1a) CATALASE TEST

Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape up any blood agar-blood cels are catalase positive and any contaminating agar could give a fake positive.

Add a few drops of H2O2 onto the smear. If needed, mix with a toothpick. DO NOT use a metal loop or needle with H2O2; it will give a false positive and degrade the metal.

A positive result is the rapid evolution of O2 as evidenced by bubbling.

A negative result is no bubbles or only a few scattered bubbles

Dispose of your slide in the biohazard glass disposal container. Dispose of any toothpicks in the pipet keeper.

b) OXIDASE TEST

To open a new reagent dispenser; Hold reagent dropper upright and point tip away from yourself. Grasp the middle with thumb and forefinger and squeeze gently to crush the glass ampule inside the dropper. Tap the bottom on the tabletop a few times. Invert the ampule and squeeze gently for drop by drop dispensing.

With a sterile swab, obtain a small amount of organism from an agar slant or plate.

Place one drop of reagent onto the culture on the swab.

Positive reactions turn the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions should be ignored.

c) TRIPLE SUGAR IRON AGAR

With a sterilized straight inoculation needle touch the top of a well-isolated colony

Inoculated TSI agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.

Leave the cap on loosely and incubate the tube at 350C in ambient air for 18 to 24 hours

d) COAGULASE TEST

Place a drop of physiological saline on each end of a slide, or on two separate slides.

With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drops to make two thick suspensions.

Add a drop of human or rabbit plasma to one of the suspensions, and mix gently

Look for clumping of the organisms within 10 seconds.

No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

e) OXIDATIVE FERMENTATIVE TEST

Inoculate two tubes of OF test medium with the test organism using a straight wire by stabbing ‘’half way to the bottom’’ of the tube.

Cover one tube of each pair with 1 cm layer of STERILE MINERAL OIL OR LIQUID PARAFFIN (it creates anaerobic condition in the tube by preventing diffusion of oxygen), leaving the other tube open to the air

Incubate both tubes at 350C for 48 hours (slow growing bacteria may take 3 to 4 days before results can be observed)

f) INDOLE TEST

Take a sterilized test tubes containing 4ml of tryptophan broth.

Inoculate the tube aseptically by taking the growth from 18 to 24 hours culture

Incubate the tube at 370C for 24-28 hours.

Add 0.5ml of Kovac’s reagent to the broth culture.

Observe for the presence or absence of ring

g) CARBOHYDRATE FERMENTATION TEST

Allow medium to warm to room temperature prior to inoculation.

Inoculate the purple broth(with carbohydrate of choice) with isolated colonies from an 18-24 hour pure culture of the organism.

Inoculate a control tube of purple broth base in parallel with the carbohydrate based media.

Incubate inoculated media aerobically at 35-37oC. For 3-5 days. NOTE; increased incubation up to 30 days may be necessary for some microorganisms.

Observe daily for development of a yellow color in the medium

h) UREASE TEST

Streak the surface of a urea agar slant with a portion of a well isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture

Leave the cap on loosely and incubate the tube at 350-370C in ambient air for 48 hours to 7 days.

Examine for the development of a pink color for as long as 7 days

1. HEMOLYSIS PATTERN ON BLOOD SUGAR TEST

Prepare the blood agar base as instructed by the manufacturer. Sterilize by autoclaving at 121oC for 15 minutes.

Transfer thus prepared blood agar base to a 50oC water bath.

When the agar base is cooled to 50oC, add sterile blood aseptically and mix well gently. Avoid the formation of air bubbles. You must have warmed the blood to room temperature at the time of dispensing to molten agar base. NOTE; If you are planning to prepare a batch of blood agar plates, prepare few blood agar plates first to ensure that blood is sterile.

Dispense 15ml amounts to sterile petri plates aseptically

Label the medium with the date of preparation and give it a batch number if necessary

Store the plates at 2-80C, preferably in sealed plastic bags to prevent loss of moisture. The shelf life of thus prepared blood agar is up to four weeks.

j) CAMP TEST

Streak a beta-lysin-producing stain of aureus down the center of a sheep blood agar plate

The streptococcal streak should be 3 to 4cm long.

Streak test organisms cross the plate perpendicular to the aureus streak within 2mm. (Multiple organisms can be tested on a single plate)

Incubate at 350-370C in ambient air for 18-24 hours

Group B streptococcal and a few other beta-streptococci produce an enhancement of the beta-lysin activity of the aureus strain.

2 a) CONVENTIONAL METHODS

The conventional method of ECM fungal identification involves noting the morphological characteristics of mushrooms such as their size, color, presence or absence of volva, stipe, ring, scales, reticulum, zonation, striation, warts, cap, araeloa and gills. Transverse sections of the sporocarps microscopically. Fungal hyphae are stained with lactophenol cotton blue solution and observed under the microscope and images captured (Olympus BX51 compound microscope attached with Olymous DP71 digital camera).

b) GMS STAIN (METHENAMINE SILVER-GROCOTT’S)

This stain is used for fungal identification. Fungal organisms contain mucopolysaccharide in their cell walls that will oxidize to react and aldehyde group. Silver nitrate reacts with the aldehyde group reducing it to metallic silver, making them visible. The fungi cell walls are outlined black/brown. Background stain is green. Reagents used in the procedure include 2% chromic acid, 1% metabisulfite, 5% borax, methenamine silver, 0.55 golf chloride, and 0.2% light green.

c) LACTOPHENOL COTTON BLUE (LPCB)

The lactophenol cotton blue wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components; 1. PHENOL; kills any live organisms 2. LACTIC ACID; it preserves fungal structures and, 3. COTTON BLUE; it stains the chitin in the fungal cell walls. Lactophenol cotton blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue.