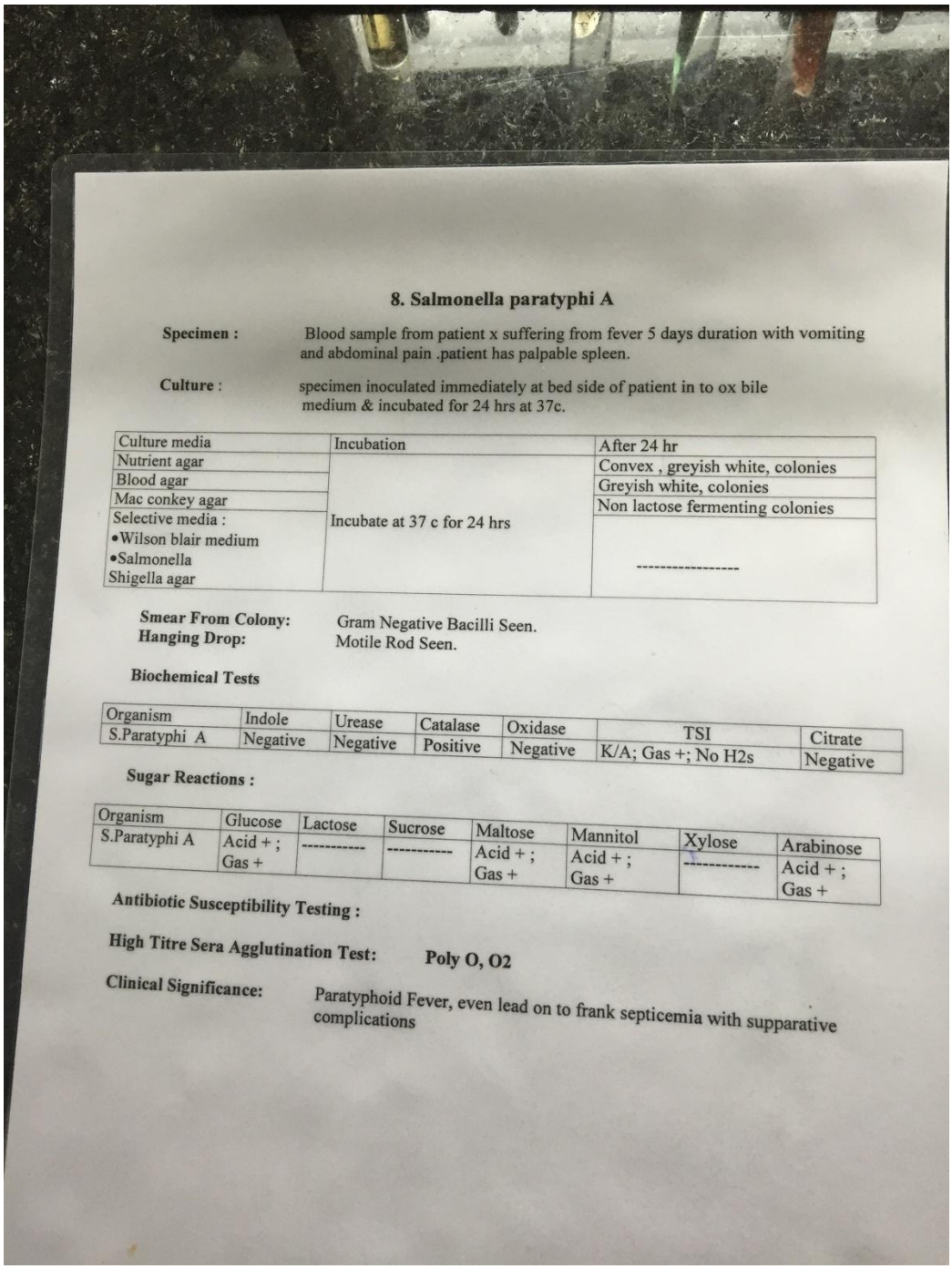
(B) *Shigella sonnei*

(C) *Salmonella enterica* subspecies *enterica* serotype Typhimurium (*Salmonella* Typhimurium)

(D) Salmonella *enterica* subspecies *enteric* serotype Typhi (*Salmonella* Typhi)

(E) Enteroinvasive *Escherichia coli (EIEC)*





8. Salmonella paratyphi A

Specimen : Blood sample from patient x suffering from fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : specimen inoculated immediately at bed side of patient in to ox bile medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli Seen.
Hanging Drop: Motile Rod Seen.

Biochemical Tests

Organism	Indole	Urease	Catalase	Oxidase	TSI	Citrate
S.Paratyphi A	Negative	Negative	Positive	Negative	K/A; Gas +; No H2s	Negative

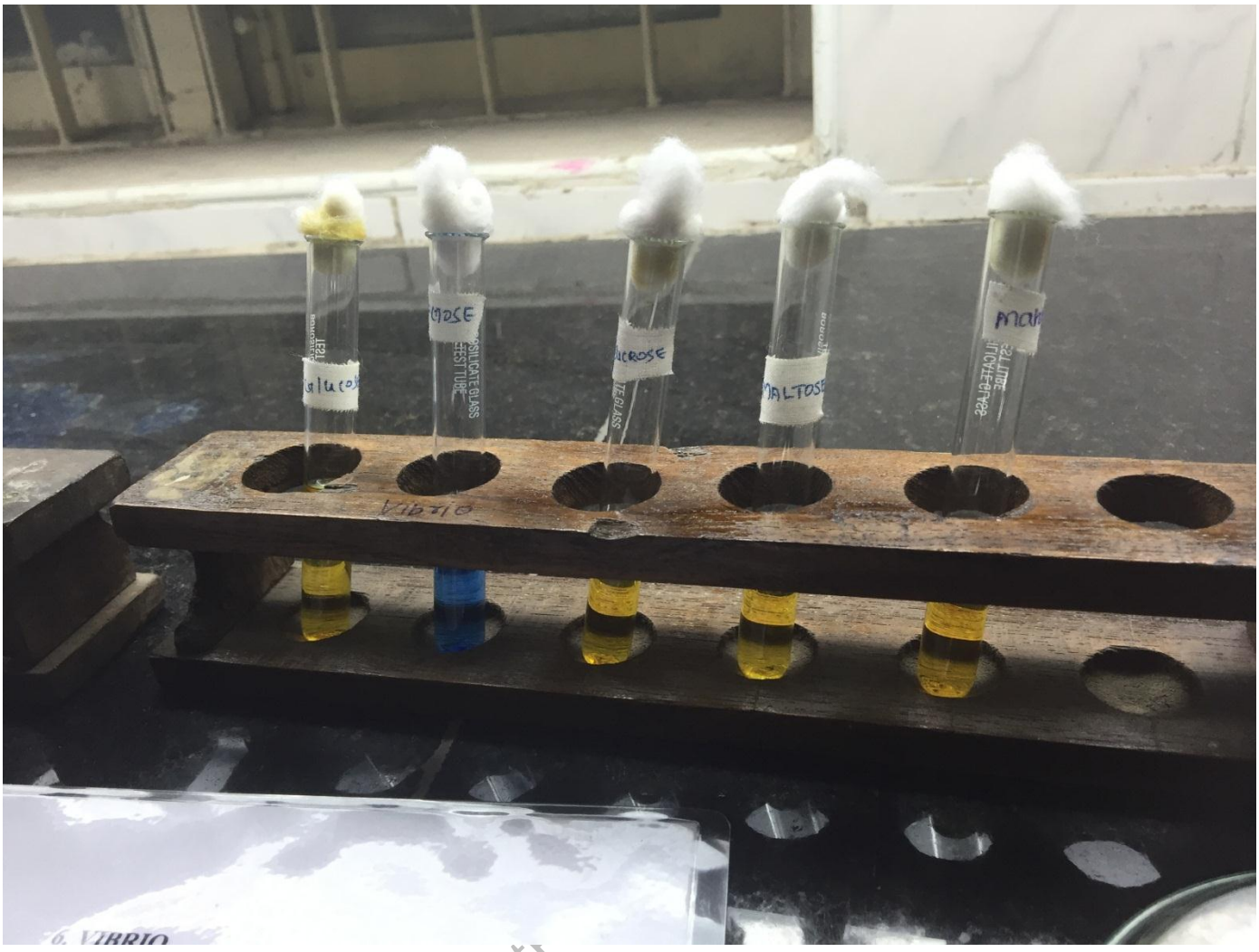
Sugar Reactions :

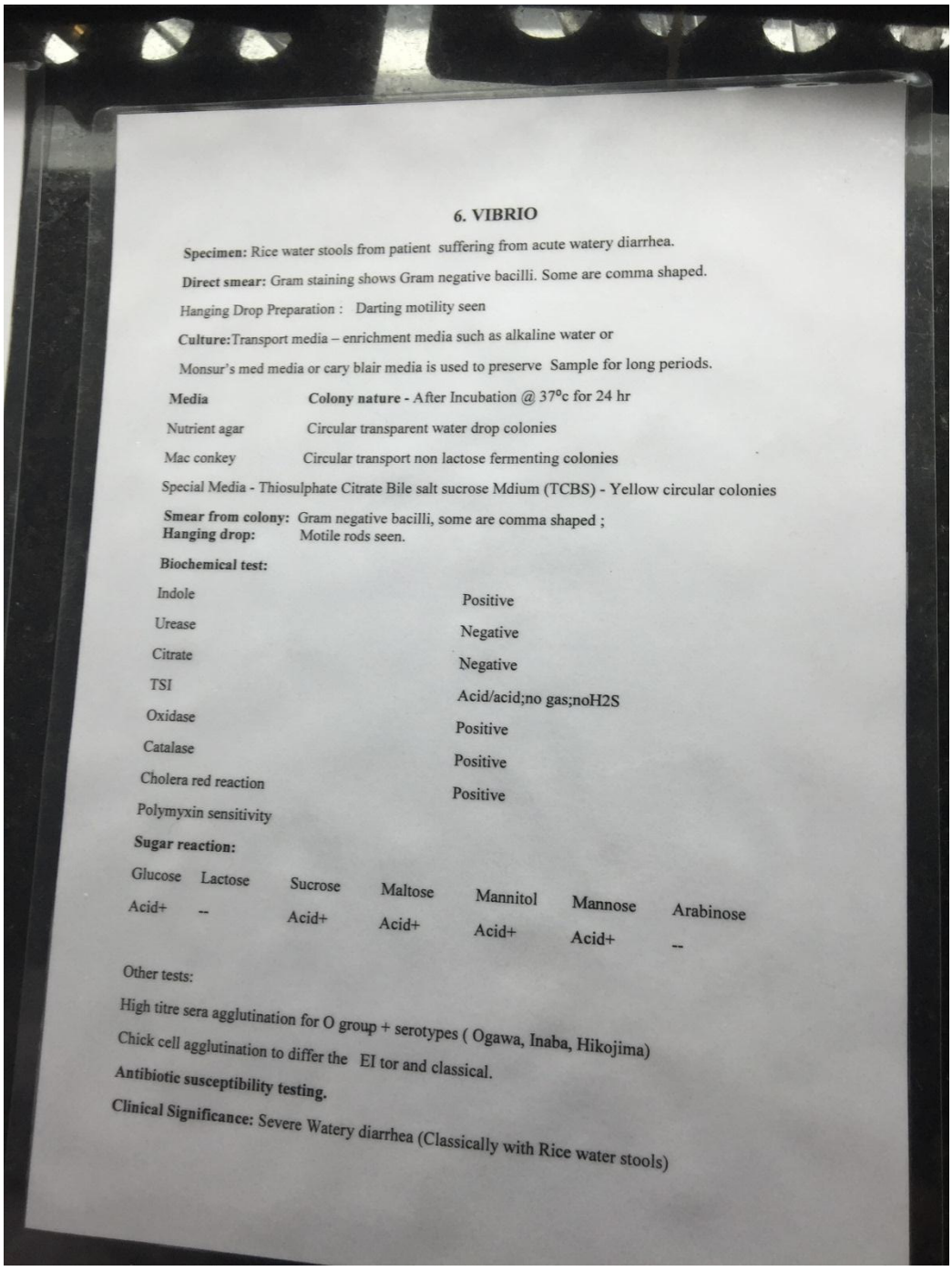
Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.Paratyphi A	Acid + ; Gas +	----------	----------	Acid + ; Gas +	Acid + ; Gas +	----------	Acid + ; Gas +

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: **Poly O, O2**

Clinical Significance: Paratyphoid Fever, even lead on to frank septicemia with supparative complications





6. VIBRIO

Specimen: Rice water stools from patient suffering from acute watery diarrhea.

