ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA: 3.64) in the Third Cycle and Graded as Category I University by MHRD- UGC]
(A State University Established by the Government of Tamilnadu)

KARAIKUDI-630 003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc. (MICROBIOLOGY)

III-SEMESTER

36434

IMMUNOLOGY, MEDICAL MICROBIOLOGY, ENVIRONMENTAL AND AGRICULTURE MICROBIOLOGY

Author :
Dr. T. Sathiyamoorthi
Assistant Professor
Department of Microbiology
Alagappa University
Karaikudi.

Reviewer:

Dr. G. Selvakumar
Assistant Professor of Microbiology,
Directorate of Distance Education,
Alagappa University,
Karaikudi -630003.

CONTENTS Page No.

1. IMMUNOLOGY 1-12

1.1 Collection of venous blood from human, separation and preservation of serum/plasma.

1. 2 Blood Grouping.

1. 3 Precipitation method - Immuno diffusion.

1.4 Latex Agglutination test.

1.5 Widal test (Tube and Slide Test).

1.6 ELISA.

1.7 Western Blotting.

2. MEDICAL MICROBIOLOGY 13-16

2.1 Isolation and identification of - Respiratory tract infections- *Pseudomonas aeruginosa*,

2.2 Isolation and identification of Urinary tract infection- *E.coli/ K. pneumonia*.

2.3 Fungal skin pathogens- Dermatophytes and Candida.

3. ENVIRONMENTAL & AGRICULTURE MICROBIOLOGY 17-37

3.1 Enumeration of microorganism from air.

3.2 Settle plate technique.

3.3 Estimation of dissolved oxygen (DO)

3.4 Estimation of BOD

3.5 Estimation of COD

3.6 Isolation of free living nitrogen fixing bacteria from soil – *Azotobacter*.

3.7 Isolation of Symbiotic nitrogen fixing bacteria from root nodule – *Rhizobium*.

3.8 Examination of Plant Bacterial diseases- Sheath blight of rice and Wilt of potato

3.8.1 Examination of Plant Bacterial diseases- Sheath blight of rice

3.8.2 Examination of Plant Bacterial diseases- Wilt of potato

3.9 Fungal diseases – Late blight of potato and Wilt of cotton

3.9.1 Fungal diseases – Late blight of potato

3.9.2 Fungal diseases – Wilt of cotton

3.10 Viral diseases- Banana bunchy top virus and Tobacco Mosaic Virus

3.10.1 Viral diseases- Banana bunchy top virus

3.10.2 Viral diseases- Tobacco Mosaic Virus

4. REFERENCES **38**

1. 1 COLLECTION OF VENOUS BLOOD FROM HUMAN, SEPARATION AND PRESERVATION OF SERUM\PLASMA

AIM

- To collect the venous blood from human.
- To separate and preserve serum\plasma.

PRINCIPLE

Blood is normally sterile, but bacteria occur transiently in the blood stream which is termed as bacteremia, during dental surgery, instrumentation of the genitourinary tract or bowel and also in infections like typhoid fever, brucellosis and meningococcal infections. A more dangerous and clinically alarming syndrome is septicemia, a condition characterized by the rapid multiplication of microorganisms with the elaboration of their toxins into the blood stream. Blood culture is requested mainly in two clinical situations.

- Where the possibility of septicemia or bactermia is suggested by the presence of fever, shock, suspected local infections, perpeural sepsis, pneumonia, meningitis, osteomyelitis or endocarditis.
- In investigation of fever difficult to diagnose because of the absence of signs of a specific infection or local infective lesion i.e., pyrexia of unknown origin.

MATERIALS REQUIRED

Iodine, Freeze vials, sterile syringe, Ethanol ether.

PROCEDURE

COLLECTION OF BLOOD

1. Using a sterile syringe of 21 gauge needle 10-12 ml of blood is withdrawn from the suitable vein, whose area has been cleansed with tincture of iodine followed by ethanol ether.
2. With care, the needle from the syringe is removed and replaced with another sterile needle of the same size and is inserted into the rubber liner of the culture bottle cap.
3. The specimen is used for microbiological analysis before it clots.

SEPARATION AND PRESERVATION OF SERUM\PLASMA

1. A 10 ml tune of whole blood will be allowed to clot for one hour at room temperature.

2. Centrifuge for 10 minutes at approximately.
3. Using clean pipette technique aliquot the serum into vials.
4. Immediately freeze vials of serum at 80 degree freezer.

INTERPRETED RESULT

Serum has been separated from blood and stored at 80 degree freezer.

1.2. BLOOD GROUPING

AIM

- To perform an experiment for blood grouping.

PRINCIPLE

Blood has been held as a mysterious fascination for humans from the dawn of time. Blood and blood transfusion became scientifically feasible only after the discovery of blood group by Karl Landsteiner (1900). Grouping of blood is based on agglutination reaction between antigen and antibody present in blood cells (RBC). When particulate antigen is mixed with its specific antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. This is known as agglutination.

MATERIALS REQUIRED

Glass slide, 70% alcohol, Lancet needle, anti-A, anti-B, anti-D

PROCEDURE

1. A clean glass slide or porcelain tile will be taken and three circles will be drawn and marked as A, B and D.
2. Wipe the left middle finger with 70% alcohol and puncture at the tip with a lancet needle.
3. The first drop blood will be wiped off and Place the subsequent drops on to the circles marked A, B and D.
4. Place a drop of anti-A, anti-B and anti-D on circles A, B and D respectively.

INTERPRETED RESULT

The test to determine your blood group is called ABO typing. Your blood sample is mixed with antibodies against type A and B blood. Then, the sample is checked to see whether or not the blood cells stick together. If blood cells stick together, it means the blood reacted with one of the antibiotics.

1.3. PRECIPITATION METHOD- IMMUNODIFFUSION

AIM

- To detect antigen-antibody complexes.
- Describe the circumstances under which antigen-antibody complexes precipitate out.
- Determine relative concentration of antigens.

