DIAGNOSIS OF INFECTIOUS DISEASES Lec. 1

The diagnosis of infectious diseases requires an understanding of the presenting clinical manifestations and a knowledge of microbiology.

The methods that used for identification of microorganisms fall into **three categories**:

**1- Phenotypic**- morphology (micro and macroscopic)

**2- Immunological**- serological analysis

**3- Genotypic**- genetic techniques

**Ι - Diagnosis of Respiratory Tract(RT) Infections :**

RT is broadly divided into:

1- Upper respiratory tract

2- Lower respiratory tract

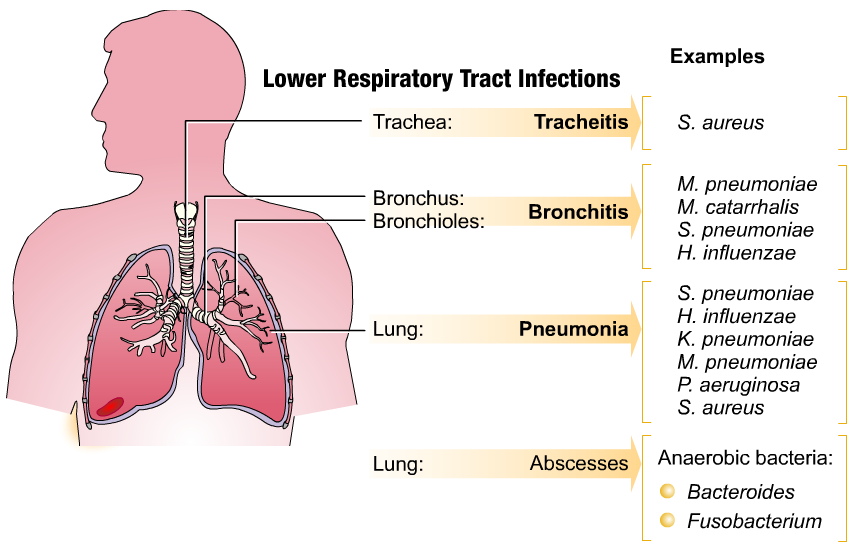
* Upper Respiratory Tract Infection

Upper respiratory tract disorder or infection (URI or URTI) is a medical condition caused by acute infection that involves upper respiratory tract such as sinuses, nose, larynx or pharynx. Pharyngitis, tonsillitis, sinusitis, laryngitis, common cold, and otitis media are some of the most common upper respiratory tract disorders. Upper respiratory tract disorders are caused by direct invasion of the mucus membrane (inner respiratory tract lining) by bacteria or virus. Rhinorrhea, sneezing, nasal congestion, nasal discharge, sore throat, cough, fever, odynophagia and malaise are some of the most common symptoms observed in the patient diagnosed with upper respiratory tract disorder. Some other symptoms of the disease are headache, foul breath, hyposmia, sinus pain, shortness of breath, conjunctivitis, vomiting diarrhea, nausea, and body ache.

Upper respiratory tract disorder can be diagnosed by physical examination, laboratory tests and review of symptoms. In physical examination, physicians look for redness and swollen inside the wall of nasal cavity, white secretions on the tonsils, redness of the eyes, redness of the throat, and enlarged lymph nodes around neck and head. Laboratory testing is usually not performed for examining upper respiratory tract disorders as most of the upper respiratory tract infections are caused by viruses and no specific treatment for different types of viral upper respiratory tract disorder is available in current scenario.

Lower Respiratory Tract Infections

As shown in the figure below



A. SPECIMENS

Collection of specimen in the case of RTI posses a number of problems because , there is enormous commensal flora that colonizes this tract .Therefore, must avoid contamination of the specimens.

1. Throat

Most “**sore throats**” are due to viral infection. Only 5–10% of “sore throats” in adults and 15– 20% in children are associated with bacterial infections. The finding of yellowish exudate or a grayish membrane must induce the suspicion that Lancefield group A β-hemolytic streptococcal, diphtherial, or candidal infection exists; such signs may also be present in infectious mononucleosis, adenovirus, and other virus infections.

Throat swabs are taken from each tonsillar area and from the posterior pharyngeal wall.

- The normal throat flora includes an abundance of viridans streptococci, Neisseriae , diphtheroids, staphylococci, small gram-negative rods, and many other organisms. Microscopic examination of smears from throat swabs is of little value in streptococcal infections, because all throats harbor a predominance of streptococci.

- Cultures of throat swabs are most reliable. Media selective for streptococci can be used to culture for group A organisms. In streaking selective media for streptococci or blood agar.

2. Nasopharynx

Specimens from the nasopharynx are studied infrequently because special techniques must be used to obtain them. Whooping cough is diagnosed by culture of *B. pertussis* from nasopharyngeal or nasal washings or, what is better, by PCR amplification of *B* .*pertussis* DNA in the specimen.

To perform a nasal wash, fill a small syringe (3-5 mL) with sodium chloride solution and attach a short length of flexible tubing. With the patient's head tilted back, instill the solution rapidly into the nostril, then immediately aspirate secretions back into the syringe and transfer the aspirate to laboratory specimen containers.

**3. Middle Ear—**Specimens are rarely obtained from the middle ear. In acute otitis media, 30–50% of aspirated fluids are bacteriologically sterile. The most frequently isolated bacteria are pneumococci, *H .influenzae, Moraxella* *catarrhalis,* and hemolytic streptococci.

* Lower Respiratory Tract

Bronchial and pulmonary secretions of exudates are often studied by examining **sputum**. The most misleading aspect of sputum examination is the contamination with saliva and mouth flora. Thus, finding candida, *S*. *aureus,* or even *Streptococcus pneumoniae* in the sputum of a patient with pneumonitis has no etiologic significance unless supported by the clinical picture.

-The presence of many squamous epithelial cells suggests heavy contamination with saliva; a large number of polymorphonuclear leukocytes (PMNs) suggests

a purulent exudate.

5. Transtracheal Aspiration, Bronchoscopy, Lung Biopsy, Bronchoalveolar Lavage

Specimens obtained by bronchoscopy may be necessary in the diagnosis of *Pneumocystis pneumonia* or infection due to *Legionella* or other organisms. Bronchoalveolar lavage specimens are particularly useful in immunocompromised patients with diffuse pneumonia.

B. MICROSCOPIC EXAMINATION

1- Smears of purulent flecks or granules from sputum stained by **Gram’s stain** or **acid-fast methods** may reveal causative organisms and PMNs.

2- Some organisms (eg, Actinomyces) are best seen in unstained wet preparations.

3- A direct “quelling” test for pneumococci can be performed with polyvalent serum on fresh sputum.

C. CULTURE

The media used for sputum cultures must be suitable for the growth of bacteria (eg, pneumococci, *Klebsiella*), fungi (eg, *Coccidioides immitis*), mycobacteria (eg, *M*. *tuberculosis*), and other organisms. Specimens obtained by bronchoscopy and lung biopsy should also be cultured on other media (eg, for anaerobes, *Legionella,* and others).

**ΙΙ - Diagnosis of Gastrointestinal Tract infections**

The intestinal microflora is a complex ecosystem containing over 400 bacterial species**.** The stability of normal flora both discourages infection by exogenous pathogens and prevents overgrowth of potentially pathogenic members.

The infections include

### 1- Peritonitis: several types of bacteria from the intestinal microflora, particularly species of *Bacteroides*, *Clostridium*, and *Peptostreptococcus*

### 2- Bacterial Diarrheas :*Enterotoxin-Mediated Diarrheas*: Enterotoxigenic bacteria, such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli* strains, colonize the upper bowel and cause watery diarrhea by producing an enterotoxin that stimulates mucosal cells to secrete fluid via an increase in intracellular CAMP and *E coli*.

**3- Other Diarrhea-Causing Toxins :** An organism that produces a different type of cytotoxin is *Vibrio parahaemolyticus*, a bacterium associated with seafood. Food-poisoning strains of *Staphylococcus aureus* and *Clostridium perfringens* both produce enterotoxins that are cytotoxic. The staphylococcal enterotoxin also has a direct effect on the vomiting center in the brain.

### 4- Gastrointestinal Disease Caused by Invasive Bacteria :Unlike the enterotoxigenic organisms, invasive bacteria exert their main impact on the host by causing gross destruction of the epithelial architecture; histological findings include mucosal ulceration and an inflammatory reaction in the lamina propria. The principal pathogens in this group are *Salmonella, Shigella, Campylobacter*, invasive *E coli*, and *Yersinia*. The enteric viruses also invade intestinal epithelial cells, but the extent of mucosal destruction is considerably less than that caused by invasive bacterial pathogens

### 5- Viral Diarrheas : Rotavirus and Calicivirus (formerly Norwalk virus) are major causes of diarrheal disease. Rotavirus diarrhea affects mostly young children; Calicivirus causes disease in all age groups

### 6- Parasitic Diarrheas : Some protozoa (especially *Entamoeba histolytica* and *Giardia lamblia*) as well as some intestinal helminths can cause diarrheal disease.