Direct smear: Gram staining shows Gram negative bacilli. Some are comma shaped.

Hanging Drop Preparation : Darting motility seen

Culture: Transport media – enrichment media such as alkaline water or

Monsur's med media or cary blair media is used to preserve Sample for long periods.

Media	**Colony nature** - After Incubation @ 37°c for 24 hr
Nutrient agar	Circular transparent water drop colonies
Mac conkey	Circular transport non lactose fermenting colonies

Special Media - Thiosulphate Citrate Bile salt sucrose Mdium (TCBS) - Yellow circular colonies

Smear from colony: Gram negative bacilli, some are comma shaped ;
Hanging drop: Motile rods seen.

Biochemical test:

Indole	Positive
Urease	Negative
Citrate	Negative
TSI	Acid/acid;no gas;noH2S
Oxidase	Positive
Catalase	Positive
Cholera red reaction	Positive
Polymyxin sensitivity	

Sugar reaction:

Glucose	Lactose	Sucrose	Maltose	Mannitol	Mannose	Arabinose
Acid+	--	Acid+	Acid+	Acid+	Acid+	--

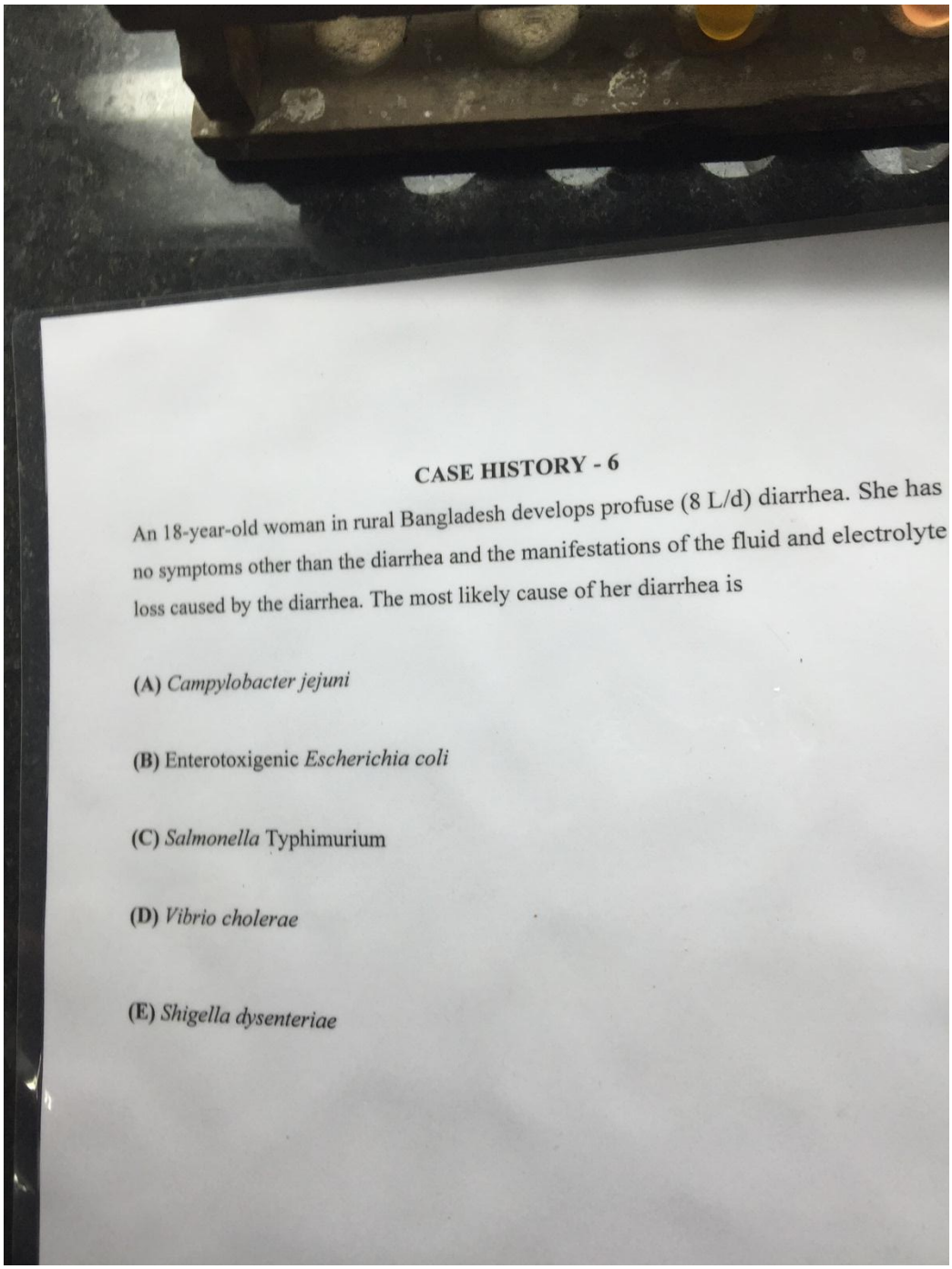
Other tests:

High titre sera agglutination for O group + serotypes (Ogawa, Inaba, Hikojima)

Chick cell agglutination to differ the EI tor and classical.

Antibiotic susceptibility testing.

Clinical Significance: Severe Watery diarrhea (Classically with Rice water stools)



CASE HISTORY - 6

An 18-year-old woman in rural Bangladesh develops profuse (8 L/d) diarrhea. She has no symptoms other than the diarrhea and the manifestations of the fluid and electrolyte loss caused by the diarrhea. The most likely cause of her diarrhea is