PRINCIPLE

Immuno-diffusion is a technique for the detection or measurement of antibodies and antigens by their precipitation which involves diffusion through a substance such as agar or gel agarose. Simply, it denotes precipitation in gel It refers to any of the several techniques for obtaining a precipitate between an antibody and its specific antigen.This can be achieved by:

a) Suspending antigen/antibody in a gel and letting the other migrate through it from a well or,

b) Letting both antibody and antigen migrate through the gel from separate wells such that they form an area of precipitation. Based on the method employed, immuno-diffusion may be:

1. Radial immunodiffusion
2. Ouchterlony Double Diffusion

PROCEDURE

1. An agar containing an appropriate antiserum (antibody) is poured in plates.
2. Carefully circular wells are cut and removed from the plates.
3. A single or series of standards containing known concentration of antigen are placed in separate wells, while control and "unknown" samples are placed in other remaining wells.
4. As the antigen diffuses radially, a ring of precipitate will form in the area of optimal antigen – antibody concentration.
5. The ring diameters are measured and noted.

INTERPRETED RESULT

Antigen antibody complex has been detected.

1.4. LATEX AGGLUTINATION TEST

AIM

- To learn the technique of latex agglutination

PRINCIPLE

Latex agglutination is observed when a sample containing the specific antigen (or antibody) is mixed with an antibody (or antigen) which is coated on the surface of latex particles. The reaction between a particulate antigen and an antibody results in visible clumping called agglutination. Antibodies that produce such reactions are known as agglutinins. The principle of Agglutination reactions are similar to precipitation reactions; they depend on the cross linking of polyvalent antigens. When the antigen is an erythrocyte it is called hemeagglutination. Theoretically all antibodies can agglutinate particulate antigens but IgM, due to its high specificity is a particularly good agglutinin.

MATERIALS REQUIRED

- Microcentrifuge, Pipette, Microtips, Laboratory refrigerator, Glycine saline buffer, Blocking buffer, Antigen for coating, Latex beads, Test antiserum, Glass slides, Beaker, Tooth pick.

PROCEDURE COATING OF LATEX

1. To 20 µl of latex beads taken in a 1.5 ml vial add 40 µl of glycine-saline buffer.
2. Add 60 µl of antigen to the latex and incubate at 37^{o}C for 2 hours.Spin down at 5000 rpm for 10 minutes and carefully aspirate the supernatant.
3. Resuspend the pellet in 1 ml of blocking buffer and spin down at 5000 rpm for 10 minutes.
4. Repeat the washing once more.
5. Add 90 µl of blocking buffer to the pellet, mix well.
6. Incubate at 4^{o}C, overnight. **Agglutination Test**
7. To 200 µl of glycine-saline buffer taken in a vial,add 4 µl of test antisera. (50 times diluted).
8. Add 50 µl of antigen to 50 µl of diluted antiserum in a 1.5 ml vial, mix well and incubate at room temperature for 10 minutes.

9. Pipette 10 µl of coated latex onto a glass slides.
10. Add 10 µl of diluted test antiserum to slide A.
11. Add 10 µl of antiserum mixed with antigen (from step 8) to B.
12. Add 10 µl of glycine-saline buffer to C.
13. Take a tooth pick and mix the content in each slide. Discard the tooth pick after using in one slide (take a new one for the next slide).
14. After mixing, wait for 2 minutes to observe the result.

INTERPRETED RESULT

Positive result will show development of an agglutinated pattern showing clearly visible clumping of the latex particles.

1.5. WIDAL TEST

AIM

- To perform a quantitative test to diagnose and determine the antibody titer of typhoid and paratyphoid fever.

PRINCIPLE

The patient's serum is serially diluted and the highest dilution at which agglutination is seen is designated as antibody titre of patient's serum. This is expressed as 1:20, 1:40, 1:80, 1:160, etc., The dilution of 1:80 is the significant titre. A daily increase of antibody titer is indicative of the disease.

PROCEDURE

1. Clean serological test tubes will be set up in a test tube rack and numbered from 1 to 10.
2. 1.9 ml of saline will be taken in the first test tube and 1 ml of saline in remaining tubes. To the first tube 0.1 ml of patient's serum will be added which gives a 1:20 dilution.
3. Serial dilution will be performed using 1 ml of 1:20 dilution serum sample to give 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280 dilutions.
4. The last tube serves as negative control that has only the saline in it.
5. Four sets of similar dilution will be made for the 4 antigens and were labeled appropriately.
6. Add one drop of O, H, AH and BH antigens to the appropriately labeled tubes.
7. Observe the agglutination pattern after incubation.

INTERPRETED RESULT:

The Widal test is positive if TO antigen titer is more than 1:160 in an active infection, or if TH antigen titer is more than 1:160 in past infection or in immunized persons

1.6. ELISA

AIM

- To estimate an multivalent antigen using the appropriate antibodies.

PRINCIPLE

Enzyme linked immuno sorbent assay is being extremely used as a tool in research as well as in analytical and diagnostic tests. The specificity, sensitivity add case to perform this technique has made this method popular. This method can be used for estimating any type of multivalent antigen using the appropriate antibodies. ELISA is so named because the technique involves the use of an immune sorbent, which is an absorbent material specific for one of three components of the reaction, the antigen or the antibody. This may be particulate such as cellulose or agarose or a solid phase such as polystyrene or micro wells. ELISA is usually done using 96 wells microtitre plates suitable for automation.

The method requires two antibodies that can react with two different epitopes or antigen. One of the antibody is immobilized on a solid support and the other one is linked to an enzyme. Antigen containing sample is first added to the immobilized antibody and allowed to react. Untreated enzyme antibody conjugate is washed out and the enzyme bound to the solid support is estimated by colorimetry. The enzyme activity is directly proportional to the antigen concentration. Also the positive reaction can be identified by means of colour development.

MATERIALS REQUIRED

- ELISA plate coated with antigen.
- Positive and negative control serum.
- Test serum, Phosphate buffered saline.
- Tween 20,Bovine serum albumin.
- Conjugate (anti IgM linked with horse radish peroxidase).

PROCEDURE

1. Wash the 96 well polyvinyl microtitre plated with sterile distilled water and 3 to 4 times with phosphate buffered saline-tween20.
2. Followed by washing the wells will be coated with 100µl of bacterial antigen and keep for overnight incubation. After overnight incubation, Wash the antigen coated wells with phosphate buffer saline-tween 20 for 4-5 times.
3. Then Add 80µl of dilution fluid (phosphate buffered saline-tween 20- bovine serum albumin solution) to each well.
4. To this 20µl of sample will be added (positive control serum, negative control and the test serum).

5. A blank will also be kept using sterile distilled water. Then cover the plate with aluminium foil and incubated at room temperature for one hour.
6. After incubation, washing will be carried out again for four times by adding phosphate buffer saline-tween 20.
7. After washing, Add 100µl of conjugate (1:200 dilutions) and incubate at room temperature for another one hour.
8. Then Discard the contents and washing will be repeated as described earlier.
9. Finally add 100µl of TMP and observe the formation of blue colour.

INTERPRETED RESULT:

Antibody testing is usually done on a blood sample, often using an enzyme-linked assay called an ELISA or EIA. If the person has been infected with HIV, the antibodies in the serum will bind to the HIV proteins, and the extent of this binding can be measured. Negative EIA results are usually available in a day or so.

1.7. WESTERN BLOTTING

AIM

- To perform the western blot technique for protein extraction.

PRINCIPLE

Western blotting (protein blotting or immuno blotting) is a rapid and sensitive assay for detection and characterization of proteins. It is based on the principle of immuno chromatography where proteins are separated into polyacrylamide gel according to their **molecular weight.** The protein thus separated are then transferred or electro transferred onto nitrocellulose membrane and are detected using specific primary antibody and secondary enzyme labeled antibody and substrate

PROCEDURE

GEL ELECTROPHORESIS

1. In this step, we will separate the individual proteins in our sample lysate **based upon their molecular weight** using a positive electrode to attract a negatively charged protein.
2. To do this, we load our previously prepared protein samples into a commercially available polyacrylamide gel. Gels are available in fixed percentages or gradients of acrylamide.
3. The higher the acrylamide percentage the smaller the pore size of the gel matrix. Therefore higher percentage of gels are better for low molecular weight proteins, low percentage of gel are useful for large proteins and gradient gels can be used for proteins of all sizes due to their varying range in pore size.
4. Prepare your gel by inserting it into the electrophoresis apparatus and filling with running buffer that is appropriate for your gel chemistry.
5. Rinse the wells of the gel with running buffer and add buffer to the chambers.
6. Load your samples into the wells and load a pre-stainedmolecular weight ladder into one well.
7. The ladder will allow you to monitor protein separation during electrophoresis and subsequently verify protein weight in your sample during later analysis.
8. Close the electrophoresis unit and connect it to a power supply. Most units typically run 45-60 minutes at 200 volts or until the loading buffer reaches the bottom of the gel.
9. During this time the negatively charged proteins in each sample will migrate toward the positively charged electrode making their way through the polyacrylamide gel matrix.

TRANSFER

In this next step, we will transfer separated proteins out of the gel into a solid membrane or blot. This is based upon the same principal as the previous step in which an electric field is charged to move the negative proteins towards a positive electrode. Transfer can occur under wet or semi-dry conditions.The steps of traditional wet transfer method are as follows:

1. Start by removing the gel from its cassette cutting the top portion containing the wells. Float the gel in transfer buffer while preparing the transfer sandwich. To make the transfer sandwich, a cassette, sponges, filter paper, the gel and PVDF or nitrocellulose membrane paper is needed.
2. Notch the top left corner of blotting paper to indicate blot orientation and incubate membranes in transfer buffer for 10 minutes.
3. Notch the top left corner to indicate gel orientation. Create a stack by placing the following components from the black negative cathode to red positive anode: sponge, filter paper, gel, membrane, filter paper and sponge (Be careful not to touch the gel or membrane with your bare hands and use clean tweezers or spatula instead.
4. Touching the membrane during any phase can contaminate the blot and lead to excessive background signal).
5. Use a clean roller with each layer to gently roll out any bubbles that may be present since bubbles will inhibit efficient protein transfer.
6. Lock the cassette and place it in the transfer apparatus containing cold transfer buffer ensuring that the cassette is properly positioned from negative to positive.
7. In order to prevent heat buildup, it is beneficial to transfer with a cold pack in the apparatus or in a cold room with the spinner bar placed at the bottom of the chamber.
8. Close the chamber and connect to a power supply.
9. Perform the transfer according to the manufacturer's instructions which is normally 100 volts for third to 120 minutes.

DETECTION:

In this final phase, we will demonstrate signal development using the most common, most sensitive and most inexpensive detection method the electro chemiluminescence or ECL reaction. This method utilizes

the HRP enzyme which was conjugated to the secondary to catalyze the ECL reaction and produce light. A light is then gathered onto x-ray film and developed or digitized with the aid of a specialized camera sensitive enough for this application**.**

Steps:

1. We start by mixing equal parts ECL reagents in a one to one ratio according to the manufacturer's instructions.
2. We will incubate the membrane for 3-5 minutes without agitation.
3. After incubation, decant ECL mixture and use a laboratory wipe to wipe off excess solution from the corner of the membrane.
4. Place the membrane in a clear plastic wrap such as a sheet protector to prevent drying.
5. We can now use a roller to push out any bubbles or any excess solution.
6. Immediately develop the membrane.
7. Both film and camera systems allow us to manually adjust the exposure time in order to ensure a picture perfect Western Blot.
8. Relative band densities can now be quantified with commercially available software.
9. Proper molecular weight can also be verified by comparing band sizes to the molecular weight ladder

INTERPRETED RESULT:

The Western blot assay is a method in which individual proteins of an HIV-1 lysate are separated according to size by polyacrylamide gel electrophoresis. A consistent sequence of antibody responses occurs after infection.

2.1 ISOLATION AND IDENTIFICATION OF RESPIRATORY TRACT INFECTIONS - *Pseudomonas aeruginosa*

AIM

- To isolate and identify the upper respiratory tract bacterial pathogen from throat.

BACKGROUND INFORMATION

Infections affecting the throat (larynx) or the main airway (trachea) or the airways going into the lungs (bronchi) are common. These infections are sometimes called laryngitis, trachitis or bronchitis. Doctors often just use the term URIT (upper respiratory tract infection) to include any or all of these. Cough is usually the main symptom. Other symptoms include fever, headache, aches and pains. Cold symptoms may occur if the infection also affects the nose. Symptoms typically peak after 2-3 days and then gradually clear. However the cough may persist after the infection has gone. This is because the inflammation in the airways caused by the infection can take a while to clear. It may take upto 4 weeks after other symptoms have hone for the cough to clear completely.

PRINCIPLE

S.pneumoniae is one of the most common gram positive cocci of the family *Streptococcaceae*. It is responsible for greater number of infectious disease. They are classified by means of two major methods based on haemolysis and based on serotype(antigens).

1. BASED ON HAEMOLYSIS

Three types of haemolytic patterens are observed on blood agar that the alpha-haemolysis, beta-haemolysis, gamma-haemolysis.