**Clinical Diagnosis**

The various ailments of the gastrointestinal (GI) tract affect food digestion and absorption. Diagnosing them is critical to effective treatment and prevention of complications. Here are 11 diagnostic tests that help diagnose various GI disorders:

**1. Clinical examination**

**2. Blood test:** [Blood tests](http://www.thehealthsite.com/diseases-conditions/blood-tests-understanding-the-different-kinds/) like blood count, liver function test, lactose tolerance test, antibodies to H. pylori, pancreatic enzyme test, etc. can help diagnose certain GI conditions like bacterial or parasitic infection, celiac disease, lactose intolerance, exact cause of diarrhoea, etc.

**3. Stool analysis**: For the analysis, a stool sample collected in a clean container is sent to the laboratory for microscopic examination and other tests.

Changes in colour, consistency and pH, and the presence of mucus, blood, white blood cells, bile, fat, sugars, etc. help diagnose GI conditions. A stool culture can help diagnose bacterial infection. Increased fat levels in the stool may be seen in celiac disease, pancreatitis, etc. High pH of the stool could indicate inflammation, cancer, etc. Some enzyme immunoassays are used to detect viral and bacterial antigen in stool sample.

**4. Renal function test**

**5. Endoscopy**

**6. Abdominal ultrasound, CT scan and MRI**

**7. Barium X-ray**

**8. Manometry**

**9. pH Monitoring**

**10. Breath Test**

The **hydrogen breath test** measures hydrogen in the breath to diagnose GI conditions like [lactose intolerance](http://www.thehealthsite.com/diseases-conditions/lactose-intolerance/001/), **Urea breath test** helps diagnose peptic ulcer. The test looks for presence of Helicobacter pylori bacteria in the stomach.

**11. Transient Elastography**

**A. SPECIMENS**

Feces and rectal swabs are the most readily available specimens. The presence of bile may reveal infection of the biliary tract. The presence of blood, mucus, leukocytes or helminths must be noted .

- Special techniques must be used to search for parasitic protozoa and helminths and their ova.

- Stained smears may reveal a prevalence of leukocytes and certain abnormal organisms, eg, candida or staphylococci, but they cannot be used to differentiate enteric bacterial pathogens from normal flora.

**B. Culture**

Specimens are suspended in broth and cultured on ordinary as well as differential media (eg, MacConkey agar, EMB agar) to permit separation of non-lactose fermenting gram-negative rods from other enteric bacteria.

- If salmonella infection is suspected, the specimen is also placed in an enrichment medium (eg, Selenite F broth) for 18 hours before being plated on differential media (eg, Hektoen enteric or Shigella-Salmonella agar).

*- Yersinia enterocolitica* is more likely to be isolated after storage of fecal suspensions for 2 weeks at 4 °C, but it can be isolated on Yersinia or Shigella-Salmonella agar incubated at 25 °C.

- Vibrios grow best on Thiosulfate Citrate Bile Salts Sucrose agar.

- Campylobacters are isolated on Campy agar or Skirrow’s selective medium incubated at 40–42 °C in 10% CO2 with greatly reduced O2 tension. Bacterial colonies are identified by standard bacteriologic methods. Agglutination of bacteria from suspect colonies by pooled specific antiserum is often the fastest way to establish the presence of *Salmonella* or *Shigella* in the intestinal tract.

**C. Non–Culture-Based Methods**

Enzyme immunoassays (EIAs) for detection of specific enteric pathogens, either directly in stool specimens or to confirm growth in broth or on plated media, are available. Enzyme immunoassays EIAs that detect Shiga toxins 1 and 2 in suspected cases of colitis caused by enterohemorrhagic *E coli* (also called shiga toxin producing *E coli* or STEC) are available and are superior to culture. Also available are EIAs for direct detection of *Campylobacter jejuni, Giardia lamblia, Cryptosporidium* *parvum,* and *Entamoeba histolytica*. The performance of these assays is variable.

Intestinal parasites and their ova are discovered by repeated microscopic study of fresh fecal specimens. The specimens require special handling in the laboratory.

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| **Disease** | **Specimen** | **Common Causative**  **Agents** | **Usual Microscopic**  **Findings** | **Culture Media** |
| **Enteric fever, typhoid** | **Blood, feces,**  **urine** | ***Salmonella Typhi*** | **Not recommended** | **MacConkey, Hektoen, bismuth sulfite agars; SS agar**  **( lactose negative. H2S produced)** |
| **Enteritis, enterocolitis,**  **bacterial**  **diarrheas, “gastroenteritis”** | **Feces** | ***Salmonella* species**  **other than**  **S. Typhi** | **Gram stain or methylene blue stain may show PMNs** | **MacConkey, Hektoen, bismuth sulfite agars; SS agar** |
| ***Shigella* species** | **Gram stain or methylene blue stain may show PMNs** | **MacConkey, Hektoen, bismuth sulfite agars; SS agar( Non-lactose-fermenting, no H2S )** |
| ***Campylobacter jejuni*** | **“Gull wing-shaped” gram negative rods and often PMNs** | **Campy BAP or similar medium**  **Incubate at 42 °C** |
| ***Vibrio cholerae*** | **Not recommended** | **Thiosulfate citrate bile**  **salts sucrose agar(TCBS); others. Taurocholate, Yellow colonies on TCBS.**  **-peptone broth for enrichment** |
| **Other vibrios** | **Not recommended** | **As for *V. cholerae***  **- Differentiate from *V. cholerae* by biochemical and culture tests** |
| ***Yersinia enterocolitica*** | **Not recommended** | **MacConkey, SS agar**  **Enrichment at 4 °C helpful; incubate cultures at 25 °C** |
| **Hemorrhagic colitis and hemolytic**  **uremic**  **syndrome** | **Feces** | ***E. coli* O157:H7** | **Not recommended** | **Sorbitol MacConkey medium, sorbitol-negative colonies; type with antisera for O antigen 157 and flagellar antigen 7** |

**ΙΙΙ-Diagnosis of Urinary tract infections Lec2**

The urinary system consists of kidneys, the drainage system and the bladder the diseases include Cystitis (infection of bladder), Pyelonephritis(infection of renal parenchyma), Asymptomatic Bacteriuria, Renal Abscess.

Urinary tract and urine are normally sterile. Numerous mechanical and biologic processes ensure that microorganisms do not enter the urinary tract. Women are more susceptible to urinary infections because the female urethra is short and because the area around the urethral opening is colonized with potential pathogens (e.g. *E coli* and *E faecalis*).

Organisms normally present in the intestinal tract cause most urinary tract infections. The commonest of these by far is *E.coli*, which is responsible for 80 percent of infections that are **acquired outside of hospitals** . Other Gram-negative rods such as *Klebsiella*, *Enterobacter*, and *Proteus* spp are relatively common, each accounting for 3 to 5 percent of infections. **Within the hospital environment**, *Pseudomonas aeruginosa*, *Serratia marscesens*, and other, more resistant, hospital-associated pathogens account for many infections.

Gram-positive organisms, particularly coagulase-negative staphylococci and enterococci, cause some infections. *Staphylococcus saprophyticus* causes about 10 percent of urinary tract infections in young women. *Candida albicans* is also a frequent pathogen in hospitalized patients, particularly if diabetes is present.

Anaerobes and fastidious organisms rarely cause urinary infections. A number of viruses, particularly mumps virus, cytomegalovirus, and coxsackie viruses, can be present in the kidneys and urine, but rarely cause symptoms or any consequences.

Urine secreted in the kidney is sterile unless the kidney is infected. Uncontaminated bladder urine is also normally sterile. The urethra, however, contains a normal flora, so that normal voided urine contains small numbers of bacteria. Because it is necessary to distinguish contaminating organisms from etiologically important organisms, only *quantitative* urine examination can yield meaningful results.

The following steps are essential in proper urine examination.

**A. PROPER COLLECTION OF SPECIMEN**

Proper collection of the specimen is the single most important step in a urine culture , using the urine cup, collect a midstream specimen. Properly label the cup.

Because many types of microorganisms multiply rapidly in urine at room or body temperature, urine specimens must be delivered to the laboratory rapidly or refrigerated not longer than overnight.

**B. MICROSCOPIC EXAMINATION**

Much can be learned from simple microscopic examination of urine. A drop of fresh uncentrifuged urine placed on a slide, covered with a cover glass, and examined under microscope can reveal leukocytes, epithelial cells, and bacteria. A Gram-stained smear of uncentrifuged midstream urine that shows gram-negative rods is diagnostic of urinary tract infection.

The presence of many squamous epithelial cells, lactobacilli, or mixed flora on culture suggests improper urine collection.

Some urine dipsticks contain leukocyte esterase and nitrite, measurements of polymorphonuclear cells and bacteria, respectively, in the urine. Positive reactions are strongly suggestive of bacterial urinary tract infection.

**C. CULTURE**

Culture of the urine, to be meaningful, must be performed quantitatively. Properly collected urine is cultured in measured amounts on solid media, and the colonies that appear after incubation are counted to indicate the number of bacteria per milliliter , if more than 105 colonies/mL are cultivated from a properly collected and properly cultured urine specimen, this constitutes strong evidence of active urinary tract infection. The presence of more than 105 bacteria of the same type per milliliter in two consecutive specimens establishes a diagnosis of active infection of the urinary tract with 95% certainty. If fewer bacteria are cultivated, repeated examination of urine is indicated to establish the presence of infection.

