LECTURE 9&10 (2022)

Laboratory Diagnosis of Microorganisms

Infectious diseases

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi and parasites. Many organisms live in and on our bodies. They're normally harmless or even helpful, but under certain conditions, some organisms may cause disease.

Some infectious diseases can be passed from person to person. Some are transmitted by bites from insects or animals. And others are acquired by ingesting contaminated food or water or being exposed to organisms in the environment.

The methods that used for identification of microorganisms include:

1- Phenotypic (morphology)
- Direct exam
- Isolation of pathogen (culture)

2- Biochemical tests

3- Immunological (serological analysis)

4- Genotypic (genetic techniques)

Blood Stream Infections (BSI)

Blood stream infection (BSI) is a serious problem that needs immediate attention and treatment. It is a cause of high mortality especially if caused by multidrug resistant bacteria.

Bacteria and other microorganisms can enter the blood stream as a severe complication of infections (like pneumonia or meningitis), during surgery (especially when involving mucous membranes such as the gastrointestinal tract), or due to catheters and other foreign bodies entering the arteries or veins (including during intravenous drug abuse). Transient bacteremia can result after dental procedures or brushing of teeth.

Conditions which increase the chances of developing bacteremia include:

- Immune suppression, either due to HIV infection or drug therapy
- Antibiotic therapy which changes the balance of bacterial types in the body.
- Prolonged or severe illness.
- Alcoholism or other drug abuse
- Malnutrition
- Diseases or drug therapy that cause ulcers in the intestines, e.g. chemotherapy for cancer.

Symptoms of bacteremia may include:

- fever over (38.3 °C)
- chills
- abdominal pain
- nausea
- vomiting
- diarrhea
- shortness of breath
- confusion

Blood culture

Blood culture is usually the most sensitive method for detection microorganisms in the blood. Blood is cultured to detect and identify bacteria or other cultivable microorganisms (yeasts, filamentous fungi). The presence of such organisms in the blood is called bacteremia or fungaemia, and is usually pathological. In the healthy subjects, the blood is sterile.

Blood collection

Blood should be taken before antibiotics are administered. The best time is when the patient is expected to have chills or a temperature spike. It is recommended that two or preferably three blood cultures be obtained, separated by intervals of approximately 1 hour (or less if treatment cannot be delayed). The advantages of repeated cultures are as follows:

— The chance of missing a transient bacteremia is reduced.

— The pathogenic role of "saprophytic" isolates (e.g. *Staphylococcus epidermidis*) is confirmed if they are recovered from multiple vein punctures.

- Because the number of bacteria per milliliter of blood is usually low, it is important to take a reasonable quantity of blood: 10 ml per vein puncture for adults; 2–5 ml may suffice for children.
- Two tubes should be used for each vein puncture: the first a vented tube for optimal recovery of strictly aerobic microorganisms, the second anon-vented tube for anaerobic culture.
- The skin at the vein puncture site must be meticulously prepared using a bactericidal disinfectant.

Blood-culture media

The blood-culture broth (brain heart infusion broth) and tryptic soy broth (TSB) should be able to support growth of all clinically significant bacteria.

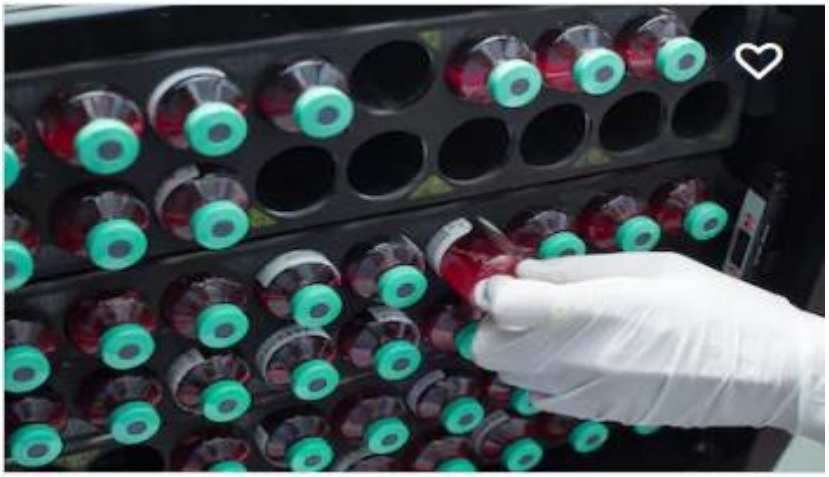
The blood should be mixed with 10 times its volume of broth (5ml of blood in 50 ml of broth) to dilute any antibiotic present and to reduce the bactericidal effect of human serum.

