Semester – III

16MBU314B **(3H – 1C)**

MICROBIAL DIAGNOSIS IN HEALTH CLINICS - PRACTICAL

Instruction Hours / week: L: 0 T: 0 P: 3 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Acquire knowledge to identify the common infectious agents with the help of laboratory procedures and use antimicrobial sensitivity tests to select suitable antimicrobial agents.

OBJECTIVES

- To provide an understanding of the natural history of infectious diseases in order to deal with the etiology, laboratory diagnosis, treatment and control of infections in the community.

EXPERIMENTS

1. Collection and processing of clinical specimen – Sputum.
2. Collection and processing of clinical specimen – Urine.
3. Collection and processing of clinical specimen – Blood.
4. Collection and processing of clinical specimen – Stool.
5. Antibiotic sensitivity testing by Kirby-Bauer method
6. Determination of minimal inhibitory concentration.

SUGGESTED READINGS

1. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013). Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication.
2. Collee JG, Fraser, AG, Marmion, BP, Simmons A (2007) Mackie and Mccartney Practical Medical Microbiology, 14th edition, Elsevier.
3. Greenwood D, Slack R, Barer M, and Irving W. (2012). Medical Microbiology, 18th Edition. Churchill Livingstone.
4. Ryan KJ and Ray CG. (2014). Sherris Medical Microbiology, 6th Edition. McGraw-Hill Professional.

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List of practicals – lecture plan

S. No	Experiment name	Hours	Support materials
1.	Collection and processing of clinical specimen – Sputum	2 weeks/ 6 hours	Manual of Medical lab technology – Dr. S. Rajan
2.	Collection and processing of clinical specimen – Urine	2 weeks/ 6 hours	Manual of Medical lab technology – Dr. S. Rajan
3.	Collection and processing of clinical specimen – Blood	2 weeks/ 6 hours	Manual of Medical lab technology – Dr. S. Rajan
4.	Collection and processing of clinical specimen – Stool	2 weeks/ 6 hours	Manual of Medical lab technology – Dr. S. Rajan
5.	Antibiotic sensitivity testing by Kirby bauer method	1 week/3 hours	Lab diagnosis of infectious disease
6.	Determination of minimal inhibitory concentration	1 week/ 3 hours	Lab diagnosis of infectious disease

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Experiment no 1 – collection and processing of clinical sample - Sputum

Scope

This experiment specifies the minimum requirements for the quality and quantity of clinical specimen – sputum sent to a laboratory for culture and conditions for transportation of specimens to the laboratory.

Definitions and abbreviations

BAL:	bronchoalveolar lavage
CPC:	cetyl pyridinium chloride
MOTT:	mycobacteria other than tuberculosis
NTP:	national tuberculosis programme

Personnel qualifications

Medical fitness

In accordance with national laws and practices, arrangements should be made for appropriate health surveillance of TB laboratory workers:

- before enrolment in the TB laboratory;
- at regular intervals thereafter, annually or bi-annually;
- after any biohazard incident;
- at the onset of TB symptoms.

Ideally, individual medical records shall be kept for up to 10 years following the end of occupational exposure.

Laboratory workers should be educated about the symptoms of TB and provided with ready access to free medical care if symptoms arise.

Confidential HIV counselling and testing should be offered to laboratory workers. Options for reassignment of HIV-positive or immuno-suppressed individuals away from the high-risk areas of the TB laboratory should be considered.

All cases of disease or death identified in accordance with national laws and/or practice as resulting from occupational exposure to biological agents shall be notified to the competent authority.

Principle

Specimen quality –from the moment of collection to the arrival of specimens at the laboratory where they will be cultured – is the responsibility of the setting in which specimens are collected, that is, either the peripheral laboratory where patients were given sputum containers or the clinics where sampling/biopsy is performed.

Since the laboratory is usually the only place where there is quality control of specimens received, laboratories at all levels must monitor quality indicators, e.g. the proportion of saliva sputum specimens, frequent late arrival of specimens, and report problems so that corrective action may be taken wherever necessary.

Specimens sent to the laboratory should be of adequate volume, as specified below, accurately labelled for identification, and accompanied by a written laboratory request form according to WHO recommendations.

Specimens should be sent to the laboratory as soon as possible after collection, in leakproof containers surrounded by absorbent material in a shock-resistant outer package that is properly labelled according to the national and/or international regulations for infectious material

Equipment and materials

Wide-mouthed, unbreakable, leakproof, screw-capped containers. Containers should have a volume capacity of 50 ml and made of translucent material in order to observe specimen volume and quality without opening the container.

Sample collection

Sputum

The large majority of specimens received for diagnosis are sputum samples.