The presence of fewer than 104 bacteria per milliliter, including several different types of bacteria, suggests that organisms come from the normal flora and are contaminants, usually from an improperly collected specimen. The presence of 104/mL of a single type of enteric gram-negative rod is strongly suggestive of urinary tract infection, especially in men. Occasionally, young women with acute dysuria and urinary tract infection will have 102–103/mL. If cultures are negative but clinical signs of urinary tract infection are present, “urethral syndrome,” urethral obstruction, tuberculosis of the bladder, or other disease must be considered.

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| **Disease** | **Specimen** | **Common Causative**  **Agents** | **Usual Microscopic**  **Findings** | **Culture Media** |
| **Urinary tract infection** | **Urine (clean catch**  **midstream**  **specimen**  **or one**  **obtained by**  **bladder catheterization**  **or suprapubic**  **aspiration)** | ***E.coli;* Enterobacteriaceae;**  **other gram-negative**  **rods** | **Gram-negative rods seen on stained smear of uncentrifuged**  **urine indicate**  **more than 105 organisms/mL** | **Blood agar; MacConkey or**  **EMB agar** |

**VΙ- Laboratory Diagnosis of Sexually Transmitted Infections (STDs)**

[**Chlamydia**](javascript:void(0))**:** A very common STD caused by a bacterial infection. Often doesn’t have symptoms, but easy to treat once it’s diagnosed.

[**Genital Warts**](javascript:void(0)): Growths on the genital area and around the anus. Caused by certain types of HPV.

[**Gonorrhea**](javascript:void(0)): A common STD caused by a bacterial infection. Often doesn’t have symptoms, but easy to treat once it’s diagnosed.

[**Hepatitis B**](javascript:void(0)): A virus that can cause liver disease, which is spread through sex or sharing personal hygiene items like razors or toothbrushes.

[**Herpes**](javascript:void(0)): A common STD that infects your mouth and/or genitals. Causes blistery sores. There’s no cure, but symptoms are treatable.

[**HIV & AIDS**](javascript:void(0)): HIV is an infection that breaks down your immune system and can lead to AIDS. There’s no cure, but treatment can help you stay healthy.

[**HPV (Human Papillomavirus)**](javascript:void(0)): A super common STD. HPV is usually harmless and goes away by itself, but some types can lead to cancer.

[**Molluscum Contagiosum**](javascript:void(0)): An infection that causes small bumps on your skin. It goes away by itself and usually isn’t dangerous.

[**Pubic Lice**](javascript:void(0))**:** AKA “crabs.” Small parasites that attach to the skin and hair near your genitals. Easy to get rid of with treatment you can get at the drugstore.

[**Scabies**](javascript:void(0)): Scabies are tiny parasites that cause itching. Passed through skin-to-skin contact, usually during sex. Can be cured.

[**Syphilis**](javascript:void(0)): Syphilis is a common bacterial infection. It’s easily cured with medicine, but it can be dangerous if you don’t treat it.

[**Trichomoniasis (Trich)**](javascript:void(0)): “Trich” is a major cause of vaginitis. It’s very common and easily treated.

The laboratory approach to the diagnosis of STDs is related to the sex of the patient , although some infections are common to both sexes like gonorrhea, syphilis and chlamydial infection but there are usually difference in the presenting symptoms , the sites and methods of specimens collection in these infections.

**Genital infections and STDs in women**

These include :

**1-Acute vaginitis:** is caused by Trichomonas vaginalis ( protozoan) and Candida albicans or other yeast.

**- Trichomoniasis** is primarily an infection of the [urogenital tract](http://en.wikipedia.org/wiki/Genitourinary_system); the most common site of infection is the [urethra](http://en.wikipedia.org/wiki/Urethra) and the [vagina](http://en.wikipedia.org/wiki/Vagina) in women, is caused by the single-celled [protozoan](http://en.wikipedia.org/wiki/Protozoan) parasite [*Trichomonas vaginalis*](http://en.wikipedia.org/wiki/Trichomonas_vaginalis)

**-Vulvovaginal candidiasis :** is caused by Candida albicans , squamous epithelial cell of vaginal wail is invaded and inflamed causing vaginal discharges and pain.

**2-Bacterial vaginosis (BV)** or very uncommonly **vaginal bacteriosis:** is caused by Gardnerella vaginalis, and anaerobic cocci , anaerobic vibrios and Mycoplasma hominis which is present in 50% of the ore sever cases of BV. Clinical manifestations include discomfort and pungent odor, a gray, thin, homogenous discharge .Characteristically, signs of inflammation are not present in the vaginal walls, although a few leukocytes may be present.

**What causes a BV?**

Oftentimes, it derives from changes in the local microflora and overgrowth of 1 or more bacterial types. This may be due in part to a reduction or loss in Lactobacillus that normally keeps the vagina slightly acidic, and/or a reduction or loss of peroxide-producing bacterial strains, which protect against BV.

**3- Cervisitis with or without Urethritis:** is caused by gonococci or *Chlamydia trachomatis*.

**4- Uterine sepsis :** is caused by Streptococcus pyogenes, Staphylococcus aureus, coliform bacilli, Bacteroides , Clostridium and Mycoplasma hominis .

**5-Toxic shock syndrome** : is caused by *Staphylococcus aureus* .

**6- Genital ulceration:** is caused by *T. pallidum , Haemophilus ducreyi* and *Chlamydia* group A.

**7- Tuberculosis of uterus**: is caused by *Mycobacterium tuberculosis*.

8-**Viruses** : Cytomegalo virus . Herpes , human papilloma can cause STDs.

**Specimens collection**

Vaginal and urethral discharges are collected by swabs made of cotton or ragon that has been treated with charcoal to adsorb toxic material to gonococci , *Mycoplasma* and *Chlamydia* .

\* For **vaginitis** or uterine sepsis the specimen is high vaginal swabs

\* For ***Trichomonas*** special specimens should be collected from vagina , urethra and cervix , the swab placed in clear *Trichomonas* transport medium or saline for microscopy and culture.

\* For **gonorrhea** the vaginal swab is unsuitable because gonococci tend to die in the acid vaginal secretion and if remaining viable , are likely to over grown by vaginal commensal bacteria, so that endocervical swab must be collected and urethral and pharyngeal swabs should be taken. Swabs for culture should be placed in tubes of Amies transport medium or modified Stuart's media and transport to the lab.(held at room temperature until inoculating)

\* For **cervisitis** endocervical swab helps to avoid contamination with vaginal flora and its useful for isolation of Herpes , *Mycoplasma* and *Chlamydia* .

\* Since ***Chlamydia*** is intracellular pathogens , it is important to remove epithelial cells with swabs from the urethral mucosa. Swabs for isolaton of***Mycoplasma*** and ***Chlamydia*** are transport in sucrose buffer with antibiotic (A useful medium has 0.2 mol/L sucrose in 0.02 M phosphate buffer, pH 7.0– 7.2, with 5% fetal calf serum. Other transport media may be equally suitable. The transport medium should contain antibiotics to suppress bacteria other than chlamydia species. Gentamicin, 10 μg/mL, vancomycin, 100 μg/mL, and amphotericin B, 4 μg/mL, can be used in combination since they do not inhibit Chlamydia ) . If specimens cannot be processed rapidly, they can be refrigerated for 24 hours; otherwise, they should be frozen at −60 °C or colder until processed.

**Microscopic examination**

* Both a wet film and gram stained film should be examined, the wet film is examined for the presence of *Trichomonas* ( motile , rounded shaped)
* flourescein conjugate monoclonal Ab reagent are quite sensitive for *Trichomonas* .
* Examine under dark field microscope for *T.pallidum*.
* Fluorescein-conjugated monoclonal antibodies for *Chlamydia* can be used for direct examination of specimens .
* Gram stained film should be examined for candidiasis and bacterial vaginitis.
* *Candid*a : G+ve yeast form and G+ve hyphe ( pseudomycelium).
* The presence of G-ve diplococcic intracellularly with limited proportion of PMNs almost diagnosis as gonorrhea.
* Bacterial vaginitis G – ve bacilli (Gardnerella vaginalis ) , squamous epithelial cells covered with many such bacilli ( clue cells).

**To make a diagnosis of bacterial vaginitis the swabs should be tested for:**

1-Thin homogenous vaginal discharge vary from white to grey in color.

2- A characteristic "fishy" odor on [wet mount](http://en.wikipedia.org/wiki/Wet_mount#Wet_mount_or_temporary_mount). This test, called the *whiff test*, is performed by adding a small amount of 10% [potassium hydroxide](http://en.wikipedia.org/wiki/Potassium_hydroxide) (KOH) solution to a [microscopic slide](http://en.wikipedia.org/wiki/Microscopic_slide) containing the vaginal discharge. A characteristic [fishy odor](http://en.wikipedia.org/wiki/Fishy_odor) is considered a positive whiff test and is suggestive of bacterial vaginosis.

