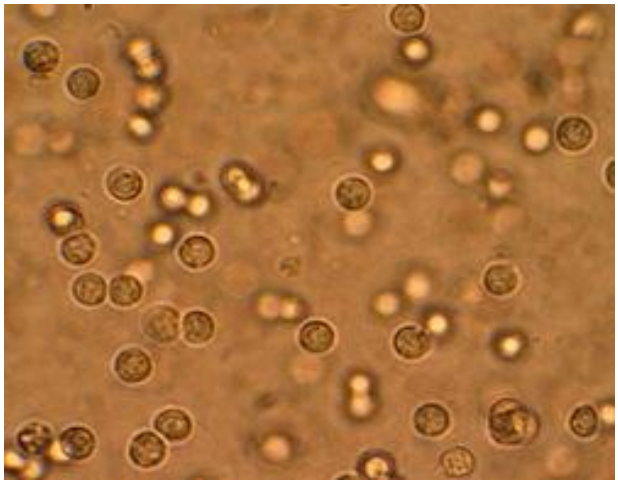
Lab – 4 Diagnosis of pathogenic bacteria

A urinary tract infection (UTI): is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as kidney infection (pyelonephritis). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder. Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI.



Multiple white cells seen in the urine of a person with a urinary tract infection using a microscopy

Cause of urinary tract infection

***E. coli* is the cause of 80–85% of community-acquired urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–10%. Rarely they may be due to viral or fungal infections. Healthcare-associated urinary tract infections (mostly related to urinary catheterization) involve a much broader range of pathogens including: *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterococcus*, *Proteus* , *Acinetobacter bauumanii* among others. Urinary tract infections due to *Staphylococcus aureus* typically occur secondary to blood-**

borne infections. The fungal pathogen *Candida albicans* also cause UTI. *Chlamydia trachomatis* and *Mycoplasma genitalium* can infect the urethra but not the bladder. These infections are usually classified as a urethritis rather than urinary tract infection.

**Escherichia coli*

***E. coli* microscopy will show gram-negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the urine. On MacConkey agar, deep red colonies are produced, as the organism is lactose-positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on EMB agar produces black colonies with a greenish-black metallic sheen. This is diagnostic of *E. coli*. The organism is also lysine positive, and grows on TSI slant with a (A/A/g+/H_2S-). Also, IMViC is {+ + – -} for *E. coli*; as it is indole-positive (red ring) and methyl red-positive (bright red), but VP-negative (no change-colorless) and citrate-negative (no change-green color).**

**Klebsiella pneumoniae*

***K. pneumoniae* is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Oxidase-negative, catalase –positive, urease- positive. *K. pneumoniae* grows on TSI slant with a (A/A/g+/H_2S-). Also, IMViC is (- - + +), it is indole-negative (no change) and methyl red- negative (yellow), but VP-positive (Red to Brown) and citrate- positive (change in color to blue).**

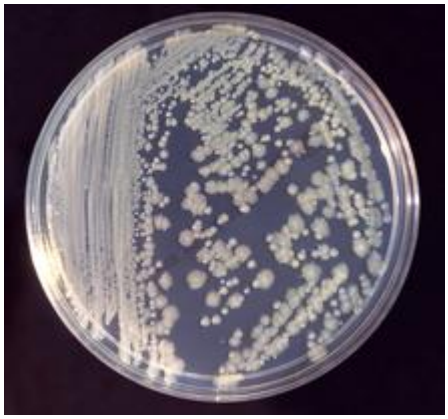
**Pseudomonas aeruginosa*

***P. aeruginosa* is a Gram-negative, aerobic, bacillus with unipolar motility, oxidase-positive, catalase- positive ,urease- positive , lactose and glucose non-fermenter , and grows on TSI slant with a (AK/no change /g-/H_2S-). Also, IMViC is {- - – +}, it is indole-negative (no change) and methyl red- negative (no change-colorless), VP-negative (no change-colorless) and citrate-positive (change color to blue). In certain conditions, *P. aeruginosa* can secrete a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and**

fluorescent), and pyorubin (red-brown). These can be used to identify the organism. *P. aeruginosa* is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odor *in vitro*. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein, as well as its ability to grow at 42 °C.