2. BASED ON SEROTYPING

Lancefield grouped *Streptococc*i based on antigen A to O. *S.pneumoniae* belongs to the group A. Members of group A Streptococci is responsible for tonsillitis, scarlet fever, cellulitis, rheumatic fever, etc.,

MATERIALS REQUIRED

Specimen- Throat swab, Chemical and media-Blood agar, Nutrient agar medium Gram staining, Biochemical test media and

NOTES

reagents. Glass wares- Glass slides, petri plates, conical flask, test tube etc.,

PROCEDURE

1. Collect the throat swab using sterile cptton without touching the tongue and lips.
2. Streak the swab on the plates of blood agar and nutrient agar after mixing with saline.
3. Incubate the plates at 37^{o}c for 24 hours.
4. Then Observe the plates for colony morphology.
5. The cultures on the above plates will be used to perform gram staining, motility and various biochemical tests.

INTERPRETED RESULT

Pseudomonas are not generally fastidious microorganisms. *Pseudomonas* gives negative Voges Proskauer, indole and methyl red tests, but a positive catalase test. While some species show a negative reaction in the oxidase test, most species, including *P. fluorescens*, give a positive result.

2.2 ISOLATION AND IDENTIFICATION OF URINARY TRACT INFECTION – *E. coli/K. pneumonia*

AIM:

- To isolate and identify microorganisms from urinary tract infection.

PRINCIPLE:

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. The main causal agent is *Escherichia coli*. Although urine contains a variety of fluids, salts, and waste products it does not usually have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, they may cause urinary tract infection. Early morning urine or midstream urine is collected for diagnosis.

MATERIALS REQUIRED:

Freshly collected urine sample, blood agar or Mac Conkey agar ,inoculation loop, etc.

PRINCIPLE:

1.24 hrs culture is made with the given sample.

2. Now the 24 hr culture is cultured in blood or Mac Conkey agar plates.

3. The plates are incubated at 37 degree C for 24 hrs.

4. The culture from the plate is then stained with Gram staining procedure.

INTERPRETED RESULT:

E. coli bacteria are among the few species of lactose (LAC)-positive, oxidase-negative, gram-negative rods that are indole positive. Due to the infrequent isolation of non-*E. coli* strains that are indole positive, the spot indole test has been used for the rapid, presumptive identification of *E. coli*

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar.

NOTES

2.3 ISOLATION AND IDENTIFICATION OF FUNGAL SKIN PATHOGENS- *Dermatophytes and Candida.*

AIM:

- To isolate and identify the fungal skin pathogens.

PRINCIPLE:

Fungi can live in the air, soil, water, and plants. There are also some fungi that live naturally in the human body. Like many microbes, there are helpful fungi and harmful fungi. When harmful fungi invade the body, they can be difficult to kill, as they can survive in the environment and re-infect the person trying to get better.

MATERIALS REQUIRED:

Sabdour dextrox agar,skin scraping for fungal culture,inoculation loop,etc.

PROCEDURE:

1. 24 hr culture is made from the skin scrapping.
2. The culture is then cultured in sabdour dextrox agar plates.
3. It is then incubated at 37 degree C for 24 hrs.
4. After incubation the staining procedure is carried out and observed under microscope.

INTERPRETED RESULT

Various *Candida species* can be detected by observing the changes in the indicator colour when the yeast cultures utilize 1% carbohydrates such as glucose, maltose, sucrose, trehalose and raffinose.

3.1 ENUMERATION OF MICROORGANISMS FROM AIR

AIM

- To enumerate the microorganisms from air.
- To enumerate microorganisms in air and study its load after UV sanitation.

PRINCIPLE

Usually microbiology labs are fumigated for killing bacteria present in lab environment which can be contaminants or pathogenic too. UV light can be used a fumigant to sanitize college laboratories. UV kills microbes by causing DNA damage by thymine dimerization. It produces mutations hampering genetic replication and protein synthesis. UV-resistant organisms, however are still able to grow even after exposure to UV. Effectiveness of UV sanitation is tested by enumerating microorganisms from air of irradiated environment. Gravity sedimentation technique is used for this. In this method, NA plate is exposed and air microbes in Laminar Air Flow (LAF) are allowed to to settle by action on gravity for 20 minutes. Plate is then incubated at room temperature for 24 hours. This process is done before performing fumigation and also after fumigation so as to compare bacterial load. As in this method, we are not considering amount of air allowed to settle, so this method is only qualitative.

REQUIREMENTS

1) St. NA plates

PROCEDURE

1) Expose a St. Nutrient Agar plate inside LAF for 20 mins.
2) Remove and close the plate.
3) Turn on the UV light and let the UV sanitize LAF for 20 mins. Then, turn off the UV.
4) Now, expose a St. Nutrient Agar plate inside LAF for 20 mins.
5) Incubate both the plates at room temp. for 23 hours.
6) Count the colonies on the plates and calculate % reduction.

NOTES

INTERPRETED RESULT

$$\% \text{ reduction} = \frac{\text{Final CFU} - \text{Initial CFU}}{\text{Final CFU}} * 100$$

Using this formula percentage of reduction on exposure to UV light can be calculated and results can be interpreted.

3.2 SETTLE PLATE TECHNIQUE

AIM

- To enumerate the microorganisms from air by settle plate technique.

PRINCIPLE

Passive air monitoring is usually performed with **settle plates** (also known as sedimentation **plates** or **settling plates**) – standard Petri dishes containing culture media that are exposed to the air for a given time and then incubated to allow visible colonies to develop and be counted.

MATERIALS REQUIRED

Nutrient agar plates, laminar air flow,etc.

PROCEDURE

1. The nutrient agar plates are made.
2. The plates are made to expose in the air and made to settle in the agar with the help of air sampler.
3. The plates are made to incubate at 37 degree C for 24 hrs.
4. After incubation the plates are observed for microbial growth.

INTERPRETED RESULT

The microbiological content of the air can be monitored by two main methods, one active and one passive. Air sampling performed during surgery is carried out to monitor the risk of microbial wound contamination, passive measurement is better than volumetric sampling at predicting the likely contamination rate at the surgical site, as it allows a direct measure of the number of microorganism settling on surface.

NOTES

3.3 ESTIMATION OF DISSOLVED OXYGEN

AIM

- To determine the dissolved oxygen in the given pond water sample.