3-Loss of [acidity](http://en.wikipedia.org/wiki/Acidity) ([pH](http://en.wikipedia.org/wiki/PH) of vaginal fluid >4.5) . To control bacterial growth, the vagina is normally slightly acidic with a [pH](http://en.wikipedia.org/wiki/PH) of 3.8–4.2. A swab of the discharge is put onto [litmus paper](http://en.wikipedia.org/wiki/Litmus_paper) to check its acidity.

4-Gram stain demonstrates a shift in vaginal flora, with a decrease in large Gram-positive rods (lactobacilli) and an increase in small Gram-variable (consisting Gardnerella vaginalis, , anaerobic vibrios such as *Bacteroides* and *Mycoplasma hominis*).

5-The presence of [*clue cells*](http://en.wikipedia.org/wiki/Clue_cell) on wet mount, the test for clue cells is performed by placing a drop of [sodium chloride](http://en.wikipedia.org/wiki/Sodium_chloride) solution on a slide containing vaginal discharge. If present, clue cells can be visualized under a microscope. They are so-named because they give a clue to the reason behind the discharge. These are [epithelial](http://en.wikipedia.org/wiki/Epithelial) cells that are coated with bacteria.

At least three of the four criteria should be present for a confirmed diagnosis so that bacterial vaginitis does not depend on the isolation of the bacteria.

**The diagnosis of acute vaginitis as followings:**

**1-Trichomonas vaginalis**

-Copious , yellow-green or discolored discharge.

- Motile trichomondas

-Vaginal pH is >4.5, and the whiff test is usually negative

-Because of the low sensitivity of direct microscopy, culture may be used, where available, to isolate the parasite from urethral swabs, urine sediments, prostate fluid and vaginal specimens.

**2- Candida albicans**

- Thick ,white ( cottage cheese ) discharge

-The vaginal pH is normal (<4.5), and the whiff test is negative.

- Wet-mount preparation with 10% KOH shows budding yeast and/or branching pseudohyphae.

**Culture:**

The specimen should be inoculated on two plates of a rich Blood agar , one incubated at 35-37c˚ in 5% CO2 with moisture & the other in an aerobic atmosphere with CO2.

*Candida albicans*  can be recognized on the aerobic Blood agar and grow well on Colombia agar base + 5% Sheep blood agar + Naldixic acid ( CAN) , Sabouraud agar or Malt extract agar , placing 50U Nastatin disc & 20 µg amphotericin disc will assist in the recognition of *Candida albicans*  ( sensitive ) from resistant Staphylococcal and other bacterial colonies .

*-****N. gonorrhoeae*** grow well on Thayer Martin ( TM ) medium wich contains the antibiotics ( Vancomycin , Colistin , Nastatin ) , although Thayer Martin medium widely used but 3 - 10% of gonococcal strains are inhibited by Vancomycin so that a modified TM medium which is Modified New York City medium (MNYC medium ) is preferred because it gives better growth and the use of Lincomycin as a selective agent avoids the problem of Vancomycin sensitivity , incubated in air plus CO2 ; but if the clinical features or the appearances in the gram smear suggest that there is gonococcal infection the specimen should be inoculated additionally on Chocolate agar and incubated at 35-37 c˚ in air plus 5-10 % CO2 .

For further diagnosis of *N. gonorrhoeae*  CF test and Indirect fluorescent Abs can be used.

*-****Gardnerella*** and **Sterptococci** grow on CAN.

-***Mycoplasma***and***Ureplasma urrelyticum***grow on Diphasic medium and SP-4 medium.

- For ***Trichomonas vaginalis***the Cysteine peptone liver infusion maltose

( CPLM) medium is used under anaerobic condition.

- Cell culture techniques are recommended for the isolation of ***Chlamydia***species. Usually involves inoculation of the clinical specimens onto cycloheximide-treated McCoy cells. One technique uses a confluent growth of McCoy cells on 13- mm coverslips in small disposable vials. The inoculums is placed in duplicate vials and centrifuged onto the monolayers at approximately 3000 × *g* followed by incubation at 35 °C for 48–72 hours and stained. To detect *Chlamydia* immunofluorescence, Giemsa’s stain, or iodine stain is used to search for intracytoplasmic inclusions. Immunofluorescent techniques are the most sensitive of the three stains but require special IF reagents and microscopy. Giemsa is more sensitive than iodine, but the microscopy is more difficult.

Enzyme immunoassays (EIAs) are used for detecting chlamydial antigens in genital tract specimens from patients and PCR( 16S RNA sequence) .

**Genital infections in men Lec3**

The infections in men are mostly caused by the same organisms as in women , include :-

**1-Urethritis** : is classified as gonococcal or non gonococcal (NGU) depending on whether or not gonococci are found in gram film or culture of discharge . Most cases of NGU are caused by *Chlamydia trachomatis* and *Ureaplasma* in 10% of cases *.*

**2-Prostatitis** : is usually caused by gonococci or Chlamydia. Sub acute or chronic prostatitis found in older men is usually associated with the presence of coliform bacilli or enterococci.

**3-Ulceration** *:* caused by Herpes simplex virus ( usually type 2) , *T. pallidum , Haemophilus ducreyi* and *Chlamydia.*

**Collection of specimens and laboratory examination**

Urethral discharge may be expressed directly on ti slide for gram stain and be inoculated immediately on Chocolate agar and selective medium for the culture of gonococci . If specimens have to be transported to the laboratory the exudates from ulcers should be collected on a swab and put into a tube of Amie ҆ s transport medium.

For isolation of *Haemophilus ducreyi* a special agar ( Mueller Hinton Chocolate Horse Blood agar) enriched with 1% Iso vitale X and Vancomycin ( 3µg/ ml) incubated in 5-7% CO2 at 37 c ˚.

The gram stained films may show small pleomorphic G –ve rods or coccobacilli arranged in chains and groups.

Herpes simplex virusexamined by immunofluorescent Abs or by ELISA.

**SYPHILIS**

Syphilis is a contagious venereal disease caused by the spirochete Treponema pallidum. The organism enters the body through a break in mucosa or epithelial layer. After a 10-60 day incubation, a painless inflammatory reaction producing a characteristic ulcerated lesion called a chancre usually appears at the site of entry.

Syphilis is usually cured by penicillin, if treated early. If untreated, a generalized skin rash and other abnormalities will begin appearing six weeks to six months following the disappearance of the chancer (secondary stage syphilis). Again, the clinical symptoms may disappear (latent stage syphilis).

The latent syphilis may continue throughout life, it may terminate with spontaneous cure, or it may advance to tertiary syphilis. Pregnant women with active syphilis (even primary stage) can transmit the organism to the unborn child (congenital syphilis).

Tertiary Stage occurs anywhere from months to years after secondary stage, typically between 10 to 30 years (gummatous syphilis ,cardiovascular syphilis ,neurosyphilis ).

**Congenital Syphilis**

-Transmitted from mother to fetus .Fetus affected during the second or third trimester

-40% result in syphilitic stillbirth

-Live-born infants show no signs during first few weeks

-60-90% develop clear or hemorrhagic rhinitis

-rash especially around mouth, palms of hands and soles of feet

-general lymphadenopathy, hepatosplenomegaly, jaundice, anemia, painful limbs & bone abnormality

**DIAGNOSIS**

Evaluation based on 3 factors

-Clinical findings

-Demonstration of spirochetes in clinical specimen

-Present of antibodies in blood or CSF

= more than one test should be performed

**CULTURE**

No cultural methods are available.

**DIRECT SMEARS**

1-Darkfield microscopy

Dark field microscopy is used to demonstrate *Treponema pallidum* in material from lesions or lymph nodes. Since *T. pallidum* is identified by characteristic morphology and motility, the preparation must be fresh and the organisms actively motile.



2-Direct fluorescent antibody (DFA-TP)

As an alternative to dark field microscopy, fixed smears from lesions, serous fluid, or lymph node aspirates may be sent to reference laboratories for staining with fluorescent-conjugated antibody to *T.* *pallidum*. The results, however, are usually not available for days to weeks and thus, may not be helpful in guiding patient management.

**SEROLOGICAL TESTS**

**1-Nontreponemal or reagin tests**

This group of common nontreponemal tests measure antibody to a nonspecific cardiolipin lecithin antigen. The tests are moderately specific for syphilis (false-positives occur), but highly sensitive.

Non-specific or non-treponemal serological test to detect reagin

= Reagin is an antibody formed against cardiolipin

= Found in sera of patients with syphilis as well as other diseases

= Non-treponemal tests become positive 1-4 weeks after appearance of primary chancre, in secondary stage may have false positive , in tertiary 25% are negative, after successful treatment will become non-reactive after 1 to 2 years.

Because they are easily performed, the nontreponemal tests are useful screening tools. The tests can be quantitative to obtain a titer and, thus, are useful in monitoring patient response to therapy.