*Enterobacter spp

***Enterobacter* is a genus of common Gram-negative, facultative anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. The urinary and respiratory tracts are the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermo tolerant coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts. Two clinically important species from this genus are *E. aerogenes* and *cloacae. The* genus *Enterobacter* ferments lactose with gas production during a 48-hour incubation at 35-37 °C in the presence of bile salts and detergents. It is oxidase-negative, indole-negative, and urease-variable. Grows on TSI slant with a (A/A/g+/H_2S-). Also, IMViC is (- - + +), it is indole-negative (no change) and methyl red- negative (yellow), but VP-positive (Red to Brown) and citrate- positive (change in color to blue).**



Enterobacter

*Acinetobacter spp

***Acinetobacter* baumannii is a gram-negative coccobacillus is a Gram-negative, aerobic, non-motile , oxidase-negative , catalase- positive ,urease- negative , lactose and glucose non-fermenter , and grows on TSI slant with a (AK/no change /g-/H_2S-). Also, IMViC is {- - – +}, it is indole-negative (no change) and methyl red- negative (no change-colorless), VP-negative (no change-colorless) and citrate-positive (change color to blue).**

**Proteus spp*

Three species—*P. vulgaris*, *P. mirabilis*, and *P. penneri*—are opportunistic human pathogens. *Proteus* includes pathogens responsible for many human infections. About 10–15% of kidney stones are struvite stones, caused by alkalinization of the urine by the action of the urease enzyme (which splits urea into ammonia and carbon dioxide) of *Proteus* (and other) bacterial species. It is gram negative, pleomorphic, facultative anaerobic, motile , oxidase-negative but catalase-positive and nitrate-positive. Urease positive and phenylalanine Deaminase positive. Indole is considered reliable, as it is positive for *P. vulgaris*, but negative for *P. mirabilis*. Lactose non-fermenter, and grows on TSI slant with a (AK/A /g+/H_2S+++). Also, IMViC is {v + – v}, and have characteristic "swarming" patterns.

**** tSaphylococcus spp***

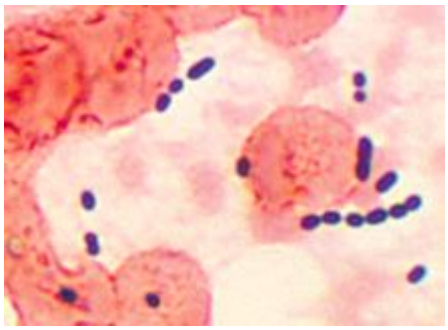
***S. aureus* is gram-positive, facultative anaerobic, cocci, in clusters, and non-motile and does not form spores. And has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. the isolate is cultured on Mannitol salt agar, which is a selective**

medium with 7–9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of Mannitol fermentation and subsequent drop in the medium's pH. *S. aureus* is catalase-positive (meaning it can produce the enzyme catalase), oxidase-negative. Coagulase-positive, DNAse- positive.

a.Sureus colonies

*Enterococcus spp

***Enterococcus* is a large genus of lactic acid bacteria of the phylum Firmicutes. Enterococci are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90–95%) and *E. faecium* (5–10%). Rare clusters of infections occur with other species, including *E. casseliflavus*, *E. gallinarum*, and *E. raffinosus*. Enterococci are facultative anaerobic organisms, they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments. Though they are not capable of forming spores, enterococci are tolerant of a wide range of environmental conditions: extreme temperature (10–45 °C), pH (4.5–10.0), and high sodium chloride concentrations, and survive at temperatures of 60 °C for 30 min. Enterococci typically exhibit gamma-hemolysis on sheep's blood agar. *E. faecalis* is a nonmotile microbe; it ferments glucose without gas production, and does not produce a catalase reaction with hydrogen peroxide. It can produce a pseudo catalase reaction if grown on blood agar. The reaction is usually weak. It produces a reduction of litmus milk, but does not liquefy gelatin. It shows consistent growth throughout nutrient broth which is consistent with being an aero tolerant anaerobe. They catabolize a variety of energy sources including glycerol, lactate, malate, citrate, arginine, agmatine, and many keto acids. They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation.**



Enterococcus

The Basics of Specimen(urine sample) Collection

Urine has a long, rich history as a source for measuring health and well-being and remains an important tool for clinical diagnosis. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling. A vast assortment of collection and transport containers for urine specimens are available. Determining which urine collection method and container should be used depends on the type of laboratory test ordered.