- If good specimens are to be obtained, patients must be instructed in how to produce sputum. Specimens should be collected in a separate, ventilated room or preferably outdoors. Keeping both hands on hips, cough forcibly and collect sputum in the mouth; spit the sputum carefully into a wide-mouthed, unbreakable, leakproof container and close the lid tightly.
- Ideally, a sputum specimen should be 3–5ml in volume, although smaller quantities are acceptable if the quality is satisfactory.
- If specimens are to be cultured using a centrifugation method (see SOP Specimen processing for culture), sputa should preferably collected directly into 50-ml centrifuge tubes to avoid the need for their transfer from one container to another.
- Label each specimen with the unique identification number from the laboratory request form.
- Collect two or three specimens from each patient according to the NTP policy (INSERT THE NTP POLICY HERE)

Laryngeal swab

Laryngeal swabs may be useful in children and patients who cannot produce sputum or may swallow it.

- Collect laryngeal swabs in the early morning, before patients eat or drink anything.
- Use a sterile absorbent cotton swab for collection.
- Transport each specimen in a container with a few drops of sterile 0.9% saline solution in order to keep the swab wet.

Other respiratory specimens

- Bronchial secretion (2–5 ml) and BAL (20–40 ml)
- Pleural effusions (20–50 ml)
- Transbronchial and other biopsies taken under sterile conditions should be kept wet during transportation by adding few drops of sterile 0.9% saline to the tissue.
- .

  Note: *Specimens are sometimes sent in formalin or bleach! It may therefore be advisable to remind the physician of collection conditions, the day before surgery*.

Gastric lavage

Gastric lavages often contain MOTT and are therefore rarely used for adults; they are indicated for children, however, who produce almost no sputum

- Make the collection early in the morning, when the patient has an empty stomach.
- Neutralize the specimen by adding 100 mg of sodium bicarbonate to the gastric aspirate and transport it immediately to the laboratory.

Extrapulmonary specimens

The laboratory may receive a variety of specimens for diagnosis of extrapulmonary TB – body fluids, tissues, urine etc. These specimens may be broadly divided into two groups which are processed in different ways:

- Aseptically collected specimens (spinal fluid, pericardial, synovial and ascitic fluid, blood, bone marrow, etc.), which are usually free from contaminating flora.
  - All liquid specimens should be collected in sterile glass containers without using any preservative..
  - Specimens can be inoculated directly into liquid vials and transported to the laboratory for culture.
  - Specimens must be transported to the laboratory immediately; they should be processed as soon as possible or kept at 2–6 ºC.
  - The optimal volumes are at least 3 ml of cerebrospinal fluid and 5–10 ml of blood, collected in citrate blood tubes.
- Specimens with resident or contamination flora.
  - A urine specimen should consist of a single, early-morning, midstream sample of, collected in a wide-mouthed sterile vessel (of at least 200 ml capacity).
  - Sperm and prostate secretions are sent without any additions.
  - Menstrual blood samples should be discouraged.

  - Stool samples should be discouraged; however, stool samples from immunocompromised patients may be used, mainly to detect MOTT.

Transport conditions

Sputa should be transported to the laboratory as soon as possible. If a delay of a few days cannot be avoided, keep specimens cool (refrigerated but *not frozen*) Up to a week in cold conditions will not significantly affect the positivity rate of smear microscopy; however, the additional growth of contaminants will result in an increased contamination rate on culture media. If the delay exceeds 3 days, an equal volume of cetyl pyridinium chloride (CPC; solution of 1% CPC in 2% sodium chloride) should therefore be added to sputum (see SOP preparation of reagents for culture). Sputum containing CPC can be kept for up to 7 days but must be kept at room temperature (>20 °C since CPC crystallizes at lower temperatures). The addition of CPC must be indicated on the accompanying documents (see form below) because CPC has to be removed before culturing.

Transport packaging

The basic packaging system for local surface transport of all specimens consists of three layers (Annex 1):

- Primary receptacle – the specimen container – packaged with enough absorbent material to absorb all fluid in case of breakage.
- Secondary packaging – a second durable, watertight, leakproof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material must be used to absorb all fluid in case of breakage.

For cold transportation conditions, ice or dry ice shall be placed outside the secondary receptacle. Wet ice shall be placed in a leakproof container;

- Outer packaging – secondary packagings are placed in outer shipping packagings with suitable cushioning material. Outer packagings protect their contents from external influences, such as physical damage, during transit.

For *surface transport* there is no maximum quantity per package.

For *air transport*, no primary receptacle shall exceed 1 l for liquids or the outer packaging mass limit for solids. The volume shipped per package shall not exceed 4 l or 4 kg.

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Experiment no 2 – collection and processing of clinical sample – Urine

Urine Specimens – an overview of collection methods, collection devices, specimen handling and transportation

This Focus Topic is the first of a two part series on urine specimen collection. Part 2 will cover sources of preanalytical artifact arising during urine collection, handling and transportation.
Urine has a long history as a specimen for analysis in clinical laboratories. After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and detection of drugs.Urine testing, using both automated and traditional manual methods, is growing rapidly.1 As with all clinical laboratory specimens, preanalytical error in urine specimens is often difficult to detect. Because of this, it is important for laboratories to have processes in place to ensure compliance with best practice in specimen collection, handling and transport – including the use of preservatives where appropriate.

