Methods isolation of microorganisms using swab

Follow several methods to collect and isolate diagnose microorganisms of various body tissues (as shown in the table), which shows some examples to facilitate isolating pathogenic microorganisms, and when failure to isolate causative organism requires to follow other methods.

1- Upper Respiratory Tract culture

For purpose of isolation pathogenic microorganisms from upper respiratory tract is taking swabs from throat or nasopharynx swabs on culture swabs on transport media to ensure transfer to laboratory before being damaged. The sample culture on surface media Sheep blood agar and incubating typically 5-10% Co_2 for purpose of isolating pathogenic microorganisms existing.

Allows blood agar to investigate hemolysis blood of alpha type by watching green color surrounding colony in blood agar media, and investigation of hemolysis of beta blood type (β –hemolysis) by observing clear areas around colony in blood agar media, and when culturing swabs from throat note that most colonies of pathogenic bacteria belonging to genus *Strep. pyogenes* seem relatively small size and hemolysis blood from beta type on blood agar media.

Note // it is improtant to be not used **human blood** for preparation of this medium as found antibodies that inhibits growth of bacteria, especially belonging to *Streptococcus ssp* but using sheep blood is for this purpose.

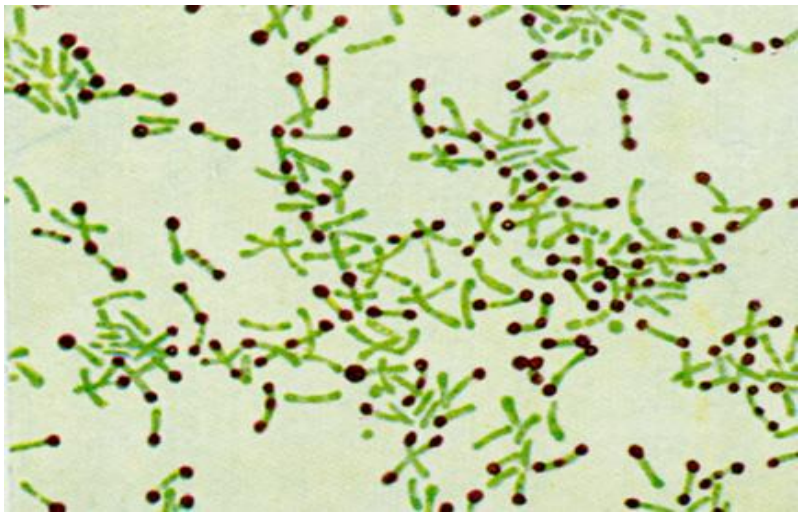
The importance β-hemolytic investigating is consided of one cause of rheumatoid fever, also be investigated for presence of *Staphylococcus aureus* that cause showing β-hemolytic on blood agar media.

Possible to investigate bacteria *Haemophilus influenzae* and *Neisseria meningitidis* and *N. gonorrhoeae* using throat swabs using different culturing media, this microorganism growth better on media of **chocolate agar** (the media preparation by heated blood agar to until color turns brown). The preferred use media to isolate *N. gonorrhoeae* also is **Thayer - martin agar** containing antibiotics which inhibit growth of normal flora of **throat**.

The upper respiratory tract infection with these microorganisms are serious diseases including **Meningitis**, so early diagnosis is important, as lead early diagnosis when suspected infection with bacteria *H. influenzae* for being cause of disease, acute epiglottitis and leading to death within 24 hours.

Using Nasopharyngeal swabs to diagnose a number of pathogenic bacteria, the most important of *Bordetella pertussis* cause whooping cough, and need to isolate these bacteria used Bordet-Gengou potato media.