Types of Collection

~~**Laboratory urine specimens are classified by the type of collection conducted or by the collection procedure used to obtain the specimen.**~~

Random Specimen: **This is the specimen most commonly sent to the laboratory for analysis, primarily because it is the easiest to obtain and is readily available. This specimen is usually submitted for urinalysis and microscopic analysis, although it is not the specimen of choice for either of these tests. Random specimens can sometimes give an inaccurate view of a patient's health if the specimen is too diluted and analyte values are artificially lowered. Pediatric specimens, which routinely undergo chemistry and microscopic analysis, are generally of this type. As the name implies,**

the random specimen can be collected at any time. Although there are no specific guidelines for how the collection should be conducted, avoiding the introduction of contaminants into the specimen is recommended. This requires explicit instructions to patients so that they do not touch the inside of the cup or cup lid.

First Morning Specimen: This is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes such as protein, if present. Also called an 8-hour specimen, the first morning specimen is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep. Since the urine can be collected over any eight-hour period, collection is practical for patients who have atypical work/sleep schedules. Proper collection practices and accurate recording of the collection time are important criteria of a first morning specimen. Note: Any urine that is voided from the bladder during the eight-hour collection period should be pooled and refrigerated, so that a true 8-hour specimen is obtained.

Midstream Clean Catch Specimen: This is the preferred type of specimen for culture and sensitivity testing because of the reduced incidence of cellular and microbial contamination. Patients are required to first cleanse the urethral area with a castile soap towelette. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter into the urine stream. The urine midstream is then collected into a clean container (any excess urine should be voided into the toilet). This method of collection can be conducted at any time of day or night.

Timed Collection: Specimen Among the most commonly performed tests requiring timed specimens are those measuring creatinine, urine urea nitrogen, glucose, sodium, potassium, or

analytes such as catechol amines and 17-hydroxysteroids that are affected by diurnal variations.

A timed specimen is collected to measure the concentration of these substances in urine over a specified length of time, usually 8 or 24 hours. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. The specimen should be refrigerated during the collection period, unless otherwise requested by the physician. Accurate timing is critical to the calculations that are conducted to determine analyte concentrations and ratios. Interpretations based on faulty calculations can result in improper diagnoses or medical treatment.

Catheter Collection Specimen: This assisted procedure is conducted when a patient is bedridden or cannot urinate independently. The healthcare provider inserts a foley catheter into the bladder through the urethra to collect the urine specimen. (Specimens may also be collected through an existing foley catheter.) Specimens may be collected directly from a foley into an evacuated tube or transferred from a syringe into a tube or cup.

Suprapubic Aspiration Specimen: This method is used when a bedridden patient cannot be catheterized or a sterile specimen is required. The urine specimen is collected by needle aspiration through the abdominal wall into the bladder.

Pediatric Specimen: For infants and small children, a special urine collection bag is adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for

laboratory testing since contamination from the diaper material may affect test results.

Laboratory Diagnosis

1-Specimen culture

***MacConkey agar**

***Mannitol salt agar**

***Blood agar**

***Nutrient agar**

2- Microscopy

Gram stain

3-Biochemical tests

Tests used to identify Gram Positive Bacteria

1. **Catalase Test**
2. **Taxos P (optochin sensitivity testing)**
3. **Taxos A (bacitracin sensitivity testing)**
4. **CAMP Test**
5. **Bile Esculin Agar**
6. **Nitrate Broth**
7. **Starch hydrolysis test**
8. **Motility Agar**

Tests used to identify Gram Negative Bacteria

1. **Oxidase Test**
2. **Sugar (eg glucose) broth with Durham tubes**
3. **Methyl Red / Voges-Proskauer (MR/VP)**
4. **Kliger's Iron Agar (KIA)**
5. **Nitrate Broth**
6. **Motility Agar**
7. **Simmon's Citrate Agar**
8. **Urease test**

9. **Sulfur Indole Motility Media (SIM)**

4- Serological methods

Test	hcsE	nEterob	belK	Pseudo	orP	nicA
esadixO	–	–	–	+	–	–
esalataC	+	+	+	+	+	+
CiVMI	++- -	-- ++	- - ++	- - - +	v + - v	- - - +
IST	A/A+ -	A/A+ -	A/A+ -	KA/no change - -	AK/A+ +	AK /no change - -
Urea Hydrolysis	–	v	+	+	+	–
Motility	+	+	–	+	+	–
Phenylalanine Deaminase	–	–	–	–	+	–
esotcaL retnemref	+	+	+	–	–	–

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