Types of Urine Collection Methods

Urine specimens may be collected in a variety of ways according to the type of specimen required, the collection site and patient type.
Randomly Collected Specimens are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids.
First Morning Specimen is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated.
Midstream Clean Catch Specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination.
Timed Collection Specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper,17-ketosteroids, and 17-hydroxysteroids.
Collection from Catheters (e.g. Foley catheter)using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.
Supra-pubic Aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means.
Pediatric Specimens present many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area.

Urine Collection Devices

An extensive array of urine collection products is available on the market. Information on features, intended use and instructions for use should be obtained from the device manufacturer and reviewed before being incorporated into a specimen collection protocol.
Urine Collection Containers (cups for collection and transport)
Urine collection container cups are available in a variety of shapes and sizes with lids that are either ‘snap-on’ or ‘screw-on’. Leakage is a common problem with low quality products. To protect healthcare workers from exposure to the specimen and protect the specimen from

exposure to contaminants, leak-proof cups should be utilized. Some urine specimen containers have closures with special access ports that allow closed-system transfer of urine directly from the collection device to the tube

Urine Collection Containers for 24-hour Collection

Urine collection containers for 24-hour specimens commonly have a 3 liter capacity. As for the urine collection cups above, closure types vary with some containers featuring anintegrated port for transfer of an aliquot of the specimen to an evacuated urine collection tube (further information). This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection). Additional precautions need to be taken when a preservative is required Urine Specimen Tubes

Urine specimens may be poured directly into tubes with 'screw-on' or 'snap-on' caps. Additionally,evacuated tubes, similar to those used in blood collection, are available.

Urine Specimen Collection and Transportation Guidelines

As for any type of clinical laboratory specimen, certain criteria for collection and transportation of urine specimens must be met to ensure high quality specimens free of preanalytical artifact are obtained consistently. Without this, accurate test results cannot be guaranteed.

Urine Specimen Preservation

For urinalysis and culture and sensitivity testing, CLSI Guidelines2 recommends testing within two hours of collection. Different time limits may apply to specimens required for molecular testing of infectious agents (e.g. testing for Neisseria gonorrhoeae, Chlamydia trachomatis). For this type of testing, laboratories should ensure they are able to comply with specimen transportation conditions prescribed by the assay manufacturers. Where compliance with these and/or CLSI recommendations is not possible, consideration should be given to the use of a preservative. Specimen collection tubes with preservatives for chemical urinalysis and culture and antibiotic susceptibility are available

Urine Specimen Handling Guidelines

Labels include the patient name and identification on labels. Make sure that the information on the container label and the requisition match. If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed on a different container. Ensure that the labels used on the containers are adherent under refrigerated conditions. Volume Ensure that there is sufficient volume to fill the tubes and/or perform the tests. Underfilling or overfilling containers with preservatives may affect specimen-to-additive ratios. Collection Date and Time Include collection time and date on the specimen label. This will confirm that the collection was done correctly. For timed specimens, verify start and stop times of collection. Document the time at which the specimen was received in the laboratory for verification of proper handling and transport after collection.Collection Method The method of collection should be checked when the specimen is received in the laboratory to ensure the type of specimen submitted meets the needs of the test ordered. An example of an optimum specimen/test match would be a first morning specimen for urinalysis and microscopic examination. Proper Preservation Check if there is a chemical preservative present or if the specimen has not been refrigerated for greater than two hours post collection. After accepting the test request, ensure that the method of preservation used is appropriate for the selected test. If the correct preservative was not used the test cannot be conducted. Light

Protection Verify that specimens submitted for testing of light-sensitive analytes are collected in containers that protect the specimen from light. This is a glimpse into the complexity of proper urine collection and handling. Since a variety of urine collection procedures and applications exist today, it becomes critical to understand how, when and where things can go wrong. As the trend toward more home-based testing and less invasive methods continues, urine will become one of the most useful specimen types collected for clinical assessment.

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Experiment no 3 – collection and processing of clinical sample – blood

Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient. See below forvenipuncture site selection "notes."
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.
10. Ask the patient to make a fist; avoid "pumping the fist." Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.
11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials/supplies in designated containers. Note: The larger median cubital and cephalic veins are the usual choice, but the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable. Foot veins are a last resort because of the higher probability of complications.

Fingerstick Procedure:

1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.

4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.
7. Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or "milking" that may squeeze tissue fluid into the drop of blood.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Heelstick Procedure (infants):

The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates the proper area to use for heel punctures for blood collection.

1. Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
2. Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
3. Hold the baby's foot firmly to avoid sudden movement.
4. Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
5. Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
6. Fill the required microtainer(s) as needed.
7. When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
8. Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
9. Remove your gloves and wash your hands.

Order of Draw:

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic vacutainer tubes is:

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top).
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:
    - SST (red-gray or gold top). Contains a gel separator and clot activator.
    - Sodium heparin (dark green top)
    - PST (light green top). Contains lithium heparin anticoagulant and a gel separator.
    - EDTA (lavender top)

- Oxalate/fluoride (light gray top) or other additives

NOTE: Tubes with additives must be thoroughly mixed. Clotting or erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

Labeling The Sample

All specimens must be received by the laboratory with a legible label containing at least two (2) unique identifiers.