**\*Venereal Disease Research Laboratory=VDRL**

The (VDRL) is a [nontreponemal](http://en.wikipedia.org/wiki/Nontreponemal) serological screening for [syphilis](http://en.wikipedia.org/wiki/Syphilis) that is also used to assess response to therapy, to detect CNS involvement, and as an aid in the diagnosis of congenital syphilis. The basis of the test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart (diphosphatidyl glycerol). It therefore detects [anti-cardiolipin antibodies](http://en.wikipedia.org/wiki/Anti-cardiolipin_antibodies) (IgG, IgM or IgA).

- Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin

- the antigen consists of cardiolipin, cholesterol and lecithin

- serum must be heated to 56 C for 30 minnutes to remove anti complimentary activity which may cause false positive

- reported as Non-reactive, weakly reactive and reactive

- used primarily to screen CSF

Many other medical conditions can produce false positive results, including some viruses (mononucleosis, hepatitis), drugs, pregnancy, rheumatic fever, rheumatoid arthritis, lupus, and leprosy.

\***The RPR (**[Rapid Plasma Reagin](http://en.wikipedia.org/wiki/Rapid_Plasma_Reagin)**) test**

uses the same antigen as the VDRL, but in that test it has been bound to several other molecules including a carbon particle to allow visualization of the flocculation reaction without the need of a microscope. The RPR test utilizes the VDRL cardiolipin antigen is modified with choline chloride to make it more stable and is attached to charcoal particles to allow macroscopic reading.

- general screening test

- cannot be performed on CSF

- serum or plasma may be used for testing, serum is not heated

- results are read macroscopically

- appears to be more sensitive than the VDRL

- easy and cheap, used for screening

- reported as a titer

- used to follow treatment ,sensitive except in late syphilis

**\* Other tests which use modified VDRL Ag**

**A. USR – unheated serum reagin test**

- modified VDRL Ag, uses choline chloride/EDTA

- microscopic flocculation test

**B. RST – reagin screen test**

- modified VDRL Ag with Sudan Black

- Sudan Black makes flocculation reaction macroscopically visible

**\*The Wassermann test**

Is based on [complement-fixation](http://en.wikipedia.org/wiki/Complement-fixation). A sample of [blood](http://en.wikipedia.org/wiki/Blood) or [cerebrospinal fluid](http://en.wikipedia.org/wiki/Cerebrospinal_fluid) is taken and introduced to the antigen - [cardiolipin](http://en.wikipedia.org/wiki/Cardiolipin) extracted from bovine muscle or heart, the Wassermann reaction of [antiphospholipid antibodies](http://en.wikipedia.org/wiki/Antiphospholipid_antibody) (APAs) ,Syphilis non-specific antibodies (co[reagin](http://en.wikipedia.org/w/index.php?title=Reagin&action=edit&redlink=1)) which react with lipid in presence of complement then indicator system ( Sheep RBCs coated with anti-Sheep RBCs) , no hemolysis consider as positive result .

The reaction is not specific to syphilis and will produce a positive reaction to other diseases, including [malaria](http://en.wikipedia.org/wiki/Malaria), [tuberculosis](http://en.wikipedia.org/wiki/Tuberculosis), and numerous other diseases. It is possible for an infected individual to produce no reaction and for a successfully treated individual to continue to produce a reaction (known as being "Wassermann fast" or "fixed").The Wassermann test it is rarely used today. Replacement tests such as the [VDRL](http://en.wikipedia.org/wiki/VDRL) test and the [RPR test](http://en.wikipedia.org/wiki/Rapid_plasma_reagin).

**2-Treponemal Tests**

These tests measure antibody specific for *T. pallidum*. They are highly specific and highly sensitive. Treponemal tests are not currently used for general screening because they are expensive and time consuming to perform. Their use is limited to confirmation of positive reagin tests (to identify false-positive diagnoses) and in the diagnosis of late syphilis when reagin tests may be nonreactive.

**●-Treponema Pallidum Immobilization Test (TPI)**

- measures ability of (patient produced) antibody and complement to immobilize live (reagent) treponemes. Live T. pallidum become immobilized by antibody in serum of infected persons , expensive test.

**●-Hemagglutination Tests**

(includes Treponema pallidum Hemagglutination – TPHA and adapted to microtechniques (MHA-TP) ) utilize tanned sheep RBC’s are coated with T. pallidum antigen from Nichol’s strain. Serum is pre-treated with non-pathologic Reiter Strain treponemes to limit non-specific reactions. Agglutination indicated is positive**.** More sensitive and more specific, even in late syphilis . Reported as positive or negative

**● Fluorescent treponemal antibody absorption (FTAABS) Test**

- an indirect fluorescent antibody test requiring diluted heat-inactivated patient serum. The serum is mixed with non-pathologic Reiter Strain treponemes to remove nonspecific cross-reactive antibodies.

The ‘absorbed’ serum is then tested with the Nichols Strain of *T. pallidum*, washed, stained with an antibody conjugate (ant immunoglobulin with a fluorescein isothiocyanate label) and examined under a fluorescent microscope.

- one of the most used confirmatory test

- requires experienced personnel to read

- highly sensitive and specific

●ELISA

- tubes coated with T. pallidum antigen, antibody in serum attaches to antigen

- following washing, add an anti-antibody tagged with enzyme alkaline phosphatase , detectable color changes occur.

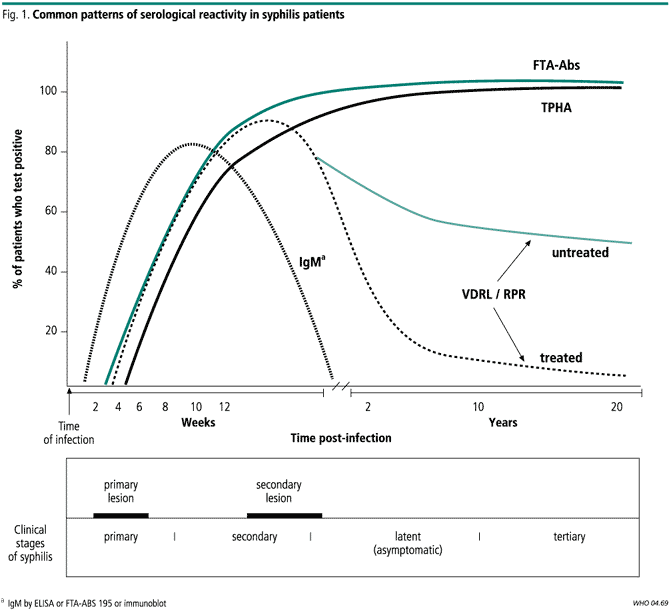
**Interpretation:**

1- If the test is negative, but the physician still suspects the infection is present, the more specific treponemal tests should be ordered.

2- Reagin tests (VDRL & RPR) are considered screening tests. If positive results are obtained, the more specific treponemal testing (FTA-ABS, MHA-TP, etc.) should be performed. Specimens giving any degree of clumping should be subjected to further serological study.

3- However it must be noted that all treponemal specific tests will remain positive for life once a person has been infected with syphilis, even if syphilis has been adequately treated. Therefore, these types of tests cannot be used to monitor the treatment of syphilis. automated RPR test(ART)is available for large scale tests.

Time course of antibody development during syphilis



4-Biologic False Positives (BFP)

A. Collagen diseases such as arthritis, LE, etc., sometimes result in increased amount of reagin

B. Certain infections : IM, malaria, leprosy

C. Other treponemal infections

5- False negatives

A. Very early in disease or latent, inactive stage

B. Immunosuppressed patients

**Leptospirosis Lec4**  
Leptospirosis is a typical zoonosis that can be transmitted from animals to humans and occurs worldwide. Leptospirosis is a bacterial disease that affects humans and animals. It is caused *Leptospira interrogans*, more than 200 serovars are known.

*L. interrogans* can enter the body through scratches or breaks in the skin. The bacteria use blood as a means of travel, traveling throughout the body and infecting different organs. It is believed that the bacteria can attach to several different receptors on the host cells which is why it can effect so many different hosts and attack different organs. The kidney is where *L. interrogans* survive and multiply the best, causing kidney infections. Having leptospirosis is usually not fatal to humans but has been known to cause death in a human at least once. There are two phases an animal goes through when being infected by *L. interrogans*, the leptosipremic acute phase and the immune leptospirosis phase. The symptoms during the first phase include fever, nausea, headaches, and muscle pain. An organism experiencing the second phase also will have fever and may develop meningitis. Organ failure and renal failure may occur in severe cases. It is during this phase that the bacteria leave the body through the urine .

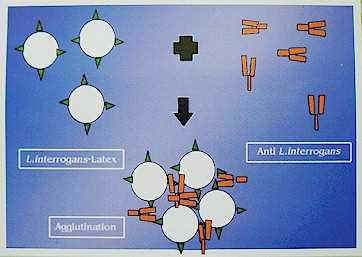
Leptospirosis is confirmed by laboratory testing of a blood or urine sample.

**-Microscopic agglutination test (MAT) :**is the standard test to detect serovar specific antibodies. However, this method cannot be performed in general laboratories because it requires cultures of reference leptospire strains of many serovars as a series of antigens for testing. The method is also complicated, time consuming and needs killed for operation

**-Lepto Latex Test( Latex Agglutination) for Leptospirosis test(LA)**: is a simple serodiagnostic method for leptospirosis by using the latex agglutination test to detect antibodies to leptospires.