Incubation time

Blood-culture bottles should be incubated at 35–37 C for 7 days. In some cases, incubation may be prolonged for an additional 7 days, e.g. if *Brucella* or other fastidious organisms are suspected, in cases of endocarditis, or if the patient has received antimicrobials. A sterile culture usually shows a layer of sediment red blood covered by a pale yellow transparent broth. Growth is evidenced by:

— **a floccular deposit on top of the blood layer**
— uniform or subsurface turbidity
— haemolysis
— coagulation of the broth
— a surface pellicle
— production of gas
— white grains on the surface or deep in the blood layer.

BacT/ALERT system: is an automated microbial detection system based on the colorimetric detection of CO2 produced by growing microorganisms. It has a blood culture bottle with supplemented brain heart infusion broth (BHI) containing activated particles that significantly increase the yield of microorganisms over standard blood culture media. In addition, resins or charcoal may be added to commercial media to absorb and inactivate antimicrobial agents within the patient's blood. BacT/ALERT system has greatly simplified handling in the microbiological laboratory and provides faster detection for many organisms in a short time than is possible with conventional systems.



Whenever visible growth appears, the bottle should be opened aseptically, a small amount of broth removed with a sterile loop or Pasteur pipette, and a Gram-stained smear examined for the presence of microorganisms.

Subcultures are performed by streaking a loopful on appropriate media: MacConkey agar, Blood agar and chocolate agar.

Some microorganisms may grow without producing turbidity or visible alteration of the broth. Other organisms, e.g. pneumococci, tend to undergo autolysis and die very rapidly. For this reason some laboratories perform routine subcultures on chocolate agar after 18–24 hours of incubation.

Common causes of bacteraemia:

Gram-negative organisms	**Gram-positive organisms**
Escherichia coli	*Staphylococcus aureus*
Klebsiella spp.	*Staphylococcus. epidermidis*
*Enterobacter*spp.	a-Haemolytic (viridans) streptococci
Pseudomonas aeruginosa	*Streptococcus pneumoniae*
Salmonella typhi	*Enterococcus. faecalis*
Salmonella spp. other than *S. typhi*	*Streptococcus pyogenes*
Proteus spp.	*Streptococcus agalactiae*

Laboratory Diagnosis of Cerebrospinal Fluid (CSF) infections

Examination of cerebrospinal fluid (CSF) is an essential step in the diagnosis of bacterial and fungal meningitis and CSF must always be considered as a priority specimen that requires prompt attention by the laboratory staff.

Normal CSF is sterile, clear, colorless liquid found in your brain and spinal cord and usually contains three leukocytes or fewer per mm3 and no erythrocytes. The chemical and cytological composition of CSF is modified by meningeal or cerebral inflammation, i.e. meningitis or encephalitis.

A CSF analysis may include tests to diagnose:

- **Infectious diseases of the brain and spinal cord**: Including meningitis and encephalitis. CSF tests for infections look at white blood cells, bacteria, and other substances in the cerebrospinal fluid
- **Autoimmune disorders**: such as multiple sclerosis (MS). CSF tests for these disorder look for high levels of certain proteins in the cerebrospinal fluid. These tests are called albumin protein and IgG/albumin.
- **Bleeding in the brain**
- **Brain tumors**

Symptoms of a brain or spinal cord infection include:

- Fever
- Severe headache
- Stiff neck
- Nausea and vomiting
- Sensitivity to light
- Double vision
- Changes in behavior
- Confusion

Collection and transportation of specimens

Approximately 5–10 ml of CSF should be collected in two sterile tubes by lumbar puncture performed by a physician. Part of the CSF specimen will be used for cytological and chemical examination, and the remainder for the microbiological examination.

The specimen should be delivered to the laboratory at once, and processed immediately, since cells disintegrate rapidly. Any delay may produce a cell count that does not reflect the clinical situation of the patient.