Need it for special steps when suspected injury with **Diphtheria**, the observation of chinese letters bacteria using microscopic examination is not related to ***Corynebacterium diphtheriae***, as there are bacteria that is not pathogenic within normal flora of throat known as ***diphtheroids*** which is assemble in the same way but it is different from bacteria ***Corynebacterium diphtheriae***. ***Diphtheria*** being thick and shoot, and when an examination of gram stain **to *Corynebacterium diphtheriae*** notes bacteria bacilli curved or straight and mostly enlarged club shape and the side grouped in Chinese letters and there is found beads, which is source of energy and called **Metachromatic granules** and consider gram positive stain, used **Alberts stain to explain these granules** as it appears **green color** inside areas stain brown representing grains, and when you see bacteria, writes in report laboratory (KLB bacteria suspected to be positive), this is usually recommended that steps above when suspected Diphtheria case by doctor who writes a swab KLB is meaning names of scientists Klebs, Loeffer, then taken swab from area of infection and tonsillitis as it is surrounded by a membrane **Pseudomembrane** composed of **macrophages** and **neutrophiles** and remains of dead tissue and bacteria with secretions of cells and membrane white envelope to **tonsils**, and block process of swallowing may extend to trachea, there by block passage of air to lungs so-called disease **diphtheria**.



Form of *Corynebacterium diphtheriae* bacteria

Microorganism General Characteristics

- Gram-positive bacillus with club-shaped swelling at each end (5)
- Non-motile
- Facultative anaerobic
- Non-endospore-forming
- Dividing cells are often observed to fold together to form V- and Y-shaped which are often described as having a Chinese character appearance

Identification Key Tests

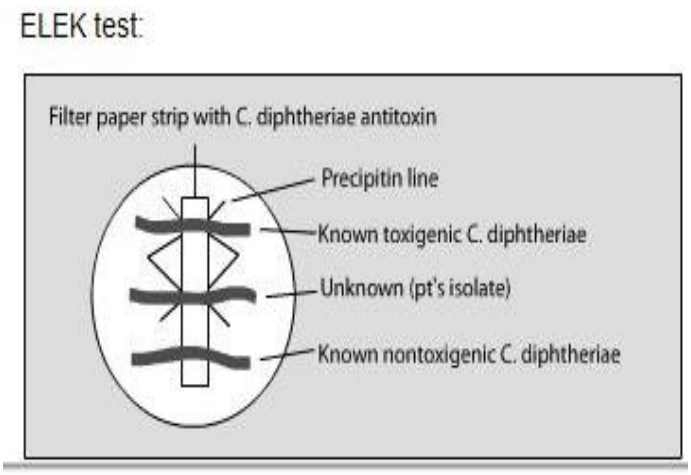
- Albert staining identifies *C. diphtheriae*. *C. diphtheriae* produces characteristic metachromatic granules that appear bluish black and the body appears green or bluish green. Methylene blue is also used for staining, which makes the granules appear red.

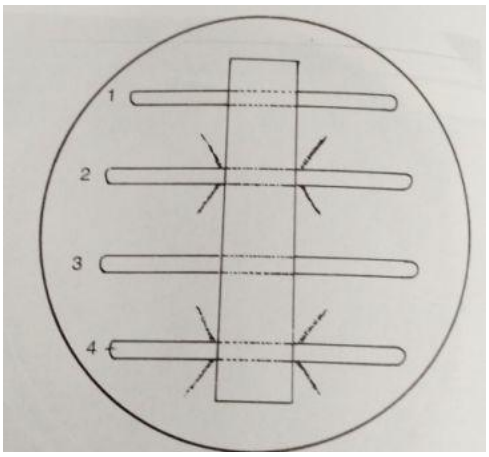
- Other diagnostic tests include isolation of *C. diphtheriae* on culture, biotype (i.e., substrain) determination, and toxigenicity testing. Toxigenicity testing is performed by using the Elek test, which determines whether the isolated *C. diphtheriae* produces toxin.

- Polymerase chain reaction (PCR) testing is performed by CDC, which can be done to confirm a toxigenic strain infection. The PCR test detects the regulatory gene for toxin production and the diphtheria toxin gene.

To confirm isolation depends on test another important as it sends isolate to determine toxicity by Eleks test is a test for toxicity of this bacteria in addition to an examination of sensitivity to antibiotics, this is existence and isolation of these bacteria from cases that require action state of emergency in laboratory as it should be sterilized air in laboratory in addition to tables and other routine examination.

Elek test is an **in vitro immunoprecipitation (immunodiffusion) test** to determine whether or not a strain of *Corynebacterium diphtheriae* is toxigenic. A test strips of filter paper containing diphtheria antitoxin is placed in the center of the agar plate. Strains to be tested (patient's isolate), known positive and negative toxigenic strains are also streaked on the agar's surface in a line across the plate and at a right angle to the antitoxin paper strip.