The specimen must be labeled with the patient's full name (preferably last name first, then first name last) and one of the following:

- GHS medical record number (MRN) - for Geisinger locations, this is the required second identifier
- Patient's full date of birth (must include the month, day, and year)
- Unique requisition identifier/label

Areas to Avoid When Choosing a Site for Blood Draw:

Certain areas are to be avoided when choosing a site for blood draw:

- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible.
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

Blood Sample Handling and Processing:

Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Vacutainer tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.

- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation – It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.
- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a "balanced load" noting the following:
    - Opposing tube holders must be identical and contain the same cushion or none at all.
    - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
    - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Centrifuge Safety

- Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.
- Centrifuges must never be operated without a cover in place.
- Uncovered specimen tubes must not be centrifuged.
- Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
- Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.
- Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.

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Experiment no 4 – collection and processing of clinical sample – Stool

Faeces for the microbiological exam must be accumulated throughout the intense degree of diarrhoea.

In a hospital with a microbiology laboratory

1.Deliver the affected person a smooth, dry, disinfectant-loos bed pan or appropriate extensive-necked box In which to bypass a specimen. The container wants now not be sterile. Ask the patient to avoid contaminating the faeces with urine.

2.Transfer a component (approximately a spoonful) of the specimen, particularly that which incorporates mucus, pus, or blood, into a smooth, dry, leak-proof container. Worms and tapeworm segments: when the specimen carries worms or tapeworm segments, transfer those to a separate box and send them to the laboratory for identification.

3 .Write on the request form the colour of the specimen and whether it's miles shaped, semiformed, unformed, or fluid. record additionally if blood, mucus, worms, or tapeworm segments are present.

4.Label the specimen and ship it with a requested shape to attain the laboratory within 1 hour (if a postpone longer than 1 hour is predicted, acquire the specimen in Cary-Blair medium.

Important: whilst the specimen incorporates blood or amoebic dysentery is suspected, deliver it to the laboratory as soon as viable. A sparkling specimen is required to illustrate actively motile amoebae and also to isolate shigellae.

In a health centre for transport to a microbiology laboratory

1 .Request a specimen from the patient as defined formerly under the medical institution series of faeces.

Be aware: Leaves, cardboard bins, plastic baggage are no longer suitable for the collection of faeces.

2 .Switch a part of the faeces to a cotton wool swab. Insert the swab in a box of sterile Cary-Blair transport medium breaking off the swab stick to allow the bottle top to be replaced tightly. Salmonella serovars, Shigella, Vibrio and Yersini species continue to exist properly in Cary-Blair medium for up to 48 hours, and Campylobacter for up to six hours.

Note: Merthiolate iodine formaldehyde (MIF) solution need to no longer be used due to the fact MIF kills dwelling organisms. MIF is used as a fixative for protozoal parasites. Whilst cholera is suspected: switch about 1 ml of the specimen into 10 ml of sterile alkaline peptone water and label. The specimen should attain the Microbiology Laboratory inside eight hours of series.

3 .Write an outline of the specimen on the request form.

Note: while worms or tapeworm segments are present, transfer those (using forceps) to a container of physiological saline and send to the laboratory for identity. Commands concerning the packaging and dispatch of specimens may be observed .

Laboratory examination of faeces

The function of a microbiological laboratory in investigating infective diarrhoeal disorder With maximum sufferers, diarrhoea is self-proscribing and can be dealt with rehydration and other supportive remedies without the want for antimicrobials and microbiological investigations. The microbiological exam of faecal specimens is especially undertaken:

- To analyse outbreaks of dysentery (mainly shigellosis), cholera, and different acute bacterial infective diarrhoeal sickness of public health concern.
- To help the valuable public health laboratory in the surveillance of endemic shigellosis and salmonellosis (along with susceptibility of pathogens to antimicrobials).

- To diagnose symptomatic amoebic dysentery, giardiasis and other regionally critical intestinal parasitic infections.

Day 1

Describe the appearance of the specimen

- the coloration of the specimen.
- whether it's far fashioned, semiformed, unformed or fluid.
- Presence of blood, mucus or pus
- The presence of worms, e.g. Enterobius vermicularis, Ascaris lumbricoides, or tapeworm segments e.g. Taenia species.

Observe the specimen microscopically Saline and eosin arrangements to detect E. histolytica and other parasites

- Area a drop of fresh physiological saline on one ease of a slide and a drop of eosin stain on the alternative, using a chunk of stick or cord loop, blend a small quantity of clean specimen (particularly mucus and blood) with every drop. Cover each practise with a cowl glass.

Important: The eosin training must no longer be too thick otherwise it will no longer be possible to peer amoebae or cysts.

- Look at the arrangements the usage of the 10_ and 40_ objective with the condenser iris closed sufficiently to offer proper evaluation.
- Look specifically for motile E. histolytica trophozoites containing red cells, motile G. lamblia trophozoites, motile Strongyloides larvae, and the eggs and cysts of parasitic pathogens.
- Vicinity a drop of methylene blue stain on a slide. Blend a small quantity of specimen with the stain, and cowl with a cowl glass.
- Study the education for faecal leucocytes using the 40_ objective with the condenser iris closed sufficiently to give good contrast.
- The report also the presence of crimson blood cells (RBC) as those are often present with pus cells in inflammatory invasive diarrhoeal sickness .