*Leptospira interrogans* serovar *pyrogenes* was cultured in neopeptone medium. Leptospiral cells was inactivated (killed) with 0.1% formalin for 2 hours. The antigen-coated latex was stable for at least 12 month when kept at 4-10 C.

The Lepto Latex Test kit is used for detection of anti-Leptospires in the human serum or plasma which capable to react with antigen of Leptospires sp. that coated on Latex bead. The reaction of antigen and antibodies will form agglutination clearly within 5 minutes by normal eye vision. When no specific antibodies are present, the latex suspension will remain homogeneous (negative result).

  
The diagnosis of Leptospirosis by latex agglutination is convenient, simple and rapid. This method is suitable for use as a laboratory screening test. LA is highly sensitive at 94.7% and specific 93.3% (of the patients confirmed as cases of leptospirosis by Microscopic agglutination test MAT)

**Skin ,Wound and Soft tissue Infections**

Many minor and superficial skin , wound & Soft tissue infections are diagnosed by the doctor based on a physical examination, [signs](http://labtestsonline.org/glossary/sign/) and [symptoms](http://labtestsonline.org/glossary/symptom/) . A clinical evaluation cannot, however, definitively tell the doctor which [microorganism](http://labtestsonline.org/glossary/microorganism) is causing a wound infection or what treatment is likely to be effective. For that, laboratory testing is required.

Skin, Wound and Soft tissue Infections include :-

1- **Folliculitis:** is a minor infection of the hair follicles.It caused by *Pseudomonas aeruginosa , Staphylococcus aureus & Candida albicans*

**2- Acne :** also involves inflammation of hair follicles and associated sebaceous glands. It caused by *Propionibacterium acnes*

3- **Furuncles:** is a small abscess that develops in the region of a hair follicle. It caused by *Staphylococcus aureus*

4- **Impetigo:** Pyoderma, also termed impetigo, is a common, sometimes epidemic, skin lesion. The initiallesion is often a small vesicle that develops at the site of invasion and ruptures with superficialspread characterized by skin erosion and a serous exudate, which dries to produce ahoney-colored crust.It caused byGroup A β-hemolytic streptococci, *Staphylococcus aureus* , rarely, *Corynebacterium diphtheriae*

5- **Erysipelas:** is a rapidly spreading infection of the deeper layers of the dermis. It is associated with edema of the skin, marked erythema, pain. It caused byGroup A β-hemolytic streptococci.

**6- Nosocomial wound infection** : caused by *S. aureus , Bacteroides fragilis, Clostridium perfringens , S. pyogenes ,*  and coliform bacilli.

**7- Soft tissue infections:**  (gangrene, necrotizing fasciitis, cellulitis): caused by a variety of aerobic and anaerobic species of bacteria may be present either singly or in combination (synergistic infections) .

**8- Burns** : *S. aureus and Pseudomonas aeruginosa* .

**9- Bone infections** ( osyeomyelitis) :

-In infants caused by *S. aureus , Streptococcus*  group Band coliform bacilli.

-In children caused by *Streptococcus*  group Aand *H. influenza* , skeletal tuberculosis may be occur.

-In adults caused by G-ve bacilli and various cocci , vertebral tuberculosis is seen.

**Laboratory tests**

Laboratory testing is primarily used to diagnose [bacterial](http://labtestsonline.org/glossary/bacterium) wound infections, to identify the microorganism responsible, and to determine its likely susceptibility to specific antimicrobial drugs. Sometimes testing is also performed to detect and identify [fungal infections](http://labtestsonline.org/understanding/conditions/fungal).

**Samples**

Sample collection may involve swabbing the surface of a wound to collect cells or [pus](http://labtestsonline.org/glossary/pus), [aspiration](http://labtestsonline.org/glossary/aspiration) of fluid or pus with a needle and syringe, and/or the collection of a tissue [biopsy](http://labtestsonline.org/glossary/biopsy). For fungal evaluation, scrapings of the skin may be collected. If the patient is febrile or in shock or the infection accompanied by bacteremia , a sample of blood should be taken for culture.

**Testing may include:**

**1-Naked eye examination:**

The appearance of pus or exudates ( color , consistency and odor ) should be noted.

**\*Staphylococcal lesion** -------- the pus is typically creamy and thick in consistency with pus cells evident on microscopy.

*\*****Streptococcus pyogenes*** **infection** -------- the pus is straw colored and watery , with lysis of pus cells seen on microscopy.

*\*****Proteus* infection** ------- the pus with fishy smell.

*\*****Pseudomonas* infection** -------- the pus with a sweet-musty odor and often a blue pigmentation.

\***Anaerobic organisms infection** ----- the pus has an offensive putrid smell.

\***Actinomycosis**( **caused by *Actinomyces***) ---- the pus contain small micro colonies that appear as sulphur granules ( the pus yellowish granular ).

Fungal infections such as yeast ( mycetoma) ------ black or brown granules may be present .

**2-Microscopic examination :**

- [Gram stain](http://labtestsonline.org/understanding/analytes/gram-stain) – This test is usually performed in conjunction with the wound culture. It is a special staining procedure that allows bacteria to be evaluated under the microscope. The results of this test are usually available the same day the sample is received in the laboratory and can give the doctor preliminary information about the microorganisms that may be causing the infection.

- Ziehl-Neelsen stain for Mycobacterium and Nocardia

- Immunofluorescent staining with specific antisera for some pathogenic clostiridia.

**3-** [**Bacterial culture**](http://labtestsonline.org/understanding/analytes/wound-culture)

This is the primary test used to diagnose a bacterial infection. Part of this evaluation involves the identification of methicillin-resistant *Staphylcoccus aureus* (MRSA) when it is present. Results of bacterial wound cultures are usually available within 24-48 hours from the time the specimen is received in the laboratory. Results of special cultures for slow-growing organisms, such as [fungi](http://labtestsonline.org/glossary/fungus/) or [mycobacteria](http://labtestsonline.org/glossary/mycobacteria/), may require several weeks.

Acid fast stain

yeast

Blood agar

Coagulase test + Mannitol salt agar

G – ve rods

Anaerobically in N2 or H2 +5-10% CO2

Mycobacterium

Biochemical tests

Closteridia

Staphylococci

– A follow-up test to the wound culture. When a [pathogen](http://labtestsonline.org/glossary/pathogen/) is identified using the wound culture, this test is used to determine the bacteria's likely susceptibility to certain drug treatments. This information helps guide the doctor in selecting appropriate antibiotics for treatment. These results are typically available about 24 hours after isolation of the microorganism that is causing the infection.

**4-Other tests that may be ordered include:**

\*[KOH prep](http://labtestsonline.org/understanding/analytes/fungal) – A rapid test performed to detect fungi in a sample. The sample is treated with a special solution, placed on a slide, and examined under a microscope.

\*[Fungal culture](http://labtestsonline.org/understanding/analytes/fungal) – Ordered when a fungal infection is suspected. Many fungi are slow-growing and may take several weeks to identify.

\*[Blood culture](http://labtestsonline.org/understanding/analytes/blood-culture) – Ordered when infection from a wound may have spread and [septicemia](http://labtestsonline.org/glossary/septicemia/) is suspected.

\*Molecular testing to detect genetic material of a specific organism.

**Bacteremia**

Bacteremia is the presence of viable bacteria in the blood stream. Bacteremia is different from [sepsis](http://en.wikipedia.org/wiki/Sepsis) (so-called blood poisoning or toxemia), which is a condition where bacteremia is associated with an [inflammatory](http://en.wikipedia.org/wiki/Inflammation) response from the body (causing [systemic inflammatory response syndrome](http://en.wikipedia.org/wiki/Systemic_inflammatory_response_syndrome), characterised by [rapid breathing](http://en.wikipedia.org/wiki/Tachypnea), [low blood pressure](http://en.wikipedia.org/wiki/Hypotension), [fever](http://en.wikipedia.org/wiki/Fever), etc.). [**Bacteremia**](http://en.wikipedia.org/wiki/Bacteremia): is the presence of viable bacteria in the blood. The term **septicemia:** means the presence of microorganisms or their toxins in the blood.

**Causes :** In the hospital, indwelling [catheters](http://en.wikipedia.org/wiki/Catheter) are a frequent cause of bacteremia and subsequent [nosocomial](http://en.wikipedia.org/wiki/Nosocomial) infections, because they provide a means by which bacteria normally found on the skin can enter the bloodstream. Other causes of bacteremia include dental procedures, [urinary tract infections](http://en.wikipedia.org/wiki/Urinary_tract_infection), [peritonitis](http://en.wikipedia.org/wiki/Peritonitis), [*Clostridium difficile* colitis](http://en.wikipedia.org/wiki/Clostridium_difficile_colitis), intravenous drug use, and [colorectal cancer](http://en.wikipedia.org/wiki/Colorectal_cancer).