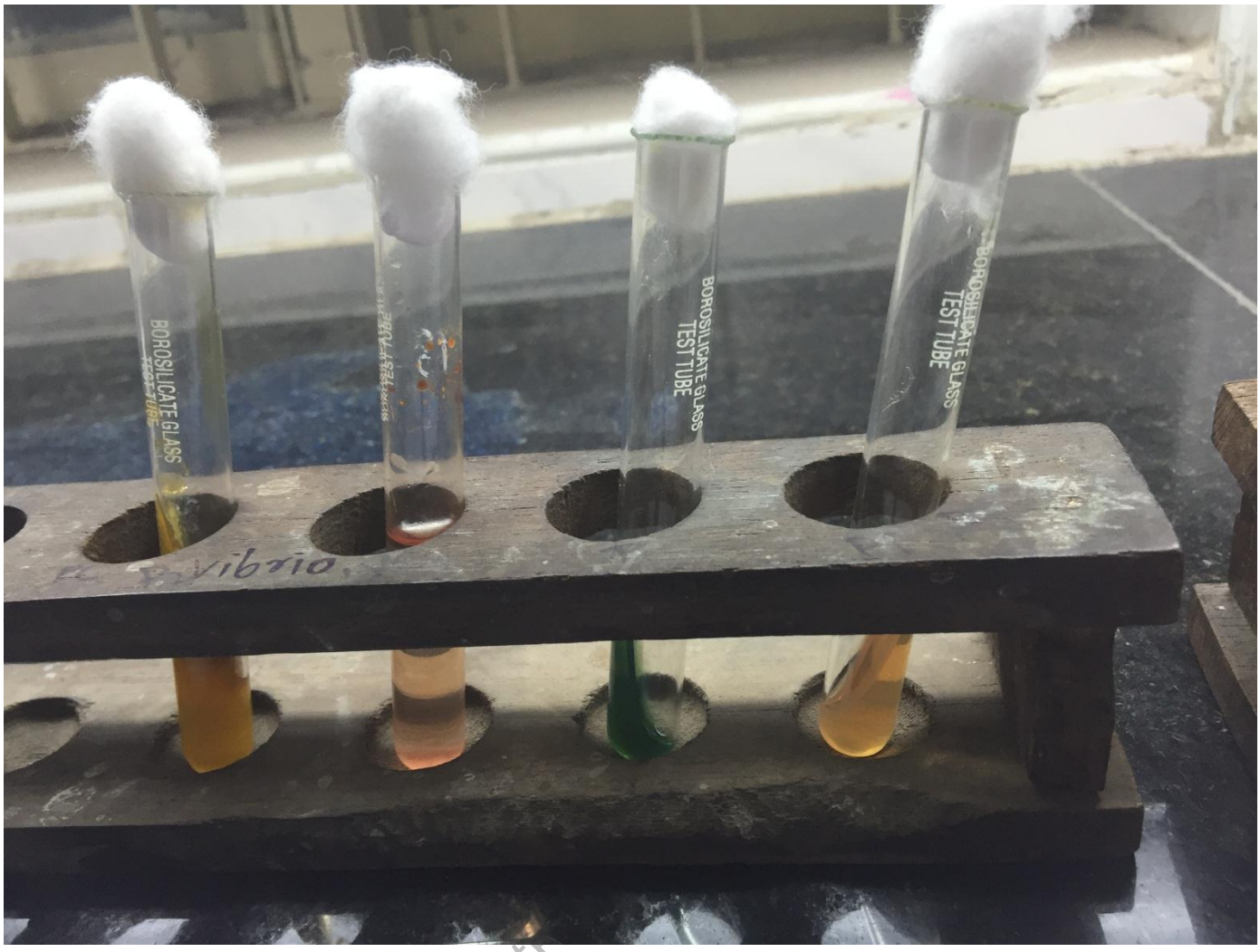
(A) *Campylobacter jejuni*

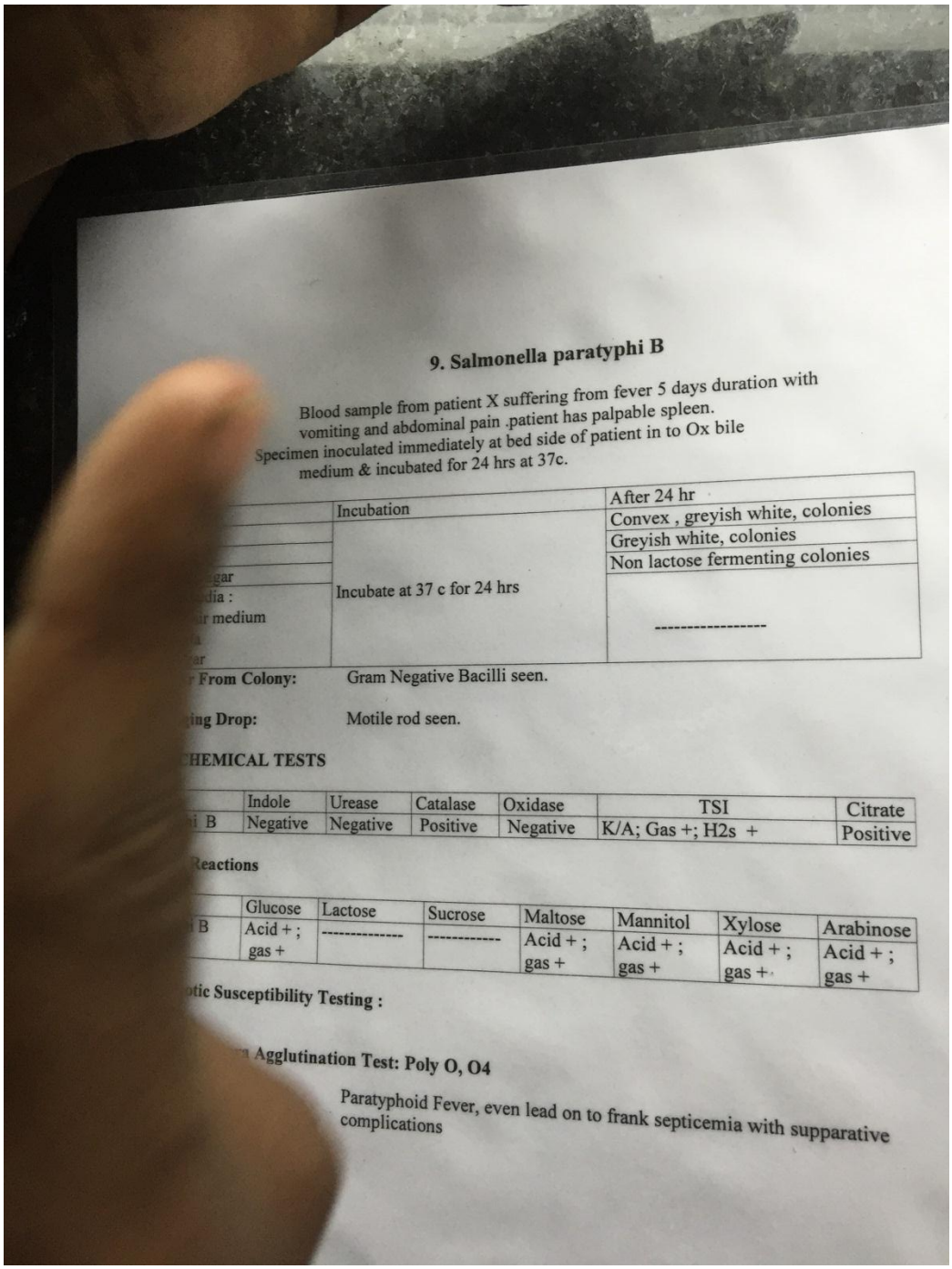
(B) Enterotoxigenic *Escherichia coli*

(C) *Salmonella* Typhimurium

(D) *Vibrio cholerae*

(E) *Shigella dysenteriae*





9. Salmonella paratyphi B

Blood sample from patient X suffering from fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.
Specimen inoculated immediately at bed side of patient in to Ox bile medium & incubated for 24 hrs at 37c.

	Incubation	After 24 hr
	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
		Greyish white, colonies
gar		Non lactose fermenting colonies
dia : r medium		----------

From Colony: Gram Negative Bacilli seen.

ing Drop: Motile rod seen.

HEMICAL TESTS

	Indole	Urease	Catalase	Oxidase	TSI	Citrate
i B	Negative	Negative	Positive	Negative	K/A; Gas +; H2s +	Positive

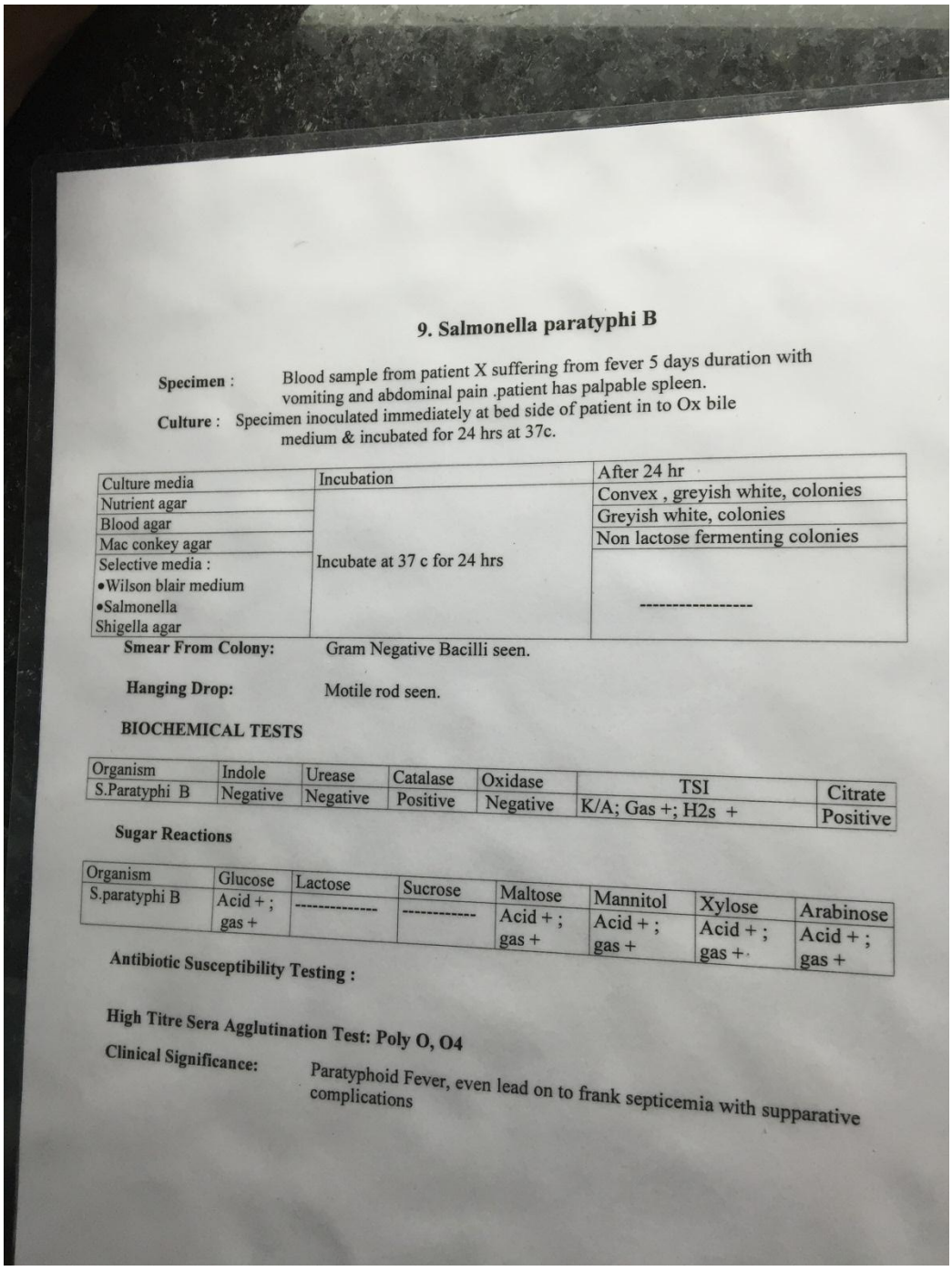
Reactions

	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
B	Acid + ; gas +	----------	----------	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +

otic Susceptibility Testing :

n Agglutination Test: Poly O, O4

Paratyphoid Fever, even lead on to frank septicemia with supparative complications



9. Salmonella paratyphi B

Specimen : Blood sample from patient X suffering from fever 5 days duration with vomiting and abdominal pain .patient has palpable spleen.

Culture : Specimen inoculated immediately at bed side of patient in to Ox bile medium & incubated for 24 hrs at 37c.

Culture media	Incubation	After 24 hr
Nutrient agar	Incubate at 37 c for 24 hrs	Convex , greyish white, colonies
Blood agar		Greyish white, colonies
Mac conkey agar		Non lactose fermenting colonies
Selective media : •Wilson blair medium •Salmonella Shigella agar		----------

Smear From Colony: Gram Negative Bacilli seen.

Hanging Drop: Motile rod seen.

BIOCHEMICAL TESTS

Organism	Indole	Urease	Catalase	Oxidase	TSI	Citrate
S.Paratyphi B	Negative	Negative	Positive	Negative	K/A; Gas +; H2s +	Positive