PRINCIPLE

Dissolved oxygen analysis can be used to determine. The health or cleanliness of a lake or stream . The amount and type biomass a fresh water system can support. The amount of decomposition occuring in the lake or stream. Ideally sample should be measured in the field immediately after collection

REAGENT LIST

2ml Manganese sulfate, 2ml Alkali -iodine –azide, 2ml Concentrated sulphuric acid, 2ml starch solution, Sodium thiosulphate (0.01N).

PROCEDURE

1. Carefully fill 300ml glass BOD stoppered bottle brim full with sample water.
2. Immediately add 2ml manganese sulphate to the collection bottle by inserting the calibrated pipette just below to the surface of the liquid.
3. Add 2ml alkali-iodized-azide reagent in the same manner.
4. Stopper the bottle with case to the sure microorganism is introduced. Mix the sample by inverting several times. Check for air bubble, discard the sample and start over if any are seen. If oxygen is present,a brownish orange colour of precipatation or floc has settle to the bottom,mix the sample by turning it upside down several times and let it settle again.
5. Add 2ml of concentrated sulpuric acid via pipette held just above the surface of the sample. Carefully stopper and insert several times to distance the floc.At this point the sample in fixed and can be store for 8 hours if kept in a cool,dark place. As an added precaution, squirt distilled water along the slipper and cap the cap the bottle with aluminium foil and a rubbet band during the storage period.

6. In a glass flask titrate 20ml of the sample with sodium thiosulphate to a pale straw colour. Titrate by slowly droping titrated solution from the calibrated pipette into the flask and continously stir the sample water.
7. Add 2ml of starch solution so blue colour forms.
8. Continue slow titrating until the sample turns clear. As this experiment reaches the end point it will take only 1 drop of the titrate to eliminate the blue colour. Be carefull that each drop in fully mixed into the sample before adding the next. It in sometimes helpful to hold the flask upto a white sheet of paper to check for absence of blue colour.
9. The concentration of DO is the sample in equivalent to the number of millimeters of titrant used.each ml of sodium thioslphate added in the step 6 and 8 equals 1mg\l DO.s

INTERPRETED RESULT

The total number of milliliters of titrant used in steps 6-8 equals the total dissolved oxygen in the sample in mg/L. Oxygen saturation is temperature dependent - gas is more soluble in cold waters, hence cold waters generally have higher dissolved oxygen concentrations. Dissolved oxygen also depends on salinity and elevation, or partial pressure.

NOTES

3.4 ESTIMATION OF BOD

AIM

To determine the amount of B.O.D. exerted by the given sample(s).

PRINCIPLE

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardised time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard. The B.O.D. test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water. It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution. The test has its widest application in measuring waste loading to treatment plants and in evaluating the efficiency of such treatment systems.

The test consists in taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles are taken for each concentration and three concentrations are used for each sample. One set of bottles is incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content (D_1) in the other set of bottles will be determined immediately. At the end of 5 days, the dissolved oxygen content (D_2) in the incubated set of bottles is determined.

$$\text{Then, mg/L B.O.D.} = \frac{(D_1 - D_2)}{P}$$

where,

P= decimal fraction of sample used.

D_1 = dissolved oxygen of diluted sample (mg/L), immediately after preparation.

D_2 = dissolved oxygen of diluted sample (mg/L), at the end of 5 days incubation.

Among the three values of B.O.D. obtained for a sample select that dilution showing the residual dissolved oxygen of at least 1 mg/L and

a depletion of at least 2 mg/L. If two or more dilutions are showing the same condition then select the B.O.D. value obtained by that dilution in which the maximum dissolved oxygen depletion is obtained.

MATERIALS REQUIRED

1. B.O.D. bottles 300mL capacity
2. B.O.D. incubator
3. Burette
4. Pipette
5. Air compressor
6. Measuring cylinder etc.

REAGENTS

1. Distilled water
2. Phosphate buffer solution
3. Magnesium sulphate solution
4. Calcium chloride solution
5. Ferric chloride solution
6. Acid and alkali solution
7. Seeding
8. Sodium sulphite solution
9. Reagents required for the determination of D.O.

PROCEDURE

1. Place the desired volume of distilled water in a 5 litre flask (usually about 3 litres of distilled water will be needed for each sample).
2. Add 1mL each of phosphate buffer, magnesium sulphate solution, calcium chloride solution and ferric chloride solution for every litre of distilled water.
3. Seed the sample with 1-2 mL of settled domestic sewage.
4. Saturate the dilution water in the flask by aerating with a supply of clean compressed air for at least 30 minutes.
5. Highly alkaline or acidic samples should be neutralised to pH 7.

NOTES

6. Destroy the chlorine residual in the sample by keeping the sample exposed to air for 1 to 2 hours or by adding a few mL of sodium sulphite solution.
7. Take the sample in the required concentrations. The following concentrations are suggested:
   Strong industrial waste : 0.1, 0.5 and 1 per cent
   Raw and settled sewage : 1.0, 2.5 and 5 per cent
   Oxidised effluents : 5, 12.5 and 25 per cent
   Polluted river water : 25, 50 and 100 per cent
8. Add the required quantity of sample (calculate for 650 mL dilution water the required quantity of sample for a particular concentration) into a 1000 mL measuring cylinder. Add the dilution water up to the 650mL mark.
9. Mix the contents in the measuring cylinder.
10. Add this solution into two B.O.D. bottles, one for incubation and the other for determination of initial dissolved oxygen in the mixture.
11. Prepare in the same manner for other concentrations and for all the other samples.
12. Lastly fill the dilution water alone into two B.O.D. bottles. Keep one for incubation and the other for determination of initial dissolved oxygen.
13. Place the set of bottles to be incubated in a B.O.D. incubator for 5 days at 20°C. Care should be taken to maintain the water seal over the bottles throughout the period of incubation.
14. Determine the initial dissolved oxygen contents in the other set of bottles and note down the results.
15. Determine the dissolved oxygen content in the incubated bottles at the end of 5 days and note down the results.

INTERPRETED RESULT

Calculate BOD value using his formula

$$\text{B.O.D} = \frac{(D_1 - D_2)}{P} =.$$

3.5 ESTIMATION OF CHEMICAL OXYGEN DEMAND

AIM

- To estimate the chemical oxygen demand for the given sample.

PRINCIPLE

Under alkaline conditions, permanganate oxidizers only organic matter present in the sample without oxidizing Cl^{-}, Br^{-} and I^{-} to Cl^{-} to Cl2',Br2 and I2 respectively. When all such organic matter is oxidized permanganate is allowed to liberate iodine from potassium iodide in acidic condition. Iodine so liberated is titrated against thiosulphate required to react with all the iodine liberated by unreduced permanganate is estimated from these two titrate values, the chemical oxygen demand of the water sample is calculated.