Elek test for demonstration of toxin production by *C. diphtheria*

Antitoxin diffuses away from the strip of filter paper where as toxin produced by toxin-producing strains diffuse away from growth. At the zone of equivalence, a precipitin line is formed.

Procedure:

1. Mix a tube of melted nutrient agar with 2 ml of sterile horse serum.
2. Rotate the tube to mix the serum and agar. Do not shake the tube.
3. Pour the mixture into a sterile petri dish.
4. Using lightly flamed forceps, lay the strip of anti-toxin impregnated filter paper across the centre of the petri dish allowing it to sink beneath the agar surface.

5. Allow the agar to set, then lift one corner of the lid and let the plate dry for 30-45 min in the incubator.
6. When dry inoculate with a toxinogenic strain of *C. diphtheriae* by streaking a single line of inoculum across the plate and paper strip at right angles to the strip.
7. Repeat this about 1 inch away from the C. *diphtheriae* inoculum with a test strain.
8. Incubate the plate for 24 hrs and observe the results.

Result:

After 24 hours of incubation at 37**°**C, plate is examined with transmitted light for the presence of fine precipitin lines **at 45-degree angle to the streaks.**

Positive Test: Precipitin lines form at zone of equivalence, test organism is toxigenic.

If toxin is produced by the test strain, it diffuses sideways from the streak. The antitoxin diffuses from the filter paper and where the toxin and the antitoxin meet (at zone of equivalence) a **precipitin line formed**.

The control strain also will cause a precipitate to form which will coalesce with the precipitate of the test strain to form a line of identity

See the image (right side) and try to interpret yourself before seeing the explanation below:

1. Line 1 is a negative control
2. Line 2 is the positive control
3. Line 3 is a test organism that is a nontoxigenic strain *C. diphtheriae*
4. Line 4 is a test organism that is a toxigenic strain of *C. diphtheriae*

2- Lower Respiratory tract culture

The isolation of pathogenic microorganisms from respiratory tract (LRT) is important and more difficult to isolate from upper respiratory tract (URT), and uses sample of sputum usually. And sputum represents exudate containing substances of lower respiratory tract, and is different in quality and must examination microscopically to determine suitability for culturing, if accepted sample containing large numbers neutrophile which shows presence of mucus that contains a small number squamous epithelial cells as presence of these epithelial cells frequently shows contamination sputum nasopharynx secretions and therefore would not be a sample for lower respiratory tract.

There are several ways to collect sputum samples, including the use of special tubes collected from trachea and lung, or use allergenic substances such as Nacl solution and solution Acety cytein. These ways are more accurate because samples will not be contaminated with nasopharynx secretions.

Sputum

Includes analysis of sputum is the president of the three tests:

1- General examination
2- Sputum culture
3- Cytology

• General examination

Sputum secretions generated from pulmonary bronchioles that come out through the respiratory tract may be found nature or pathogenic according to presence materials of sputum. Can be divided into tests of the sputum to:

A - Macroscopic: can be observed with the naked eye and include the following:

1 - Volume

This is the measurement of the amount of sputum collected during the period of (24) hours for the infected person and this test important implications depending on the type of condition as utilized in the diagnosis of acute bronchitis, as well as an abscess in the lungs and some cases of asthma .

2- Consistency

Sputum multiple forms of what is serous or liquid may be mucous containing pus cell (purulent) may be a mixture sero-mucoid.

3- Colour

The sputum colorless transparent net in the natural state has turned to the color yellow container pus cells this watching in cases of

pulmonary infection may become sputum color to green when the infection with the bacteria *Pseudomonas* may show rust-colored as a result of broken blood cells and exit of hemoglobin with sputum as well as in the incidence of *Pneumococcal* bacteria, or in cases of gangrene lungs. The color is bright red sputum, which refers to the blood of a modern mixed with sputum and that those seen in cases of angina pectoris or cardiac infarction.