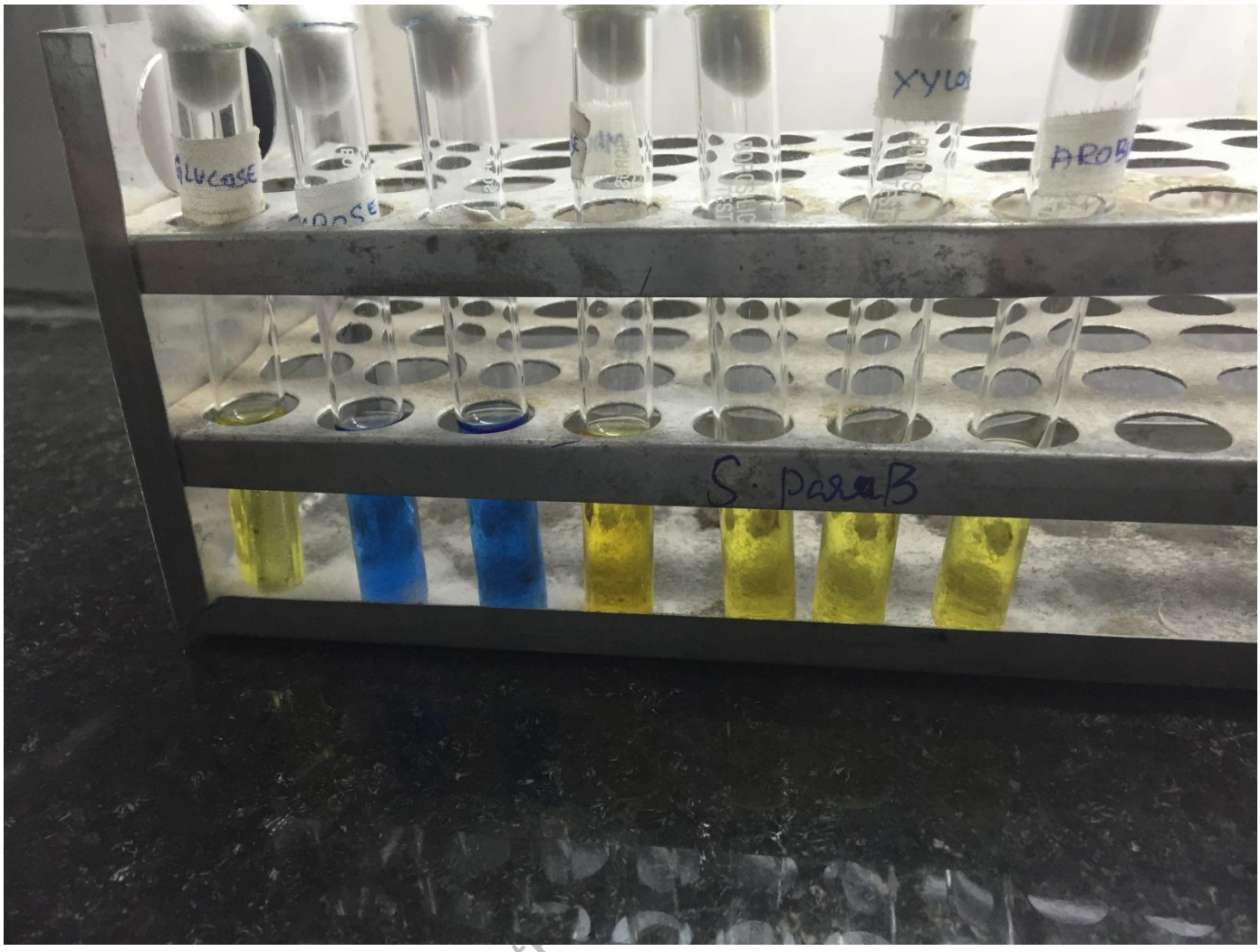
Sugar Reactions

Organism	Glucose	Lactose	Sucrose	Maltose	Mannitol	Xylose	Arabinose
S.paratyphi B	Acid + ; gas +	----------	----------	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +	Acid + ; gas +

Antibiotic Susceptibility Testing :

High Titre Sera Agglutination Test: Poly O, O4

Clinical Significance: Paratyphoid Fever, even lead on to frank septicemia with supparative complications



4. PSEUDOMONAS

Specimen: Wound swab from patient X

Direct smear: Gram staining shows plenty of pus cells with Gram negative bacilli seen.

Culture: After Incubation @ 37°c for 24 hr

Culture media	Colony Characteristics
Nutrient agar	Opaque irregular colonies with earthy smell. Pseudomonas pyogenes produce green pigment
Blood agar plate	Opaque irregular colonies surrounded by zone of hemolysis.
Macconkey agar	Non lactose fermenting colonies

Smear from colony: Gram negative bacilli.

Hanging drop: Motile rods seen

Biochemical Tests:

Indole	Negative
Urease	Negative
Citrate	Positive
TSI	k/no change No gas/no H2S
Oxidase	Positve
Catalase	Positive

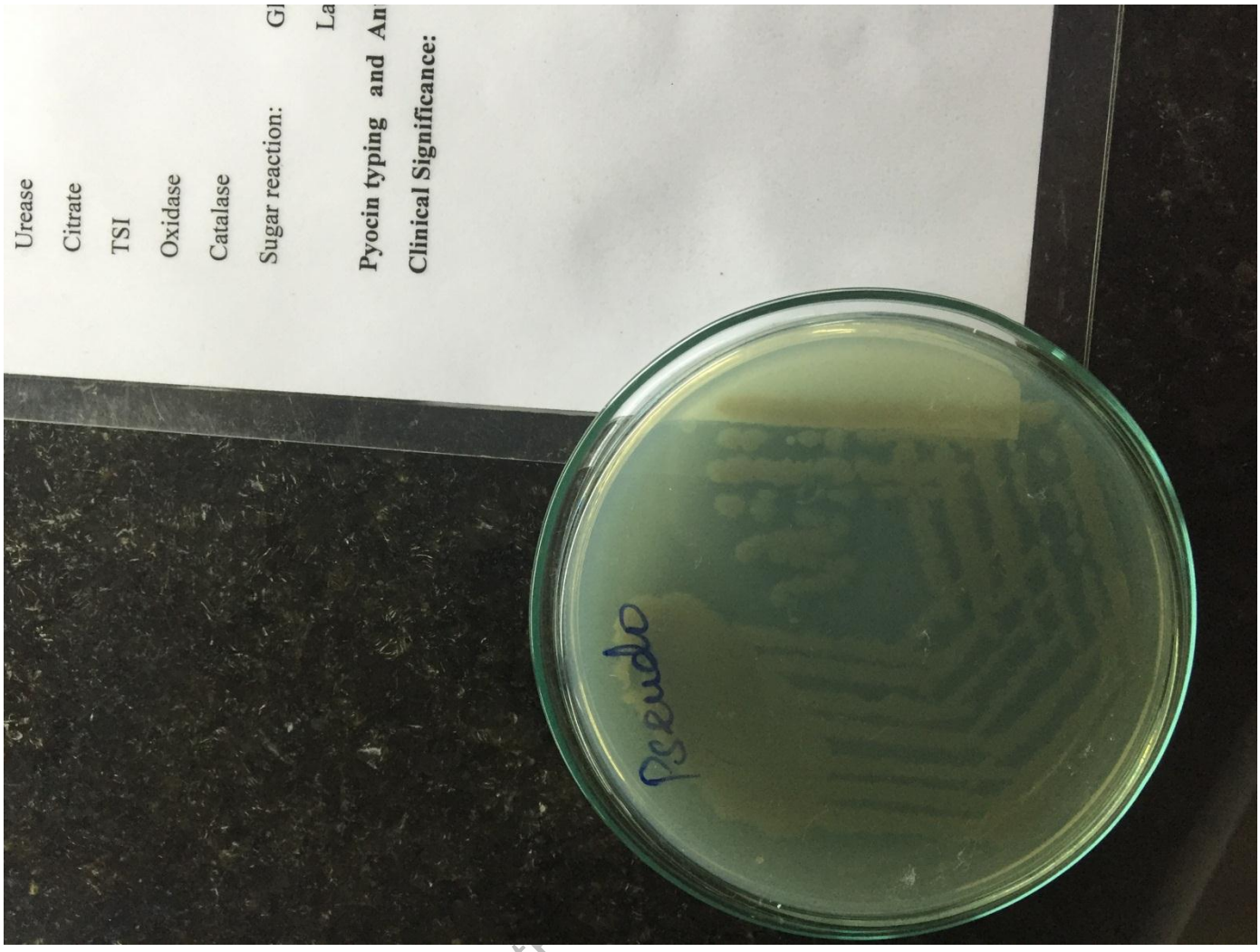
Sugar reaction: Glucose – is utilized oxidatively, form acid only;

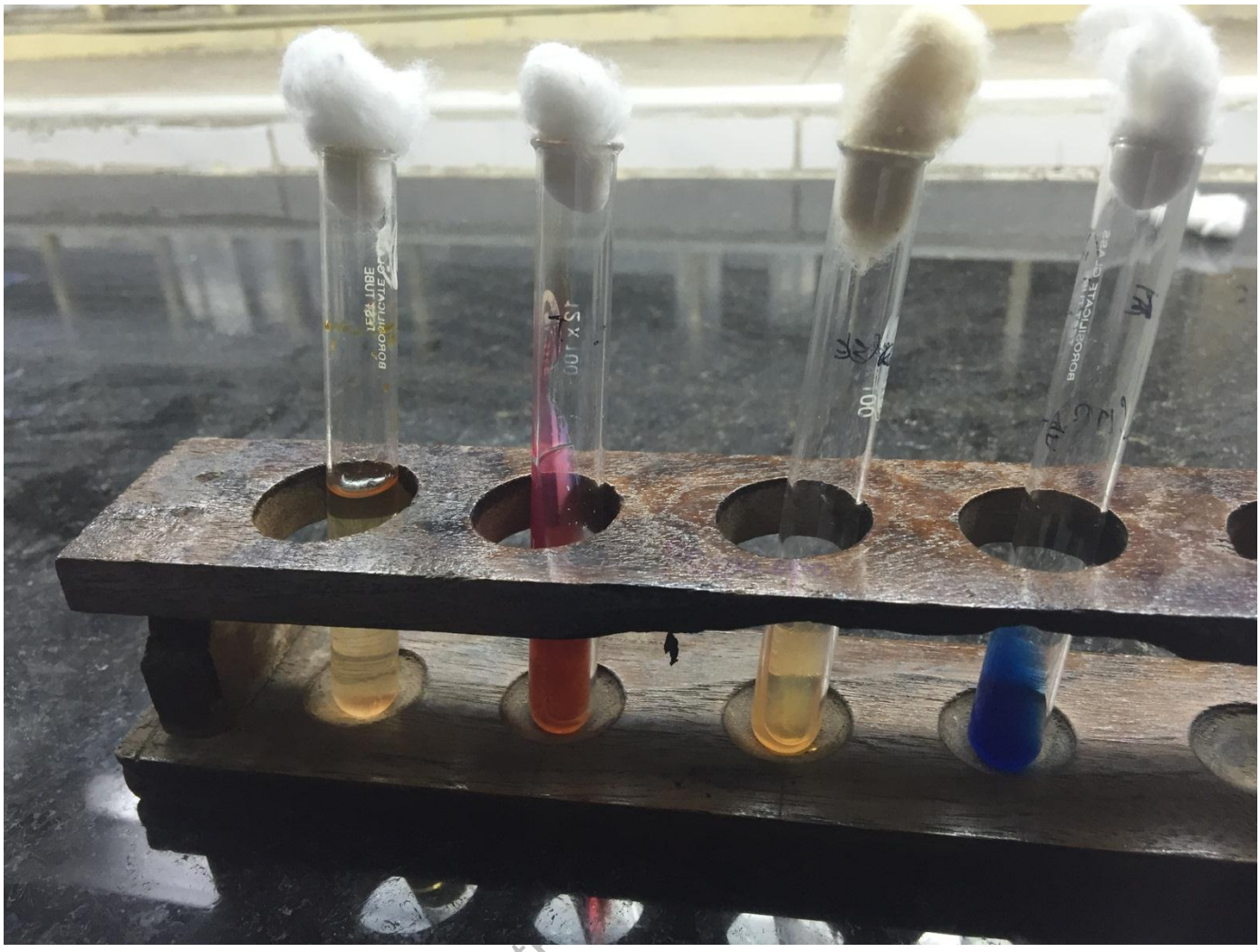
Lactose; sucrose; maltose; mannitol – not fermented