REAGENTS

- Potassium permanganate solution (0.01)
- Sulphuric acid (25%)
- NaOH solution (5%)
- Potassium iodide solution solution(0.1M)
- Sodium thiosulphate solution (0.02N)
- Starch solution (1%)

PROCEDURE

1. Take 20 mL of sample in a conical flask and add 1mL of NaOH and add 4mL of KMnO4.
2. Mix the solution and heat on water bath for 20 minutes and cool it by running cold water.
3. Now add 1mL sulphuric acid and 2mL potassium iodide solution.
4. Titrate it against sodium thiosulphate using starch as indicator.Blue colour is formed.
5. At the end the blue colour will be disappeared.Note the titrate value.

NOTES

INTERPRETED RESULT

Calculate the COD in the sample in mg/L as follows:
A = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the blank,
B = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for of the sample,
N = normality of the $Fe(NH_4)_2(SO_4)_2$ solution, and
S = milliliters of sample used for the test.

3.6 ISOLATION OF FREE LIVING NITROGEN FIXING BACTERIA FROM SOIL-*Azotobacter*

AIM

- To isolate *Azotobacter* from soil.

INTRODUCTION

Azotobacter is a genus of free-living diazotrophic bacteria whose resting stage is a cyst. It is primarily found in neutral to alkaline soils, in aquatic environments, and on some plants. It has several metabolic capabilities, including atmospheric nitrogen fixation by conversion to ammonia.

MATERIALS REQUIRED

Media: Nitrogen free mannitol broth, nitrogen free mannitol agar. Equipments: Bunsen burner, inoculation loop, conical flask, marker etc.

PROCEDURE

1. Add 1 gm of soil sample to 50ml of sterile N-free mannitol broth. Shake vigorously.
2. Incubate the culture for 4-7 days at room temperature (250C).
3. Examine the surface of the culture for the presence of a film .Do not shakes the film.
4. Using sterile inoculating technique, transfer a loop full of surface film to an appropriately labeled N-free mannitol agar plate. Perform quadrant streaking for isolation of colonies.
5. Incubate at 25 degree C for 4-6days. Observe for pigmentation of colonies.

INTERPRETED RESULT

Azotobacter chroococcum produces brown to black coloured colonies.*Azotobacter vinelandii* fluoresces green under UV light

NOTES

3.7 ISOLATION OF SYMBIOTIC NITROGEN FIXING BACTERIA FROM ROOT NODULE- *Rhizobium*

AIM

- To isolate *Rhizobium sp* from the root nodules of leguminous

INTRODUCTION

Rhizobium forms a symbiotic relationship with certain plants such as legumes, fixing nitrogen from the air into ammonia, which acts as a natural fertilizer for the plants. Current research is being conducted by agriculture research scientist microbiologists to discover a way to use *Rhizobium*'s biological nitrogen fixation. This research involves the genetic mapping of various rhizobial species with their respective symbiotic plant species, like alfalfa or soybean. The goal of this research is to increase the plants' productivity without using fertilizers.

MATERIALS REQUIRED

Sample: Root nodules.

Media: Yeast extract mannitol agar (YEMA)

Reagent: 0.1%Mercuric chloride, 75% ethyl alcohol.

Equipments: Sterile Petri plates, scalpel, forceps, Bunsen burner etc.

PROCEDURE

1. Select well formed, healthy pinkish nodule from the tap root of leguminous plants.
2. Surface sterilize by immersing in 0.1% mercuric chloride for five minutes and wash repeatedly with sterile water to remove the adhering chemicals.
3. Again sterilize with 75%ethanol for 3 minutes. Wash repeatedly in sterile water.
4. Cut the sterile nodule into 2 halves.
5. Rub the exposed, pinkish brown portion on the surface of yeast extract mannitol agar medium using sterile forceps. Incubate the plates at 37oC for 24-72 hrs.

INTERPRETED RESULT

Observe for the presence of mucoid, white colonies indicate the presence of rhizobium in the root nodules.

3.8 EXAMINATION OF PLANT BACTERIAL DISEASES- SHEATH BLIGHT OF RICE AND WILT OF POTATO

3.8.1 PLANT BACTERIAL DISEASE- SHEATH BLIGHT OF RICE

INTRODUCTION

Rice is one of the important food crops and provides an essential part of the daily dietary intake for nearly half of the world's population. Sheath blight is a fungal disease of rice caused by a necrotrophic soil-borne fungus *Rhizoctonia solani* with telomorpic stage *Thanatephorus cucumeris* (Frank) Donk, and comes under AG-1 as anastomosis group. It was first identified as a parasite of potato in 1898 by Kuhn. Sheath blight disease of rice (Oryza sativaL.), caused by *Rhizoctonia solani* Kuhn has assumed economic importance in the last two decades with the introduction of modern semi dwarf nitrogen responsive cultivars. It is one of the most destructive rice diseases worldwide and can lead to severe losses in rice productivity and grain quality by infecting and destroying rice sheath and leaves. It occurs in all rice production areas worldwide.

SYMPTOMS

Sheath blight is named of its primary infection on leaf sheath. The most critical stage for the infection to occur was at maximum tillering stage, while leaf sheath becomes discoloured at or above water level. The disease appears as oval or elliptical with greenish spots brown margin. Presence of many such spots on the leaf sheath gives the appearance of snake skin. Under favorable conditions, the infection spreads rapidly to the upper plant parts and also to the neighbouring plants by means of normal emergence and expansion of the ears and results in poor filling of the grains. The pathogen is also known to cause panicle infection resulting in production of unfilled or partially filled discoloured seed bearing brownish black spots or black to ashy gray patches.The disease attacks the leaf sheath, leaf blades and in severe cases symptoms also observed on emerging panicles. However, at maximum tillering and ear head phase, the rice crop is most vulnerable to sheath blight pathogen. Sheath blight pathogen in advanced stage of infection and disease development forms brown sclerotia, which are easily detached from the affected plant parts.

NOTES

TREATMENT

1. Use a reasonable level of fertilizer adapted to the cropping season.
2. Use reasoned density of crop establishment (direct seeding or transplanting).
3. Control weeds on leaves
4. Use fungicide to treat seeds.
5. Improve canopy architecture by reducing seeding rate or providing wider plant spacing.