Faecal leucocytes (WBCs): search for mononuclear cells and polymorphonuclear cells (pus cells). Mononuclear cells incorporate a nucleus which isn't lobed whereas polymorphonuclear cells comprise a nucleus which has or more lobes .On occasion, the cells are too broken to be identified (do not attempt to identify). Pus cells are related to the micro organism that motive irritation of the big gut regularly red cells also are discovered. Mononuclear cells are determined specifically in typhoid and in some parasitic infections, along with amoebic dysentery. reasons of inflammatory diarrhoeal ailment.

- Shigella species
- Campylobacter species
- Salmonella (non-typhoid serovars)
- histolytica
- EIEC much less not unusual:
- coli
- enterocolitica
- difficile
- perfringens (causing pigbel)
- Aeromonas species

Fundamental fuchsin smear to detect campylobacters put together whilst the specimen is unformed and, or, incorporates mucus, pus, or blood and is from a child underneath 2 y.

- Make a skinny smear of the specimen on a slide. While dry, lightly heat-restoration. Stain by way of masking the smear with 10 g/l primary fuchsin* for 10–20 seconds. Wash well with water and allow to airdry. *Dissolve 1 g basic fuchsin in one hundred ml of water, and filter out.
- Have a look at the smear for campylobacters the use of the 100_ oil immersion objective. Campylobacter organisms: search for plentiful small, delicate, spiral curved bacteria (frequently likened to gull wings), S-shapes, and short spirochaetal forms .

Note: Exam of stained faecal smears for campylobacters has been shown to be a sensitive method for the presumptive prognosis of campylobacter enteritis. Motility takes a look at and Gram stained smear when

cholera is suspected take a look at an alkaline peptone water tradition (pattern from the surface of the lifestyle) for vibrios displaying a rapid and darting motility. The practice is first-rate examined the usage of darkish-field microscopy but the

vibrios also can be visible the use of transmitted mild.

Possible pathogens

Gram-positive bacteria

- *Clostridium perfringens*
- *Clostridium difficult*
- *Bacillus cereus* (toxin)

Gram-negative

- *Shigella* species
- *Salmonella* serovars
- *Campylobacter* species
- *Yersinia enterocolitica*

Precautions while Collection of stool

1. Stools samples should be collected in clean leak-proof containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids.
2. Unpreserved stool should be refrigerated, if possible, and processed within a maximum of 2 hours after collection.
3. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium and refrigerated immediately.

Caution: *Specimens should not be collected from bedpans, because the bed pans may contain residual disinfectant or other contaminants.*

Transport media for fecal specimens

Media appropriate for the transport of fecal specimens that are suspected to contain *Shigella, Vibrio cholerae*, or *Salmonella* (including serotype Typhi) specimens are:

1. **Cary-Blair transport medium**
   High pH (8.4)
   Medium of choice for transport and preservation of *V. cholerae*
   Cary-Blair transport medium can be used to transport many bacterial enteric pathogens, including*Shigella, Salmonella,* and *Vibrio cholerae*
2. **Amies'and Staurt's transport media**
   Acceptable for *Shigella* and *Salmonella* (including ser. Typhi), but they are inferior to Cary-Blair for transport of *V. cholerae.*

3. **Alkaline peptone water**
   This medium may be used to transport *V. cholerae*, but this medium is inferior to Cary-Blair and should be used only when Cary-Blair medium is not available. Alkaline peptone water should not be used if subculture will be delayed more than 6 hours from the time of collection, because other organisms will overgrow vibrios after 6 hours.
4. **Buffered glycerol saline (BGS)**
   It's a liquid medium which can be used for S*higella* but this transport medium is unsuitable for transport of *V. cholerae*.

Rejection of fecal Specimens

Stool specimens are unacceptable if any of the following conditions apply;

- The information in the label does not match the information in the requisition
- The specimen has not been transported in the proper medium
- The quantity of specimen is insufficient for testing

2017

Experiment no 5 – Antibiotic sensitivity test- Kirby bauer method

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests(or their modification)too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the

information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
* It gives satisfactory growth of most nonfastidious pathogens.
* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Preparation of antibiotic stock solutions

Antibitiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents (Annexure III) to yield the required concentration, using sterile glassware. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitbiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc.