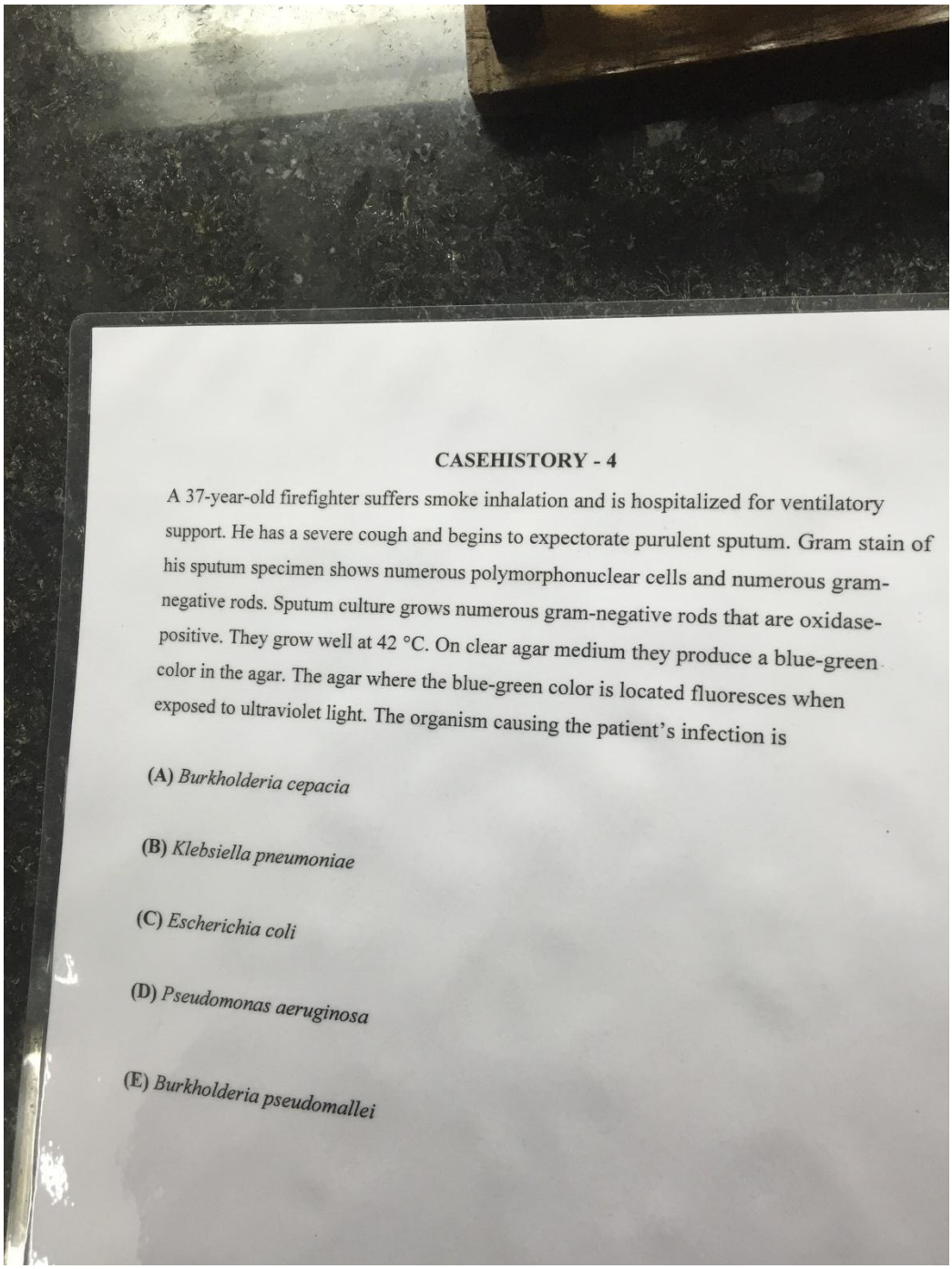
Pyocin typing and Antibiotic susceptibility testing.

Clinical Significance: Pneumonia (Cystic fibrosis patient, Immunocompromised), Burns woun infection, bed sore infection, Skin and soft tissue infection, Urinary tract infection, Malignant otitis externa, Corneal ulcer for contact lens wearer or following trauma, Endocarditis for iv drug users, Septicemia.

Most common cause for nosocomial infection







CASEHISTORY - 4

A 37-year-old firefighter suffers smoke inhalation and is hospitalized for ventilatory support. He has a severe cough and begins to expectorate purulent sputum. Gram stain of his sputum specimen shows numerous polymorphonuclear cells and numerous gram-negative rods. Sputum culture grows numerous gram-negative rods that are oxidase-positive. They grow well at 42 °C. On clear agar medium they produce a blue-green color in the agar. The agar where the blue-green color is located fluoresces when exposed to ultraviolet light. The organism causing the patient's infection is