In cases of angina pulmonary or in cases of rupture of a blood vessel in the respiratory tract (bronchitis or pulmonary bronchioles) leading to the exit of fresh blood with sputum.

4-Odour

Sputum natural odorless but in the presence of pus cells will be a stench as well as the smell is unpleasant in cases of pulmonary tuberculosis by bacteria *Mycobacterium tuberculosis* as well as gangrene pneumonia.

B- Microscopic

And are made using two pigments gram stain to verify the presence of pus cells and diagnosis of bacteria causing the injury or inflammation. As well as pigmentation of sputum samples stained Zeil-neelson (Acid fast stain) to check for tuberculosis pulmonary infection caused by bacteria, *Mycobacterium tuberculosis* and items are not always the work of this routine in the examinations only at the request of a doctor when sent to the laboratory (sputum for AFB). Can also identify some fungal infections in the lungs through the examination of slides stained with activities such as nature, which may cause serious diseases.

- **Sputum culture**

Grown sputum on the following medium:

1- Blood agar
2- Chocolate agar
3- MacConkey agar

Repeat grown in Blood agar and Chocolate agar twice any two dishes for each sample incubation the dish first in aerobic conditions incubation, either dish the second incubation found of gas Co2 mean anaerobic conditions. But MacConkey agar incubation aerobic conditions only,

examine the dishes at the end of the incubation period as can identify the most important bacteria causing pneumonia or respiratory infection:

1- *Streptococcus pyogens*
2- *Staphylococcus aureus*
3- *Haemophilus influenzae*
4- *Streptococcus pneumoniae*
5- *Pseudomonas aeruginosa*
6- *Klebsiella pneumonia*

And the characteristics of each sex and type of bacteria to hold dye gram, as well as work tests biochemical test and serological tests using serums antisera and after full diagnostic of the types of bacteria developing countries are examined sensitivity to antibiotics, which is condition for each grown laboratory. Of effective antibiotics in cases of pneumonia or respiratory infections:

1- Gentamacin, 2- Ciprofloxacin, 3- Doxcycyclin, 4- Licomycin
5- Rifampicin, 6- Ampiclox (Ampicillin+ cloxacillin)
7- Erythromycin, 8- Tetracycline, 9- Cephalexin, 10- Amoxcillin

As we mentioned earlier that tests the task that requires to be held on the sputum is to examine of bacteria, *Mycobacterium tuberculosis*, or TB bacilli resistant Acid fast bacteria as treated sputum here special treatment before the growth and include treatment of sample sputum volume of similar material base weak.

Called way petroffs method is to take place for the disposal of the fiber in the mucous sputum and to edit the bacilli of external mucous layer peripheral because the preparation of this bacterium in the sputum may be few, so these numbers are being concentrated to give an opportunity for bacterial isolates for growth.

Mixed sample with material base such as NaoH and placed the mixture in the incubator (37C°) for half an hour and a shake sputum several times during this period for the purpose of consistency and complete melting of the material mucous, then be a centrifuge on the model (3000 cycles / minute for half an hour), which leads to obtain the bacteria in the bottom of the tube as it is to get disposal of liquid net and taken sludge basal and dyeing dye Zeil-neelson as well as growth the form on the medium special for the bacteria tuberculosis (Lowerstein-jansen

media) which is perparation already in the form of slant, after the incubation period show colony bacteria yellow color can keep this medium in the incubator for more than a month, and examines each (2-3) days to verify the bacteria and the appearance of colonies, because the bacteria TB slow growth and generation time have a long (18-20 hours) compared to bacteria *E.coli* (20 minutes) so we need sufficient time for the appearance of growth bacteria.

Of other tests that are to accompany the diagnosis of tuberculosis is to examine the ESR as well as radio photography X-ray-X, which may show foci of injury, which enhances laboratory diagnosis.

In case of suspected infecting fungi by microscopic examination culturing samples on the media Sabouraud dextrose agar added his antibiotics for diagnosis of *Candida albicans* and *Coccidioids immitis* and *Histoplasma capsulatum.*

- **Cytology**

This examination is in the preparation of a slide of the patient's sputum and dyeing dye (Paps stain), which is special to pigment the cells in the sputum and to identify abnormal cells that indicate the presence of lung cancers.