Storage of commercial antimicrobial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Discs should be stored as follows:

* Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.
* The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.
* Once a cartridge of discs has been removed from its sealed package, it should be placed in a tightly sealed, desiccated container. When using a disc-dispensing apparatus, it should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. Excessive moisture should be avoided by replacing the desiccant when the indicator changes color.
* When not in use, the dispensing apparatus containing the discs should always be refrigerated.
* Only those discs that have not reached the manufacturer's expiration date stated on the label may be used. Discs should be discarded on the expiration date.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles

appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer

6. The barium sulfate standards should be replaced or their densities verified monthly.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective. Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Procedure

1. Select a pure culture plate of one of the organisms to be tested.
2. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.
3. Repeat until the turbidity of the saline solution visually match that of the standard turbidity.
4. Take a sterile swab and dip it into the broth culture of organism.
5. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.
6. Take a sterile Mueller-Hinton agar (MHA) plate or a nutrient agar (NA) plate.
7. Use the swab with the test organism to streak a MHA plate or a NA plate for a lawn of growth.
8. After the streaking is complete, allow the plate to dry for 5 minutes.
9. Antibiotic discs can be placed on the surface of the agar using sterilized forceps.
10. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.
11. Carefully invert the inoculated plates and incubate for 24 hours at 37° C.
12. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.
13. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.
14. Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.

2017

Experiment no 6 – Determination of minimum inhibitory concentration

Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

(a) Broth dilution method

(b) Agar dilution method.

Dilution methods can be carried out in 2 ways

A. Broth dilution

Broth dilution testing allows the option of providing both quantitative (MIC) and qualitative (category interpretation) results. MIC can be helpful in establishing the level of resistance of a particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents.

Broth dilution can again be performed by 2 ways

1. Macro dilution: Uses broth volume of **1 ml** in standard test tubes .
2. Microdilution: Uses about **0.05 to 0.1 ml** total broth volume and can be performed in a microtiter plate or tray .

The procedure for both macro and microdilution are same except the volume of the broth.

B. Agar dilution

MIC of an antibiotic using broth dilution method is determined by using the following procedure

1. Preparation of antibiotic stock solution
2. Preparation of antibiotic dilution range
3. Preparation of agar dilution plates
4. Preparation of inoculum
5. Inoculation
6. Incubation
7. Reading and interpreting results

Preparation of antibiotic Stock solution.

Antibiotic stock solution can be prepared by commercially available antimicrobial powders (with given potency). The amount needed and the diluents in which it can be dissolved can be calculated by using either of the following formulas to determine the amount of antimicrobial powder (1) or diluent (2) needed for a standard solution:

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 μg/mL (example: 1280 μg/mL) or 10 times the highest concentration to be tested, whichever is greater.

Because microbial contamination is extremely rare, solutions that have been prepared aseptically but not filter sterilized are generally acceptable. If desired, however, solutions may be sterilized by**membrane filtration**. Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (*preferably at −60 °C or below, but never at a temperature warmer than −20 °C and never in a self-defrosting freezer).* Vials may be thawed as needed and used the same day.

Preparation of antibiotic dilution range

- Use sterile 13- x 100-mm test tubes to conduct the test. If the tubes are to be saved for later use, be sure they can be frozen.
- Close the tubes with loose screw-caps, plastic or metal closure caps, or cotton plugs.
- Prepare the final two fold (or other) dilutions of antimicrobial agent volumetrically in the broth. A minimum final volume of 1 mL of each dilution is needed for the test.

Note: For, microdilution,only 0.1 ml is dispensed into each of the 96 wells of a standard tray.

Preparation of inoculum

1. Prepare the inoculum by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour agar plate *(use a non-selective medium, such as **blood agar**).*
2. Adjust the suspension to achieve a turbidity equivalent to a **0.5 McFarland turbidity standard**. This results in a suspension containing approximately 1 to 2 x 10^8 colony forming units (CFU)/mL for*Escherichia coli* ATCC®a 25922.
3. Compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.
4. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL.**Note:** *This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10^6 CFU/mL. The subsequent 1:2 dilution in step 3 brings the **final inoculum to 5 x 10^5 CFU/mL.***

Minimum Bactericidal Concentrations(MBC)

The main advantage of the 'Broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37^oC overnight. The tubes are also incubated overnight at 37^oC. Read the MIC of the control organism to check that the drug concentrations are correct.

Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37^{o}C overnight. Compare the amount of growth from the control tube before incubation,which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct, whether or not this organism is killed is immaterial.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only.
- A reduced number of colonies-indicating a partial or slow bactericidal activity.
- No growth- if the whole inoculum has been killed
- The highest dilution showing at least 99% inhibition is taken as MBC

Micro-broth dilution test

This test uses double-strength Müeller-Hinton broth, 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of $2x10^6$/ml. In a 96 well plate, 100 µl of double-strength MHB, 50 µl each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth will be considered the MIC of the organism.

Reading of result

MIC is expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself,the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC, run with the test is used as the control to check the reagents and conditions.

Minimum inhibitory concentration (MIC) is determined when a patient does not respond to treatment thought to be adequate, relapses while being treated or when there is immunosuppression.

MDHC Spotters

1. 

Biohazard refers to any biological materials(microorganisms, plants, animals, or their byproducts) that pose a threat to the health of living organisms.