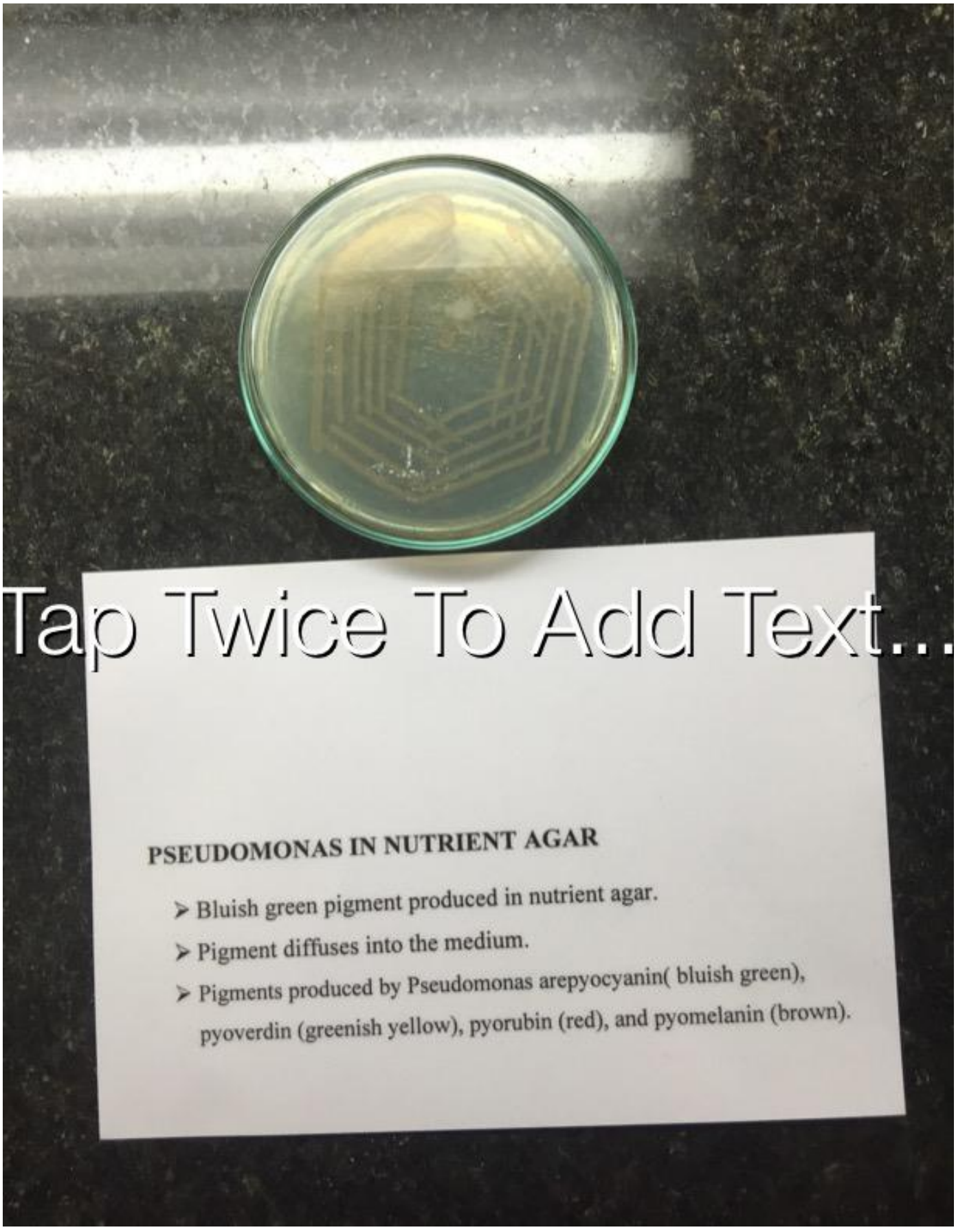
(A) *Burkholderia cepacia*

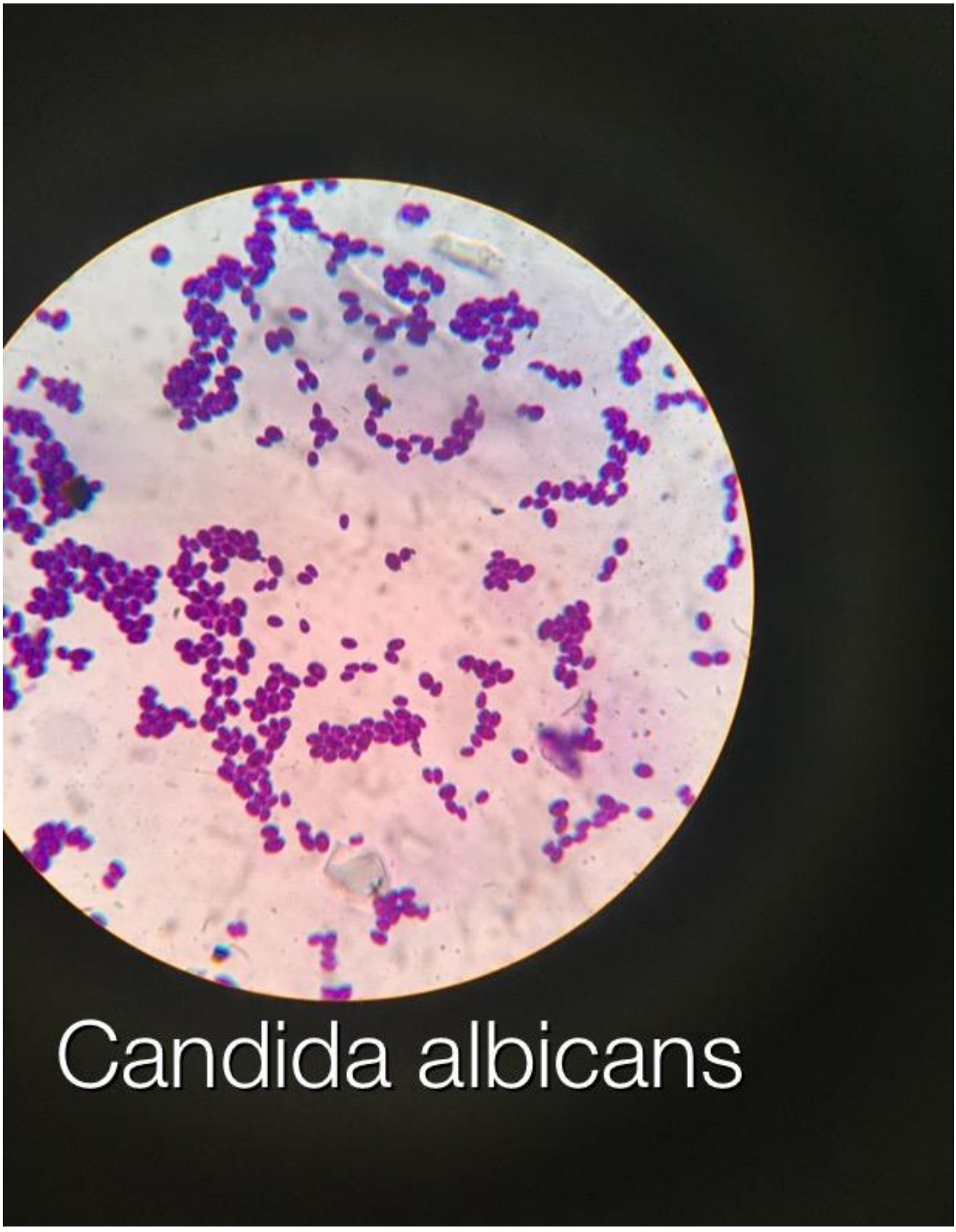
(B) *Klebsiella pneumoniae*

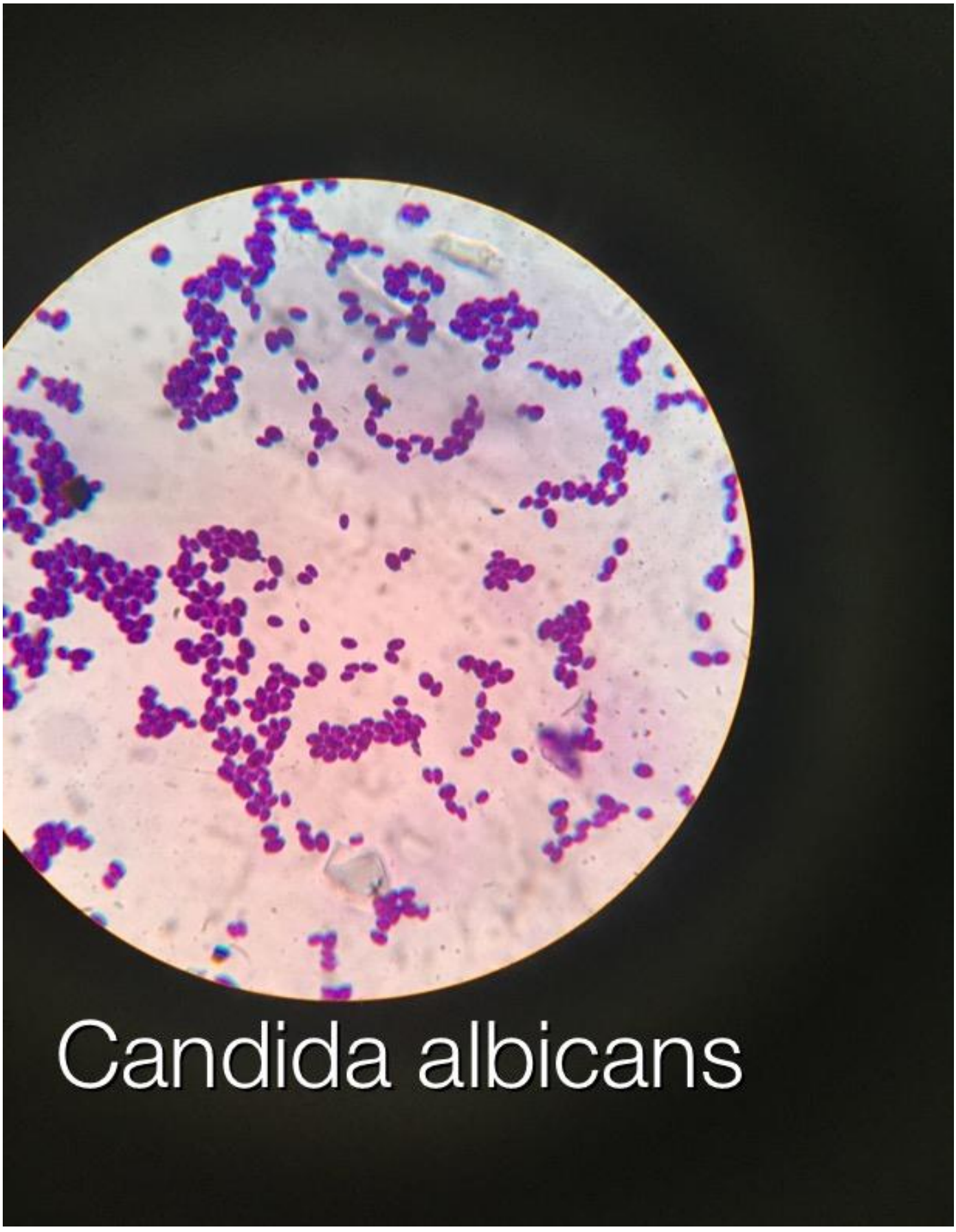
(C) *Escherichia coli*

(D) *Pseudomonas aeruginosa*

(E) *Burkholderia pseudomallei*







ASPERGILLUS NIGER

- ➤ It has hyaline septate hyphae.
- ➤ Phialides arebiseriate covering entire vesicle, form radial head.
- ➤ Conidiophores are wide hyaline changes into brown tint .
- ➤ Vesicles are spherical.
- ➤ Conidia are black in colour.

Aspergillus niger

Macroscopic : Coarse black granules present against creamy colony

Reverse white to yellow.

Microscopic:

- Broa[illegible] hyaline septate hyph[illegible]
- Co[illegible] ar[illegible]de with a [illegible]own[illegible] in upper [illegible]
- [illegible] are sp[illegible]
- [illegible]ali[illegible]re [illegible]iate
- The conidia are globose and are jet black

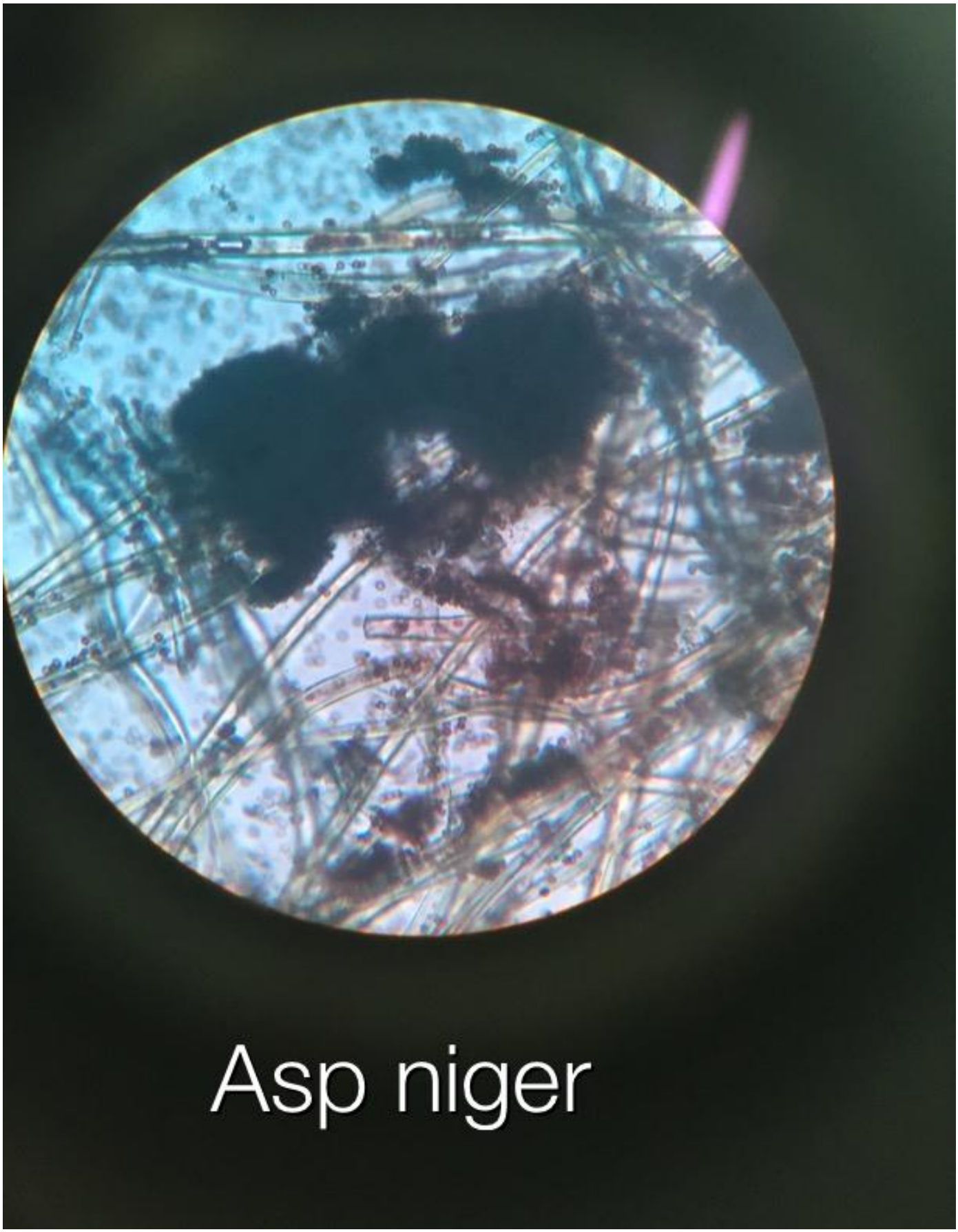
Clinical Significance:

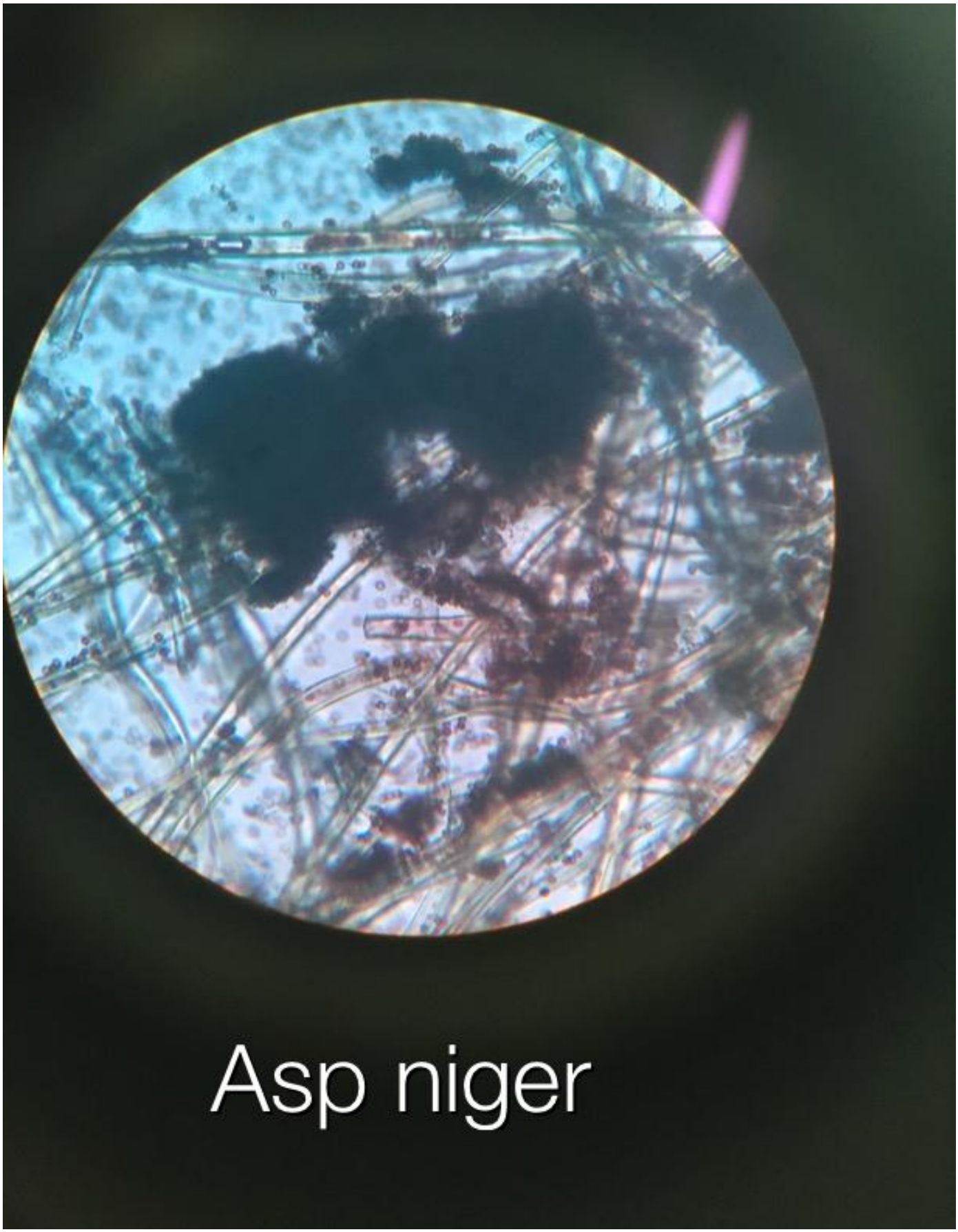
It causes aspergillus infection affecting otitis externa, (Swimmer's Ear) , a chronic local inflammation which is characterized by itching, pain, scaling.

Culture of *Aspergillus niger*. *Obverse* ***Reverse***

a

Note: Conidial head of *A. niger*. conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.





MUCOR

- It has non septate hyphae.
- Sphorangiophores are sympodially branched.
- Sporangia are globose and brown to grey incolour and columella are ovoid and containsporangiospores.
- Rhizoids are absent.

Mucor sp.

Macroscopic: Colonies are very fast growing, cottony to fluffy, white, becoming dark-grey, with the development of sporangia.

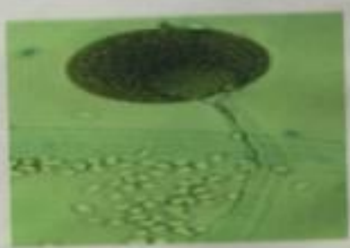
Microscopic:

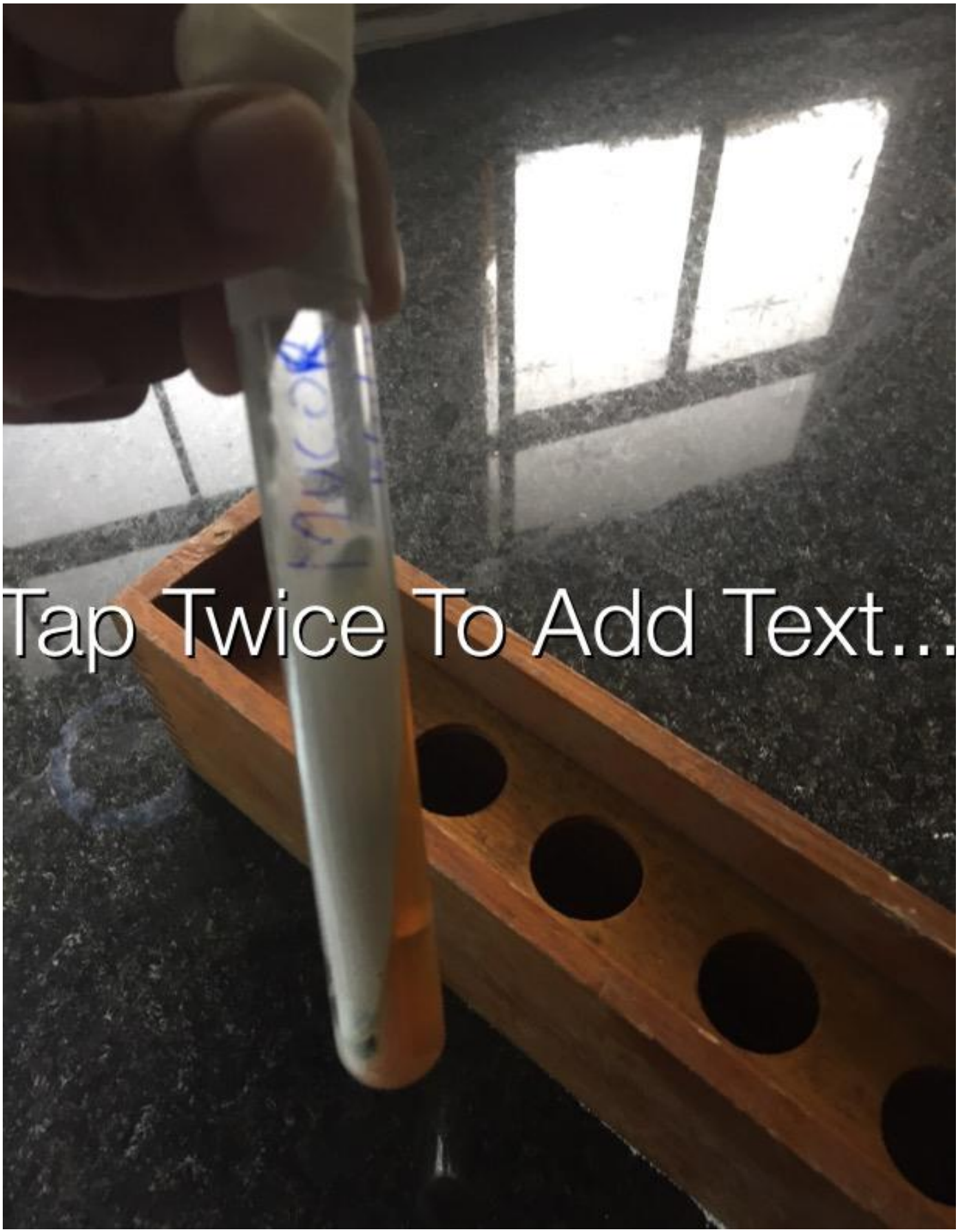
- Broad ,irregular, aseptate hyaline hyphae seen.
- The sporan[illegible] are long, straigh[illegible] with irregular bra[illegible]ing.
- Sp[illegible] enla[illegible]tal end [illegible]ulla[illegible]
- Br[illegible] with colla[illegible] su[illegible]ortin[illegible] spora[illegible] are [illegible] with sporangiospores
- The sporangia are globose
- No rhizoids are seen

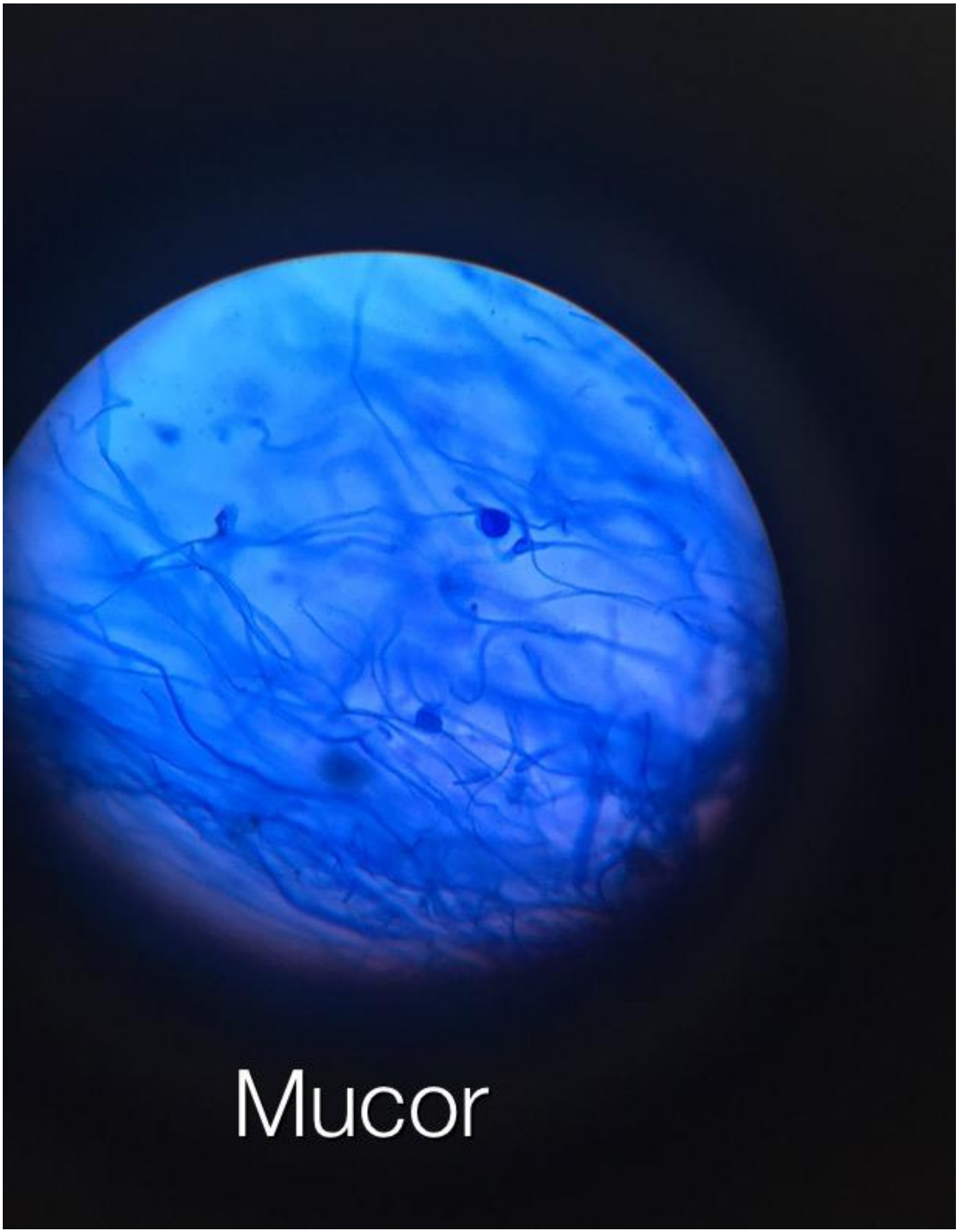
Tap Twice To Add Text...