3.8.2 PLANT BACTERIAL DISEASE- WILT OF POTATO

INTRODUCTION

Bacterial wilt is one of the most destructive diseases of the potato, which has a very wide host range. On potato, the disease is also known as brown rot, southern wilt, sore eye or jammy eye. Bacterial wilt of potato is generally favoured by temperatures between 25°C and 37°C. It usually does not cause problems in areas where mean soil temperature is below 15°C. Under conditions of optimum temperature, infection is favoured by wetness of soil. However, once infection has occurred, severity of symptoms is increased with hot and dry conditions, which facilitate wilting. Bacterial wilt is a serious problem in many developing countries in the tropical and sub-tropical zones of the world. It is usually found between the latitudes 45°N and 45°S. Bacterial wilt is responsible for causing considerable losses to the potato industry where the disease exists.The disease can cause total loss of a crop and prevent the use of land for potato production for several years. Bacterial wilt is caused by a soil-borne bacterium named *Ralstonia solanacearum* (earlier known as *Pseudomonas solanacearum).* Based on the type of host plants it attacks it is divided into three races, and based on its biochemical properties it is divided into four biovars. The most widespread strain in Australia is race 3/biovar ll. Bacterial wilt attacks more than 200 species. These include economically important hosts such as tobacco, potato, tomato, eggplant, pepper, banana, peanut and beans. Thorn apple and nightshade are two common weed hosts that are attacked by the disease.

SYMPTOMS

Symptoms are wilting, yellowing which finally die right back. Wilting is first seen as a drooping of the tip of some of the lower leaves similar to that caused by a temporary shortage of water. At

first only one branch in a hill may show wilting. Affected leaves later become permanently wilted and roll upwards and inwards from the margins. The wilting then extends to leaves further up the stem and is followed by a yellowing of the leaves. This yellowing, wilting and in-rolling of the leaves makes diseased plants very obvious, especially when surrounded by healthy plants. The leaves finally turn brown and fall off, beginning at the base of the stem and continuing upwards.

Symptoms in the tuber are very specific: brownish-grey areas are seen on the outside, especially near the point of attachment of the stolon. Cut tubers may show pockets of white to brown pus or browning of the vascular tissue which, if left standing, may exude dirty white globules of bacteria. As the disease progresses bubbly globules of bacteria may exude through the eyes; soil will often adhere to the exuded bacteria, hence the name 'sore eyes' or 'jammy eyes'.

MINIMISING THE SPREAD (TREATMENT)

1. Use of certified seed from reliable sources. Exclusion of the disease may be exercised by quarantine or other legislative measures
2. Planting in areas where bacterial wilt has not occurred previously.
3. Control self-sown potatoes.
4. Control weed hosts such as nightshade, thorn apple, Narrawa burr around dames, along channels and in the paddocks after cropping potatoes.
5. Avoid deep ploughing – the organisms survive in the deep, cool layers of soil.
6. Irrigation water should never be allowed to run freely over or below the soil surface. It should never be allowed to return to the dam or stream from which it is pumped, nor to any other irrigation source.
7. Regular crop inspection for disease symptoms and remove and destroy diseased plants, tubers and immediate neighbours.
8. Use stock to clean up chats, discarded tubers and crop debris, but do not allow the stock back onto clean paddocks.
9. Do not return potato waste, e.g. oversized, misshapen and diseased tubers to paddocks.

NOTES

10. Machinery taken onto a diseased paddock should be left on the paddock while it is being worked.
11. Machinery removed from the paddock should then be washed clean with a disinfectant solution in a dedicated area for equipment wash down.
12. Use high-pressure wash to clean machinery, sheds etc to remove soil adhering to any surfaces.
13. After harvest, all diseased and discarded tubers should be collected and buried at least one metre underground.
14. Load and unload vehicles only in designated areas with sealed or hard ground or bare paddocks away from potato paddocks.
15. Choose transport roots that minimise travel through potato paddocks and regions.
16. If second-hand bags or half tonne bins have been used to hold potatoes, these should be thoroughly washed and disinfected before being used again. Bags should be disinfected or discarded.

3.9 FUNGAL DISEASES – LATE BLIGHT OF POTATO AND WILT OF COTTON.

3.9.1 FUNGAL DISEASE- LATE BLIGHT OF POTATO

INTRODUCTION

Potato is a major food crop after wheat, rice and maize. Over next three decades when the world population is expected to grow by around 100 million a year and put further pressure on land, water and other resources, farmers in developing countries have to double their output to feed the growing numbers.

Late blight is caused by *Phytophthora infestans*. It belongs to order Peranosporales of class Oomycetes. The fungus is characterized by lemon shaped detachable, papilliate sporangia produced on sympodially branched sporangiophores of indeterminate growth. The sporangiophores exhibit a characterized swelling at junction where sporangia are attached with the sporangiophores

Fungal and Bacterial Diseases of Potato frequently, develops oospores and sporulation on tubers and is more inclined to develop resistance to fungicide metalaxyl. Population of *P.infestans* in most countries has changed dramatically and original A1have almost been displaced by more virulent A2strain. Occurrence of both A1 and A2 strains at the same location has opened up the possibility of development of thick walled oospores which could survive either extreme winter or summers conditions. The oospores may act as another source of primary inoculum, in addition to the already known sources such as infected seed tubers; waste heaps, volunteer plants etc.

SYMPTOMS

Late blight appears first as water- soaked irregular pale green lesions mostly near tip and margins of leaves. During morning hours a white mildew, which consists of sporangia and spores of the pathogen, can be seen on lower surface of infected leaves especially around the edges of the necrotic lesions. Light to dark brown lesions appear on stems or petiole which elongate and encircle the stems. The affected stems or petiole become week at these locations and may collapse. Under disease favorable conditions entire crop gives blackened blighted appearance and may be killed within a week. Tubers in soil become infected by rain borne sporangia from the diseased foliage. The infected tubers show irregular reddish brown to purplish slightly depressed areas which extend deep into internal tissues of the tubers.