- **Human blood and blood products.** This includes items that have been contaminated with blood and other body fluids or tissues that contain visible blood.
- **Animal waste.** Animal carcasses and body parts, or any bedding material used by animals that are known to be infected with pathogenic organisms.
- **Human body fluids.** Semen, cerebrospinal fluid, pleural fluid, vaginal secretions, pericardial fluid, amniotic fluid, saliva, and peritoneal fluid.
- **Microbiological wastes.** Common in laboratory settings, examples of microbiological wastes include specimen cultures, disposable culture dishes, discarded viruses, and devices used to transfer or mix cultures.
- **Pathological waste.** Unfixed human tissue (excluding skin), waste biopsy materials, and anatomical parts from medical procedures or autopsies.
- **Sharps waste.** Needles, glass slides and cover slips, scalpels, and IV tubing that have the needle attached.

There are 4 levels of biohazards, according to the Center for Disease Control:

- **Biohazard Level 1:** Agents that pose minimal threat to humans and the environment. Examples include *E. coli, Bacillus subtilis* and *Naegleria gruberi*.
- **Biohazard Level 2:** Agents that can cause severe illness in humans and are transmitted through direct contact with infected material. Examples include HIV, hepatitis B and salmonella.
- **Biohazard Level 3:** Pathogens that can become airborne and cause serious diseases. Examples include tuberculosis and *Coxiella burnetii*.
- **Biohazard Level 4:** Pathogens that pose a high risk of life-threatening disease for which there are no treatments. Examples include the Ebola virus and Lassa virus.

2. 

Blood collection tube is a sterile glass tube with a colored rubber stopper creating a vacuum seal inside of the tube facilitating the drawing of a predetermined volume of liquid. Vacutainer tubes may contain additives designed to stabilize and preserve the specimen prior to analytical testing. Tubes are available with a safety-engineered closure (rubber stopper), with a variety of labeling options and stopper colors as well as a range of draw volumes. Vacutainer tubes may contain additional substances that preserve blood for processing in a medical laboratory. Using the wrong tube may make the blood sample unusable for the intended purpose. These additives are typically thin film coatings applied using an ultrasonic nozzle.

The additives may include anticoagulants (EDTA, sodium citrate, heparin) or a gel with density between those of blood cells and blood plasma. Additionally, some tubes contain additives that preserve certain components of or substances within the blood, such as glucose. When a tube is centrifuged, the materials within are separated by density, with the blood cells sinking to the bottom and the plasma or serum accumulating at the top. Tubes containing gel can be easily handled and transported after centrifugation without the blood cells and serum mixing.

The meanings of the various colors are standardized across manufacturers.

The term *order of draw* refers to the sequence in which tubes should be filled. The needle which pierces the tubes can carry additives from one tube into the next, so the sequence is standardized so that any cross-contamination of additives will not affect laboratory results.

Containers containing coagulants

- Gold or "tiger" red/black top: clot activator and gel for serum separation
- Red top (plastic, not glass): clot activator but no serum separation gel
- Orange or grey/yellow "tiger" Top: thrombin, a rapid clot activator, for stat serum testing

Containers containing anticoagulants

- Green: sodium heparin or lithium heparin used for plasma determinations inclinical chemistry (e.g. urea and electrolyte determination). Sodium heparin collection tubes are the classically preferred tube for peripheral blood or bone marrow for cytogenetic studies. Lithium heparin is considered suboptimal for cytogenetics.
- Light green or green/gray "tiger": For plasma determinations.
- Purple or lavender: K_2 EDTA. This is a strong anticoagulant and these tubes are usually used for complete blood counts(CBC). Lavender top tubes are generally used when whole blood is needed for analysis. Can also be used for some blood bank procedures such as blood type and screen. EDTA tubes are preferred by most molecular genetics laboratories for molecular genetic studies (DNA or RNA).
- Grey: sodium fluoride and oxalate. Fluoride prevents enzymes in the blood from working, by preventing glycolysis so a substrate such as glucose will not be gradually used up during storage. Oxalate is an anticoagulant.
- Light blue: sodium citrate. Citrate is a reversible anticoagulant, and these tubes are used for coagulation assays.
- Dark Blue: EDTA.These tubes are used for trace metal analysis.
- Black - Used for Erythrocyte Sedimentation Rate (ESR).

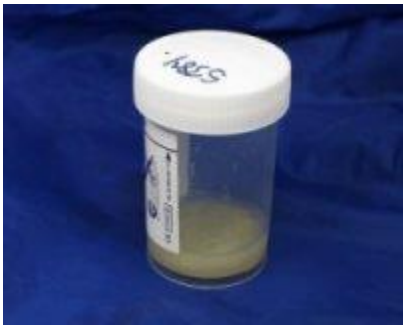
Other

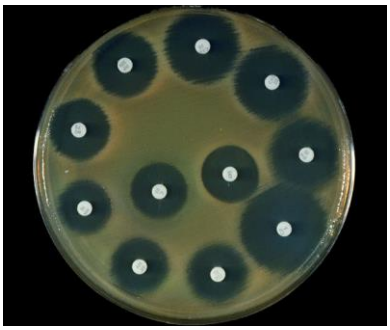
- Red (glass): Contains no additives.
- Light yellow: Contains sodium polyanethol sulfonate (SPS). Used for blood culture specimens or acid-citrate-dextrose(ACD), used for blood bank studies, HLA phenotyping, and paternity testing.
- Tan (glass or plastic): Contains either sodium heparin (glass) or K_2EDTA (plastic). Used for lead determinations. These tubes are certified to contain no lead.