**Fungemia**

is the presence of [fungi](http://en.wikipedia.org/wiki/Fungus) or [yeasts](http://en.wikipedia.org/wiki/Yeast) in the [blood](http://en.wikipedia.org/wiki/Blood). It is most commonly caused by [*Candida*](http://en.wikipedia.org/wiki/Candida_%28fungus%29) species (also known as **Candidemia**, **Candedemia**, and **Invasive Candidiasis**), but can be caused by other fungi as well, including [*Saccharomyces*](http://en.wikipedia.org/wiki/Saccharomyces), [*Aspergillus*](http://en.wikipedia.org/wiki/Aspergillus) and [*Cryptococcus*](http://en.wikipedia.org/wiki/Cryptococcus_%28fungus%29). It is most commonly seen in [immunosuppressed](http://en.wikipedia.org/wiki/Immunosuppressed) or [immunocompromised](http://en.wikipedia.org/wiki/Immunocompromised) [patients](http://en.wikipedia.org/wiki/Patient) with severe [neutropenia](http://en.wikipedia.org/wiki/Neutropenia), [oncology](http://en.wikipedia.org/wiki/Oncology) patients, or in patients with intravenous [catheters](http://en.wikipedia.org/wiki/Catheter).

## Diagnosis

Bacteremia is most commonly diagnosed by [blood culture](http://en.wikipedia.org/wiki/Blood_culture), in which a sample of blood is allowed to [incubate](http://en.wikipedia.org/wiki/Incubator_%28microbiology%29) with a [medium](http://en.wikipedia.org/wiki/Microbiological_medium) that promotes bacterial growth.

**Blood culture** is a [microbiological culture](http://en.wikipedia.org/wiki/Microbiological_culture) of [blood](http://en.wikipedia.org/wiki/Blood). It is employed to detect [infections](http://en.wikipedia.org/wiki/Infections) that are spreading through the bloodstream (such as [bacteremia](http://en.wikipedia.org/wiki/Bacteremia), [septicemia](http://en.wikipedia.org/wiki/Septicemia) amongst others). This is possible because the bloodstream is usually a sterile environment.

A minimum of 10 ml of blood is taken through [vein puncture](http://en.wikipedia.org/wiki/Venipuncture) and injected into two or more "blood bottles" with specific [media](http://en.wikipedia.org/wiki/Growth_medium) for [aerobic](http://en.wikipedia.org/wiki/Aerobic_organism) and [anaerobic organisms](http://en.wikipedia.org/wiki/Anaerobic_organism). A common media used for aerobies is Tryptic soy broth and for anaerobes is [thioglycollate broth](http://en.wikipedia.org/wiki/Thioglycollate_broth).

**Collect enough blood**

* 1-2ml in neonate
* 2-3ml in infants
* 3-5ml in children
* 10-20ml in adolescent

-The blood is collected using aseptic technique. This requires that both the tops of the culture bottles and the vein puncture site of the patient are cleaned prior to collection with swabs 70% [isopropyl alcohol](http://en.wikipedia.org/wiki/Isopropyl_alcohol).

- 2-3 blood cultures should be taken separated by 1 hr. intervals or less if treatment can not be delayed . Ordering multiple sets of cultures increases the probability of discovering a pathogenic organism in the blood and reduces the probability of having a positive culture due to skin contaminants , so that the chance of missing a transient bacteremia ( caused by *S.epidermidis* ) is reduced and the pathogenic role of *S.epidermidis* is confirmed if they are recovered from multiple vein punctures ( bacteremia in users of intravenous drugs) .

The blood should be mixed with 10 times its volume of broth( 5 ml blood in 50 ml broth) to dilute any antibiotic present and to reduce the bactericidal effect of serum . After inoculating the culture bottles incubated at 37 c for 7 days . A sterile culture shows a layer of sediment RBCs covered by a peal yellow transparent broth , microbial growth is evidenced by : a floccules deposit on top of the blood layer , turbidity , hemolysis , coagulation , gas production and white grains on the surface or deep in the blood layer.

If a culture bottle is positive, a microbiologist will perform a Gram Stain on the blood for a rapid identification of the bacteria. The blood is also subcultured onto agar plates to isolate the pathogenic organism for culture and suceptibility testing, which takes up to 3 days. This culture & sensitivity process identifies the species of bacteria. Antibiotic sensitivities are then assessed on the bacterial isolate to inform clinicians on appropriate antibiotics for treatment.

- It is necessary to determine the significance of a positive blood culture. The following criteria may be helpful in differentiating “true positives” from contaminated specimens:

(1) Growth of the same organism in repeated cultures obtained at different times from separate anatomic sites strongly suggests true bacteremia.

(2) Growth of different organisms in different culture bottles suggests contamination.

(3) Growth of normal skin flora, eg, *Staphylococcus epidermidis,* diphtheroids (corynebacteria and propionibacteria),or anaerobic gram-positive cocci, in only one ofseveral cultures suggests contamination. Growth of suchorganisms in more than one culture enhances thelikelihood that clinically significant bacteremia exists.

(4) Organisms such as viridans streptococci or enterococci are likely to grow in blood cultures from patients suspected to have endocarditis, and gram-negative rods such as *E coli* in blood cultures from patients with clinical gram-negative sepsis. Therefore, when such “expected” organisms are found, they are more apt to be etiologicallysignificant.

Blood culture bottles

Gram stain and subculture

Streptococci

G +ve cocci

G –ve rods

Macconkey agar

Staphylococci

CAMP test , Bile esculin agar

Blood agar

Kligler iron agar , motility , indol, urease medium and citrate test

Mannitol salt agar

Blood agar with Optochin , Tellurite and Bacitracin discs

**Meningitis**

**Lec5**

Meningitis is an infection of the membranes (meninges) surrounding the brain and spinal cord. Meningitis is usually of multiple etiology-bacterial, fungal or viral yet bacteria remain the common etiological agent. Meningitis can be acute, with a quick onset of symptoms, or chronic, lasting a month or more, or can be mild or aseptic, but the emphasis should be on identification of cause so that appropriate interventions can be applied.

Types of bacteria that cause bacterial meningitis vary by age group,

-In infants ( to 2 months ) ------------ E. coli , Salmonella spp. , Citrobacter spp. , group B Streptococci and Listeria monocytogenes .

-In all other age groups

\*purulent meningitis ( CSF is turbid , 100-3000 PMNs / mm3 ----- *H. influenzae , N. meningitides , S. pneumoniae and Listeria monocytogenes.*

\*A septic meningitis ( CSF is clear or slightly turbid 10-500 leukocyte / mm3  mostly lymphocytes ) ------------ *Cryptococcus neoformans , Candida albicans , Leptospira* , amoebae ( *Naegleria* or *Hartemanella* ) and virus ( *Polio , Echo , Coxsackie , Arbo* viruses) and *M. tuberculosis* .

* Viral meningitis is generally less severe and clears up without specific treatment. Viral ("aseptic") meningitis is serious but rarely fatal in people with normal immune systems. Usually, the symptoms last from 7 to 10 days and the patient recovers completely.
* **Tuberculous meningitis (TBM)**

The non-specific clinical and cerebrospinal fluid (CSF) features have made TBM often difficult to diagnose with certainty, especially at early stages and has to be differentiated from a plethora of other infectious and non-infectious meningitis such as viral, bacterial cryptococcal or carcinomatous meningitis.

In cases of TBM, the CSF pressure is typically higher than normal, appear clear or slightly turbid. If the CSF is left to stand, a fine clot resembling a pellicle or cobweb may form. This faintly visible "spider's web clot" is due to the very high level of protein in the CSF, typical of this condition.

**Collection, transportation, receipt and storage of CSF**

Direct testing of CSF is the most accurate way to confirm the diagnosis of bacterial meningitis. CSF should be collected from all the cases with suspected meningitis before commencement of antimicrobial therapy, unless lumbar puncture (LP) is contraindicated.

Petechial fluid can be another specimen in cases with meningococcal meningitis. Petechial lesions, if present, may be gently irrigated by injecting 0.2 ml of sterile saline solution using a small syringe with a fine needle and the fluid collected for smear and culture. Early diagnosis is essential and is best established by laboratory examination of CSF. However, therapy should not be dependent or delayed pending lumbar puncture or laboratory results (WHO). To initiate the definitive identification of a bacterium responsible for meningitis, CSF specimens should be obtained from patients with clinical signs and symptoms of meningitis and should be transported to the laboratory without delay. N.meningitidis, S.pneumoniae, and H.influenzae are fastidious organisms that may not survive long transit times. The processing of a CSF specimen is one of the few clinical microbiology procedures that must be done immediately. Laboratorians should always record the date and time a specimen was received. Usually, three or more tubes of CSF are collected during a LP procedure. The tubes should be numbered in sequential order with tube number one containing the first sample of CSF obtained. The CSF in tubes 1, 2, and 3 most often are examined for chemistry, microbiology, and cytology, respectively. However, the

particular tests performed on tubes 2 and 3 are subjective and probably best determined by the laboratarians.