NOTES

TREATMENT

1. Plant resistant cultivars when available.
2. Remove volunteers from the garden prior to planting and space plants far enough apart to allow for plenty of air circulation.
3. Water in the early morning hours, or use soaker horse, to give plants time to dry out during the day — avoid overhead irrigation.
4. Destroy all tomato and potato debris after harvest

3.9.2 FUNGAL DISEASES- WILT OF COTTON

INTRODUCTION

Fusarium wilt is a major disease in the cotton. Causal organisum : *Fusarium oxysporum* f.sp. *Vasinfevtum.* The fungus is present both inter and intra cellularly in the host tissue. The mycelium plugs the xylem vessels partially or completely. The macro conidia are 1-5 septate, hyaline, thin walled, linear to falcate, the tepering the micro conidia are hyaline, thin elliptical to spherical, single or two celled. Commonly found throughout the United States, Fusarium wilt is a soil-borne pathogen that attacks potato, tomato, eggplant and pepper plants. *Fusarium oxysporum* enter through the roots and get interacted with the phloem. As the infection spreads up into the stems and leaves it restricts water flow causing the foliage to wilt and turn yellow.

Symptoms for diseases are first found on the older leaves. As the disease progresses, the younger leaves will also be affected and the plant eventually dies. In many cases, only one branch or side of the plant shows symptoms. It can survive in soil for years. The fungal disease develops during hot weather and is most destructive when soil temperatures approach 80˚F.

SYMPTOMS

The disease affects the crop at all stages. on cotyledon the changes of color observed from yellow to brown . The base of petiole shows brown ring, followed by wilting and drying of the seedlings. Discoloration starts from the margin and spreads the midrib. The leaves lose their turgidity, gradually turn brown, droop and finally drop off. Symptoms start from the older leaves at the base, followed by younger ones towards the top, finally involving the branches and the whole plant. The defoliation or wilting may be complete leaving the stem alone standing in the field. Sometimes partial wilting occurs; where in only one portion of the plant is affected, the other remaining free. The taproot is usually stunted with less abundant

laterals. Browning and blackening of vascular tissues. In severe cases, discoloration may extend throughout the plant starting from roots extending to stem, leaves and even bolls.

TREATMENT

1. Plant resistant varieties when available.
2. Remove stricken growth from the garden and sterilize pruning clippers between cuts.
3. High nitrogen fertilizers may increase susceptibility to the disease. Test your soil and use a slow-release, organic fertilizers in the vegetable garden.
4. Hand pull or spot treat weeds using a weed flamersor natural habitats many weed species host the disease pathogen.
5. Mycostop is a biological fungicide that will safely protect crops against wilt caused by *Fusarium*.
6. Apply sufficient water during application to move Mycostop into the root zone.
7. If the disease persists, it is best to remove the entire plant and solarize the soil before planting again.

NOTES

3.10 VIRAL DISEASES- BANANA BUNCHY TOP VIRUS AND TOBACCO MOSAIC VIRUS.

3.10.1 VIRAL DISEASES- BANANA BUNCHY TOP VIRUS

INTRODUCTION

Banana bunchy top is a viral disease caused by a single-stranded DNA virus called the banana bunchy top virus. It is a nanovirus, single stranded DNA virus with isometric virions 18–20 nm in diameter BBTV infects most banana cultivars, retards the growth of infected plants, and causes economic losses to banana production.

SYMPTOMS

Symptoms of BBTV include dark green broken streaks on leaf veins,

midribs, petioles and pseudostem looks bunchy top appearance. When the disease get progressed the leaf get curled. Banana plants infected inthe late development produced small fruits, distorted and tip of male bud are bird mouth shape like appearance Although BBTD symptoms are usually very distinctive across all Musa spp., in some cases symptomless BBTD have been reported in Taiwan. In India, hill banana cultivation at higher elevation more than 7500 msl feet, symptomless BBTD in hill banana were detected.

TREATMENT

By destroying the infected plants disease can be controlled. Infected plants were sprayed by insecticide sevin.to get rid of alphid population.

3.10.2 VIRAL DISEASES- TOBACCO MOSAIC VIRUS.

INTRODUCTION

Tobacco mosaic virus (TMV) is a positive-sense single stranded RNA virus in genus Tobamo virus that infects a wide range of plants, especially tobacco and other members of the family Solanaceae. The infection causes mosaic like mottling and discoloration. TMV symptoms include mosaic, mottling, necrosis, stunting, leaf curling, and yellowing of plant tissues. TMV can be transmitted when an infected leaf rubs against a leaf of a healthy plant, by contaminated tools. The virus also contaminates the seed coats, and the plants germinating from these seeds can become infected.

SIGNS AND SYMPTOMS

Symptoms first appear about 10 days after infection. Stunted growth. Non uniform coloring and delayed ripening. Specific symptoms depend on the host plant, age of the infected plant, environmental conditions, the virus strain and the genetic background of the host plant. Common signs includes mosaic like patches on the leaves, curling of leaves and the yellowing of plant tissues.

CONTROL MEASURES

1. Seed beds should be located at a distance from the tobacco house.
2. Seed bed soil should be sterilized by steam.
3. To avoid contamination proper care should be taken. Since pipe tobacco, cigarettes and chewing tobacco are all sources of primary inoculum, smoking or chewing of any kind of tobacco should be avoided.
4. Susceptible hosts, weed or otherwise in which virus may harbour, should be destroyed.
5. Diseased plants should be removed and should be burned to stop further spread of the disease.
6. Growing resistant varieties makes a good way of preventing the disease.

NOTES

REFERENCES

1. Baily and Scott. (2017). Diagnostic Microbiology (14th Edition), Mosby London.

2. Collins and Lyne. (2004). Microbiological methods (8th Edition), Arnold publishers, New York.

3. Demain, A.L, and Davis, J.E. (1999). Manual of Industrial Microbiology and Biotechnology (2nd edition), American Society for Microbiology, Washington.

4. Hudson, L. and Hay, F.C. (1989). Practical Immunology (3rd Edition), Blackwell scientific Publications, Oxford.28

5. Lippincott Williams and Wilkins. (2006). Koneman's Color Atlas and Text book of Diagnostic Microbiology. Philadelphia, Baltimore.

6. Monica Cheesbrough, (2000). District Laboratory Practice in Tropical Countries, Part – 2, Cambridge University Press, Cambridge, U.K.

7. Myers, R.L. (1989). Immunology: A Laboratory Manual, Wm. C.Brown Publishers, Dubuque, Lowa.

8. Rastogi, S.C. (2012). Immunodiagnostics Principles and Practice, New Age International (P) Ltd., New Delhi.

9. Talwar, G.P. (1983). A Hand Book of Practical Immunology, Vikas Publishing House Pvt. Ltd., New Delhi.

10. Cappuccino, J.G and Sherman, G. (2014). Microbiology: A Laboratory Manual Addison (4th Edition), Wesley.