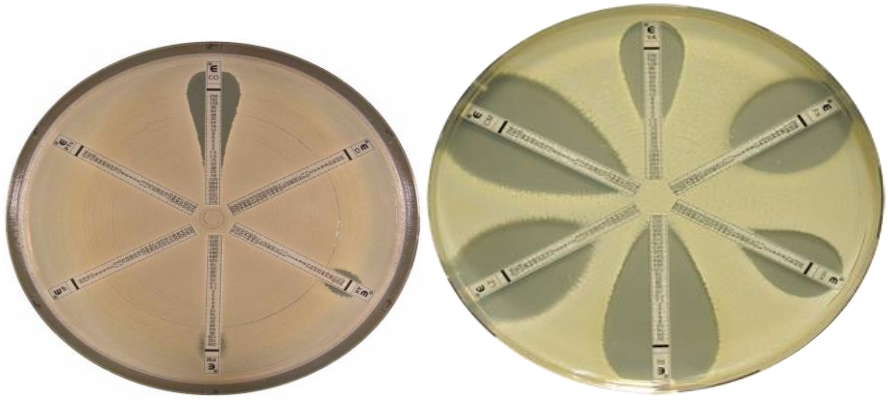
3.  sample containers

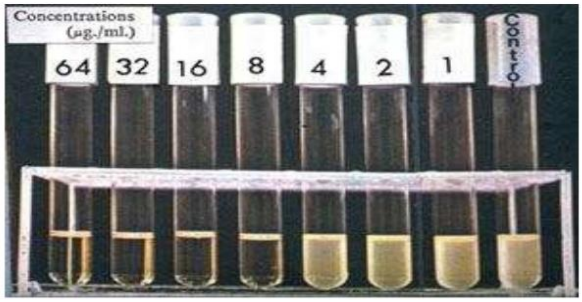
4.  sample container with stool sample

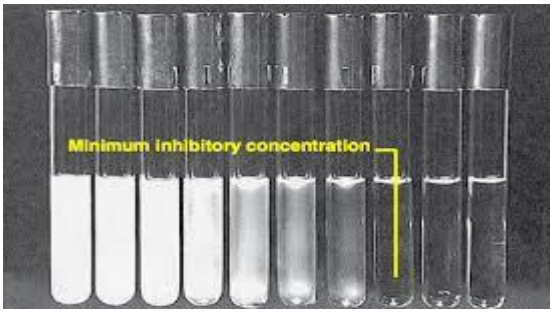
5.  Sample container with urine sample

6.  Sample container with Sputum sample

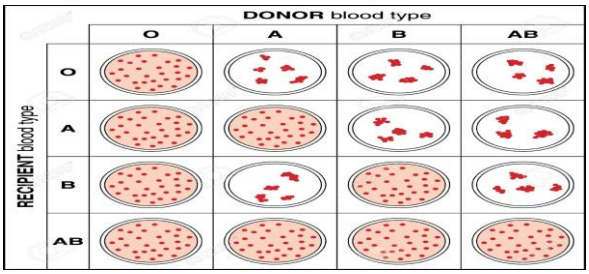
7.  Antibiotic Susceptibility test

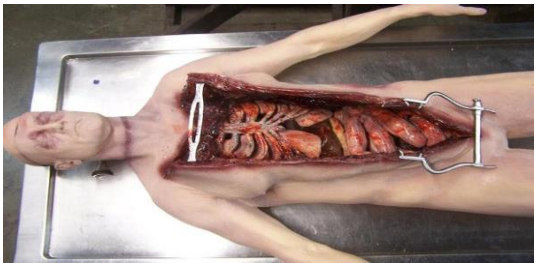
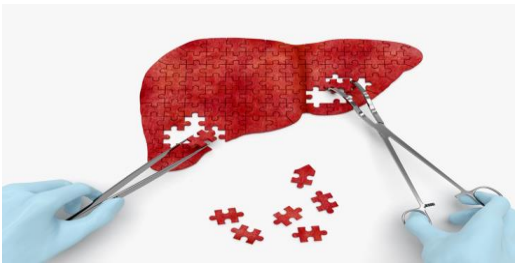
8.  MIC – E test

9. 



MIC

10. Differential media - MacConkey agar with lactose fermenting colonies
11. Selective media – EMB agar with Metallic Sheen colonies
12. Selective media – cetrimide agar with green colonies
13. Selective media – TCBS agar with yellow colonies
14. Selective media – SS agar with black colonies
15. Selective media – XLD agar with black colonies
16. Basal media – Nutrient agar
17. Basal media – Nutrient agar with quadrant streaking
18. Inoculation loop
19. Sterile swabs
20. Antibiotic discs, powders
21. Sterile discs
22. Widal kit
23. Blood grouping kit 

24. Autopsy and biopsy  
25. Gloves, aprons, masks