Laboratory Diagnosis of Meningitis

Contamination with skin flora and disinfectant will be ruled out after the first tube of CSF is collected. The probabilities of detecting microorganisms by staining and by culturing are related to the volume of specimen that is concentrated and examined CSF volumes of 2 to 3 ml are usually sufficient to detect bacteria, but for mycological and mycobacterial investigations a minimum of 5 ml (preferably 10 to 15 ml) of CSF is required. If only a small amount of CSF is received with requests for multiple assays, the order of priority of the tests is determined after discussion with the physician. The specimen should not be refrigerated before subjecting to microbiological tests as it may prevent the recovery of the organisms; fastidious organisms may not survive variations in temperature, CSF specimens should be stored at room temperature or at 37oC if they cannot be processed immediately or till microscopy and bacterial cultures are performed, after which it can be refrigerated for further use.

**Laboratory diagnosis of bacterial meningitis**

Bacterial meningitis is a significant cause of mortality and morbidity worldwide.

Neurological outcome and survival depend largely on damage to CNS prior to effective antibacterial treatment. Quick diagnosis and effective treatment is the key to success. The diagnostic dilemma in acute pyogenic meningitis is due to large spectrum of signs and symptoms.

**Examination of CSF**

The CSF should arrive still warm and either be examined immediately or placed in an incubator for examination within an hour. If delay is anticipated either in transportation to the laboratory or for examination, CSF should be divided into two containers: one in a plain bottle and the other in a bottle having a few drops of glucose broth. In the laboratory, CSF from the plain bottle can be used for making smears for staining whereas cultures are done from containers having CSF in glucose broth. The residual CSF should be preserved frozen in the CSF bank for further assessment and evaluation with evolving /additional contributory findings.

An examination of CSF involves the following:

1. Macroscopic examination.

2. Cytological examination.

3. Examination of Gram stained smear.

4. Culture and antimicrobial susceptibility testing.

5. Latex agglutination test for antigen detection.

6. Other diagnostic methods.

**3.1.1 Macroscopic examination**

By appearance, the CSF is normally clear like water; cloudy, purulent, bloody or pigmented CSF as per the disease states.

Meningitis, Hazy, cloudy, turbid CSF indicates either metastatic spread of tumors into the CNS or pleocytosis or severe meningeal infection; Opalescent CSF may be suggestive of cryptococcal meningitis. The turbid nature of the CSF is attributable to both the bacteria and leukocytes present. Hemorrhagic CSF may be indicative of Anthrax meningitis with supportive clinical findings.

Frank clots or pellicles in CSF occur only if protein concentration exceeds 15g/L.

**Table : Typical Cerebrospinal Fluid Findings in Various Central Nervous System Diseases**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diagnosis** | **Cells**  **(per μL)** | **Glucose**  **(mg/dL)** | **Protein**  **(mg/dL)** | **Opening**  **Pressure** |
| **Normal** | **0–5 lymphocytes** | **45–85** | **15–45** | **70–180 mm H2O** |
| **Purulent meningitis (bacterial)** | **200–20,000 PMNs** | **Low (< 45)** | **High (> 50)** | **++++** |
| **Granulomatous meningitis**  **(mycobacterial, fungal)** | **100–1000, mostly lymphocytes** | **Low (< 45)** | **High (> 50)** | **+++** |
| **Aseptic meningitis, viral or Meningoencephalitis** | **100–1000, mostly lymphocytes** | **Normal** | **Moderately**  **high (> 50)** | **Normal to +** |
| **Spirochetal meningitis (syphilis, leptospirosis)** | **25–2000, mostly lymphocytes** | **Normal or low** | **High (> 50)** | **+** |

**1: CSF glucose level must be considered in relation to blood glucose level. Normally, CSF glucose level is 20–30 mg/dL lower than blood glucose level, or 50–70% of blood glucose normal value.**

* CSF glucose decreased in meningitis .
* CSF protein elevated in meningitis .

**Culture :**

CSF

Blood agar chocolate agar blood agar with Lowenstein Jensen

a streak of *S. aureus* media

*N. meningitides H.influenzae M. tuberculosis*

( satellite colonies )

Oxidase test ,

Slide aggl.

slide aggl.

G + ve cocci G + ve rod G- ve rod

CAMP test Catalase test + ve, Macconkey agar ,

& optochin sensitivity Motility +ve , biochemical tests for

for Streptococci bile esculin agar for Enterobacteriaceae

*Listeria monocytogenes .*

**Latex Agglutination Test (LAT) for antigen detection**

Agglutination with the respective latex reagents indicates presence of corresponding antigen in CSF and is diagnostic. Antigen testing may result in few indeterminate, false negatives, and false-positives. True positive results do not appear to modify the decision to administer antimicrobial therapy, therefore reserved for specific clinical circumstances. The suggested indications are-

- Initial negative CSF Gram staining and CSF cultures;

- Partially-treated and pretreated patients with negative CSF culture

The false-negative LAT could be possibly because of low antigen titres in the CSF. It is possible that the antiserum in diagnostic LAT kits does not detect all the capsular serotypes prevalent in the particular geographical area or probably as yet unrecognized serotypes are the causative agents in such cases.

The reported sensitivities of LAT of CSF samples from patients with bacterial meningitis ranged from 78 to 100% for H. influenzae type b meningitis, 59 to 100% for pneumococcal meningitis, and 22 to 93% for meningococcal meningitis.

In negative CSF cultures with clinical presentation and CSF parameters compatible with bacterial meningitis, CSF latex agglutination has a lower sensitivity for detecting bacteria.

In CSF specimens positive for the causative microorganism, the positivity of the LAT can be upto 100%.

A strong decline in the sensitivity of LAT is expected among patients with antibiotic pretreatment prior to lumbar puncture. The additional value of LAT is therefore limited.

**Other laboratory tests**

1. Blood cultures 2. Polymerase Chain Reaction (PCR) 3. Flow cytometry

4. Inflammatory markers

**Mycology**

Mycology is the study of fungi. Approximately 80,000 species of fungi have been described, but fewer than 400 are medically important, and less than 50 species cause more than 90% of the fungal infections of humans and other animals. Fungal infections are **mycoses.** Most pathogenic fungi are exogenous their natural habitats being water, soil, and organic debris. The mycoses with the highest incidence—candidiasis and dermatophytosis— are caused by fungi that are part of the normal microbial flora or highly adapted to survival on the human host. For convenience, mycoses may be classified as superficial, cutaneous, subcutaneous, systemic, and opportunistic.

**LABORATORY DIAGNOSIS**

**- Direct Examination**

Fungi often demonstrate distinctive morphologic features on direct microscopic examination of infected pus, fluids, or tissues owing to their large size. The simplest method is to mix the specimen with a 10% solution of potassium hydroxide (KOH) and place it under a coverslip.

The strong alkali digests the tissue elements (epithelial cells, leukocytes, debris), but not the rigid cell walls of either yeasts or molds. After digestion of the material, the fungi can be observed under the light microscope with or without staining .

A few yeasts take the Gram stain, including *C albicans* (Gram positive).

**- Culture**

Fungi can be grown by methods similar to those used to isolate bacteria. Growth occurs readily on enriched bacteriologic media commonly used in clinical laboratories (eg, blood agar and chocolate agar). Many fungal cultures, however, require days to weeks of incubation for initial growth; bacteria present in the specimen grow more rapidly and may interfere with isolation of a slow-growing fungus. Therefore, the culture procedures of diagnostic mycology are designed to favor the growth of fungi over bacteria and to allow incubation to continue for a sufficient time to isolate slow-growing strains.

The most commonly used medium for cultivating fungi is Sabouraud’s agar, which contains only glucose and peptones as nutrients. Its pH is 5.6, which is optimal for growth of fungi.

Blood agar or another enriched bacteriologic agar medium is used when pure cultures would be expected. It can be made selective for fungi by the addition of antibacterial antibiotics such as chloramphenicol and gentamicin. Cycloheximide, an antimicrobial that inhibits some saprophytic fungi, is sometimes added to Sabouraud’s agar to prevent overgrowth of contaminating molds from the environment, particularly for skin cultures.

Selective media are not needed for growing fungi from sterile sites such as cerebrospinal fluid or tissue biopsy specimens. In contrast to most pathogenic bacteria, many fungi grow best at 25°C to 30°C, and temperatures in this range are used for primary isolation.

Once a fungus is isolated, identification procedures depend on whether it is a yeast or a mold. Yeasts are identified by biochemical tests analogous to those used for bacteria, including some that are identical (eg, urease production). The ability to form pseudohyphae is also taxonomically useful among the yeasts.

Molds are most often identified by the morphology of their conidia and conidiophores. Other features such as the size, texture, and color of the colonies help characterize molds.

These methods become less important with the development of specific DNA probes for the major systemic pathogens. These probes are rapid and can be applied directly to the mycelial growth of the readily grown mold phases of these fungi.

**- Antigen and Antibody Detection**

Serum antibodies directed against a variety of fungal antigens can be detected in patients infected with those agents. Except for some of the systemic pathogens, the sensitivity, specificity, or both, of these tests have not been sufficient to recommend them for use in diagnosis or therapeutic monitoring of fungal infections. Immunoassays and oligonucleotide probes to detect fungal antigens have been used for some time. The major targets are mannans, mannoproteins, glucan, chitin, or some other structure unique to the fungal pathogen(s). The only established test of this type is one that detects the polysaccharide capsule of *C neoformans*.