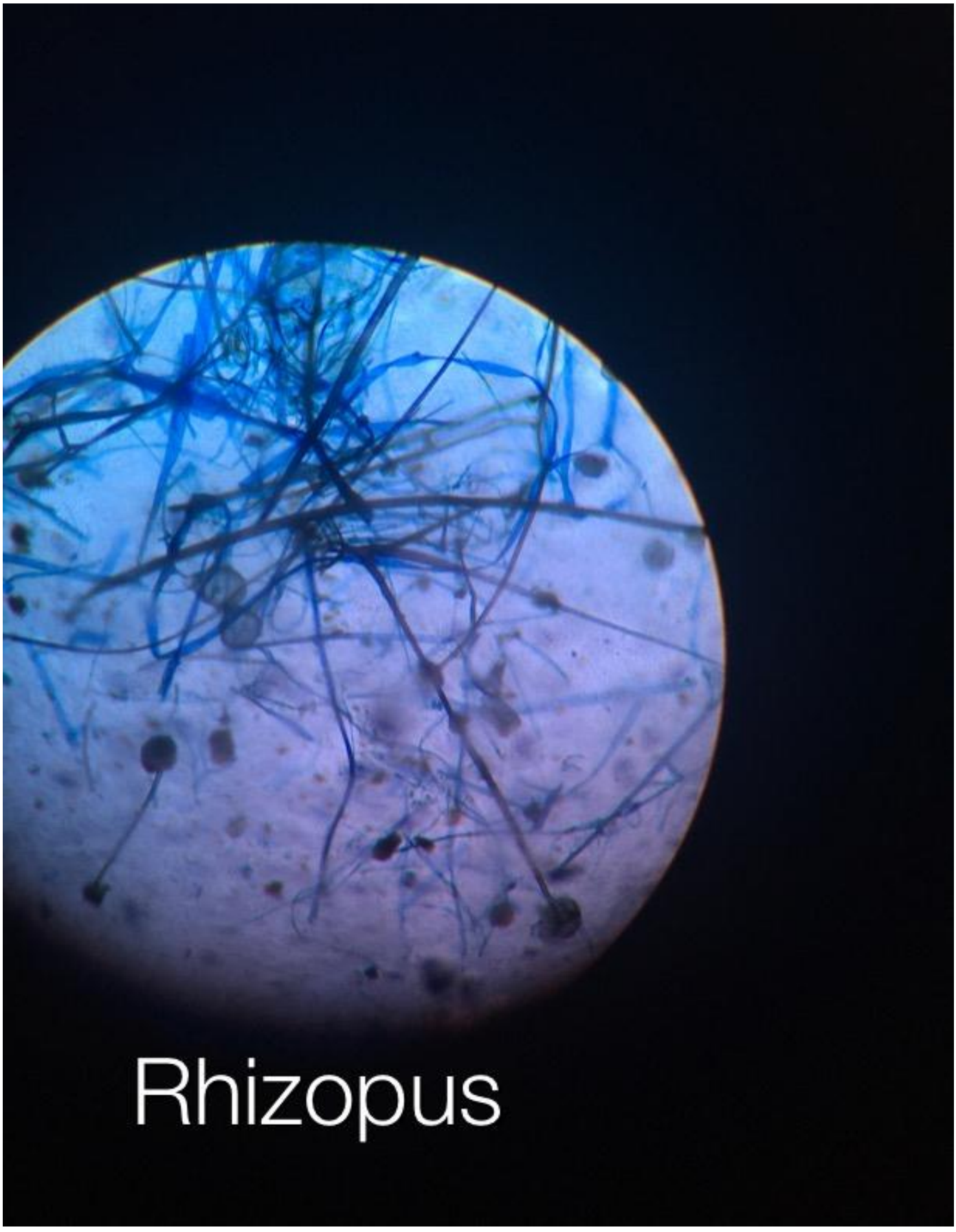
Clinical Significance:

Mainly in patients with uncontrolled diabetes or trauma can cause opportunistic, and often spreading infections known as mucormycosis.









RHIZOPUS

- Rhizoids with four to eight radial branches are seen.
- Sporangiophores are seen singly and are unbranched and may be yellowish brown to dark brown in colour.
- Sporangia may be gray beige to black in colour.
- Sporangia containssporangiospores.

Rhizopus sp.

Macroscopic: Colonies growth is rapid, with cotton texture. Salt and Pepper appearance. Colony appearing white initially, turns grey to yellowish brown in time.

Reverse is white to Pale.

Microscopic:

- Rhizoids present.
- Collumullae hemispherical.
- Apophyses absent.
- Hyaline or brown coloured round to ovoid Sporangiospores.

Clinical Significance:

Caused by Diabetes and Immunosuppression, can cause Zygomycosis is an angio invasive disease. It can be of several types mucocutaneous, rhinocerebral, genitourinary, gastrointestinal, pulmonary, and disseminated infections.

Obverse Reverse

Rhizopus Microscopic – Rhizoid Present

Aspergillus fumigatus

Macroscopic: Dark green velvety colonies are seen

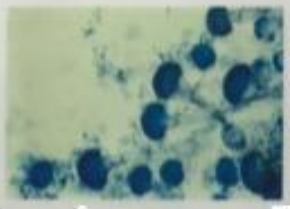
Reverse white to Tan

Microscopic:

- Broad, hyaline septate hyphae seen
- Conidiophores are smooth walled and are light green or brown in colour
- At the teriminal end of conidiophores, flask shaped vesicle is seen
- Uniseriate phialides are present at the upper half (2/3rd) of the vesicle
- Each phialide bears a chain of conidia

Clinical Significance:

It can cause Allergic broncho pulmonary aspergillosis, Aspergilloma (fungal ball infection developing in a preexisting cavity), Invasive Pulmonary Aspergillosis. In immunocompromised can cause Disseminated Aspergillosis.

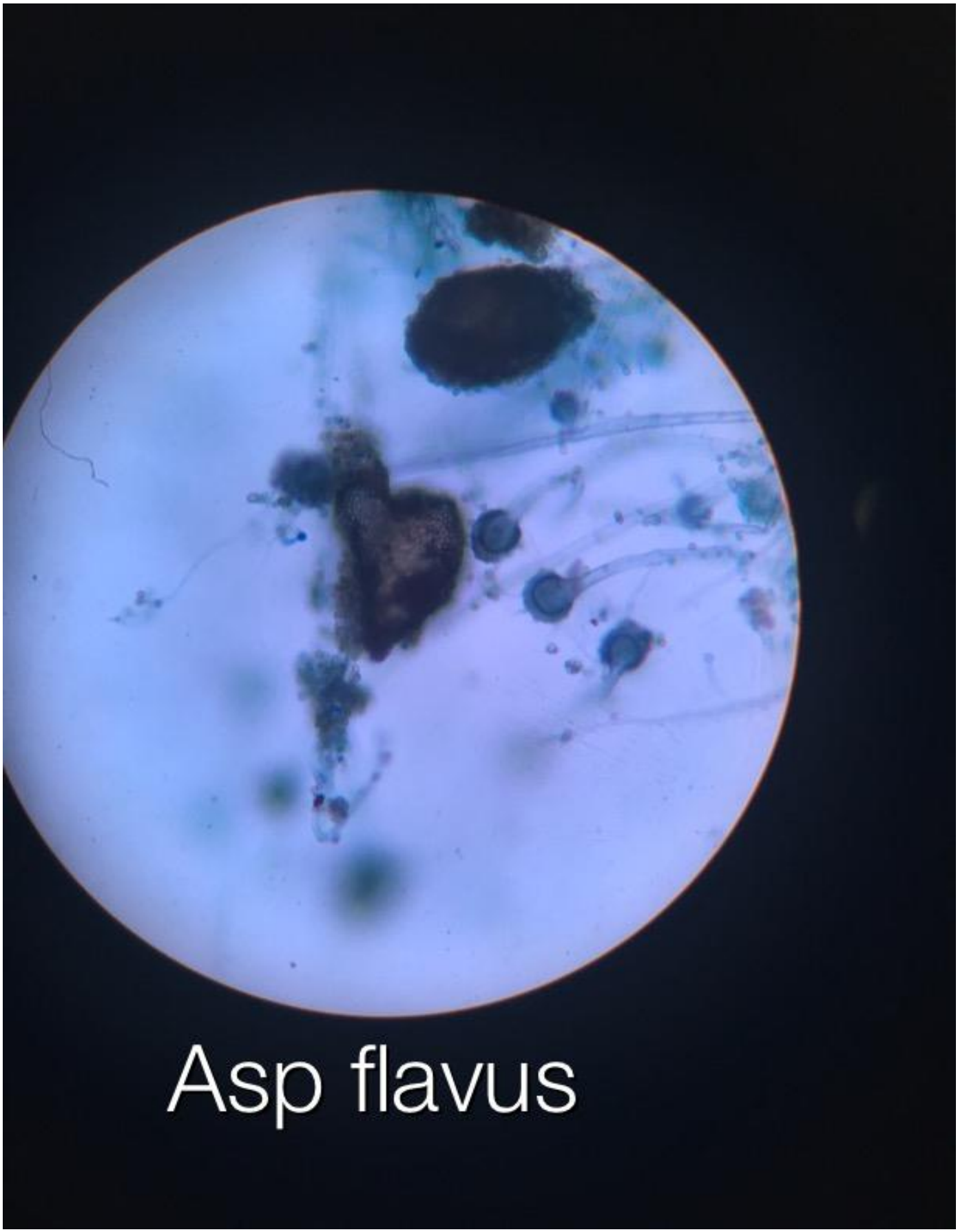


Conidial head of *A. fumigatus*

Culture of Aspergillus fumigatus. Obverse *Reverse*

ASPERGILLUS FUMIGATUS

- It has hyaline septate hyphae.
- Phialides are single(uniseriate), usually cover upper half of the vesicle, parallel to the axis of stalk.
- Conidiophores are smooth walled, they may be light green or brown.
- Vesicle is flask shaped.



Aspergillus flavus

Macroscopic: Yello to Yellow green colonies seen

Reverse goldish to red brown.

Microscopic:

- Broad , hyaline septate hyphae
- Conidiophores are thick walled , hyaline
- Vesicles are large and globose
- Phialides are biseriate and present over the entire surface of the vesicle
- The conidia are unicellular & globose

Clinical Significance:

It occurs in immunocompromised host, can cause Allergic broncho pulmonary aspergillosis. Less commonly it causes Invasive Pulmonary Aspergillosis

Culture of *Aspergillus flavus*. Obverse

Reverse

Conidial head of *A. flavus*.

Note: conidial heads with both uniseriate and biseriate arrangement of phialides may be present over the entire surface.

ASPERGILLUS FLAVUS

- It has hyaline septate hyphae.
- Conidiophores are thick walled, hyaline and coarsely roughened.
- Vesicles are large and globose.
- They are produced over most of the vesicle.
- Conidia are unicellular and typically globose.