Common causes of bacterial and fungal meningitis

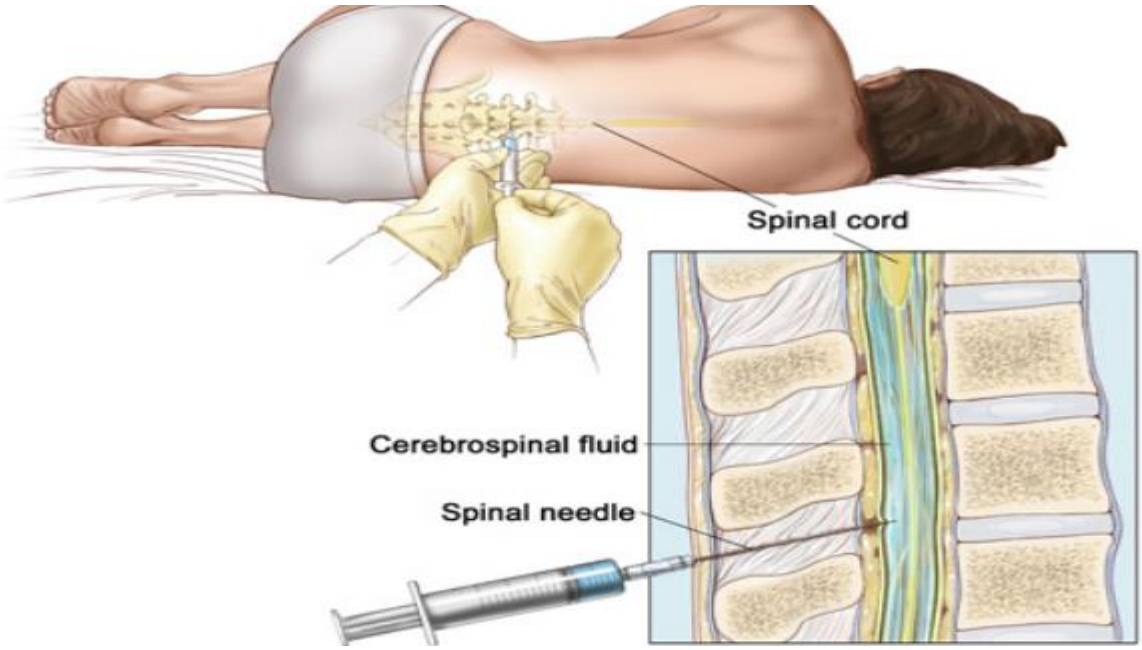
In neonates (from birth to 2 months):-

Escherichia coli, Listeria monocytogenes, Streptococcus agalactiae, Other Enterobacteriaceae, *Salmonella* spp. and *Citrobacter* spp.

In all other age groups:-

Haemophilus influenza, Neisseria meningitides, Streptococcus pneumonia, Mycobacterium tuberculosis, Listeria monocytogenes, Staphylococci and *Cryptococcus neoformans.*

- *Haemophilus influenza* the main cause meningitis in children.
- *Neisseria meningitides* and *Streptococcus pneumonia*the main cause meningitis in adult.



Microscopic examination

Preparation of specimen

If the CSF is purulent (very cloudy), it can be examined immediately without centrifugation. In all other cases, the CSF should be centrifuged in a sterile tube. Remove the supernatant and transfer it to another tube for chemical and/or serological tests. Use the sediment for further microbiologicaltests.

Direct microscopy

Examine one drop of the sediment microscopically for:

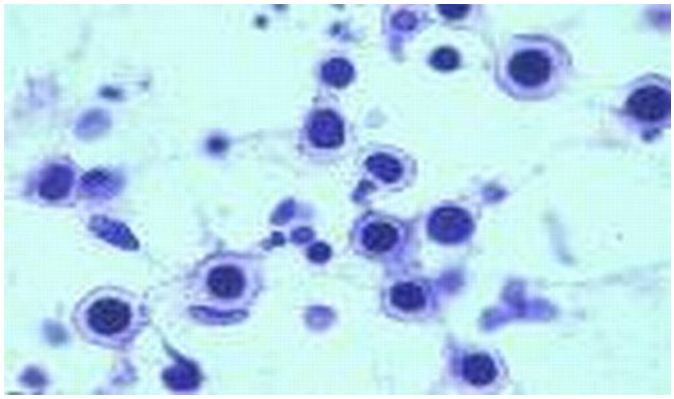
— leukocytes (polymorphonuclear neutrophils or lymphocytes)

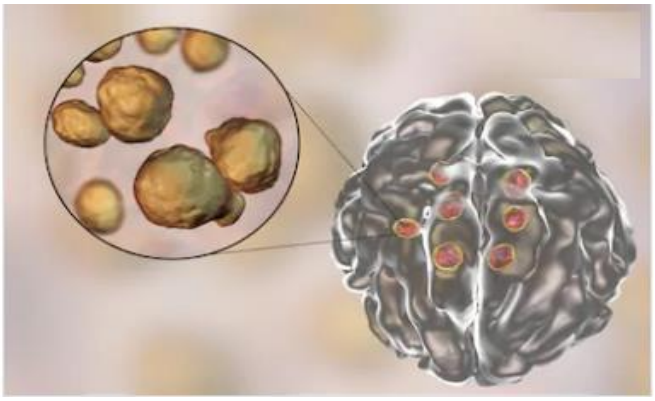
— erythrocytes

— bacteria

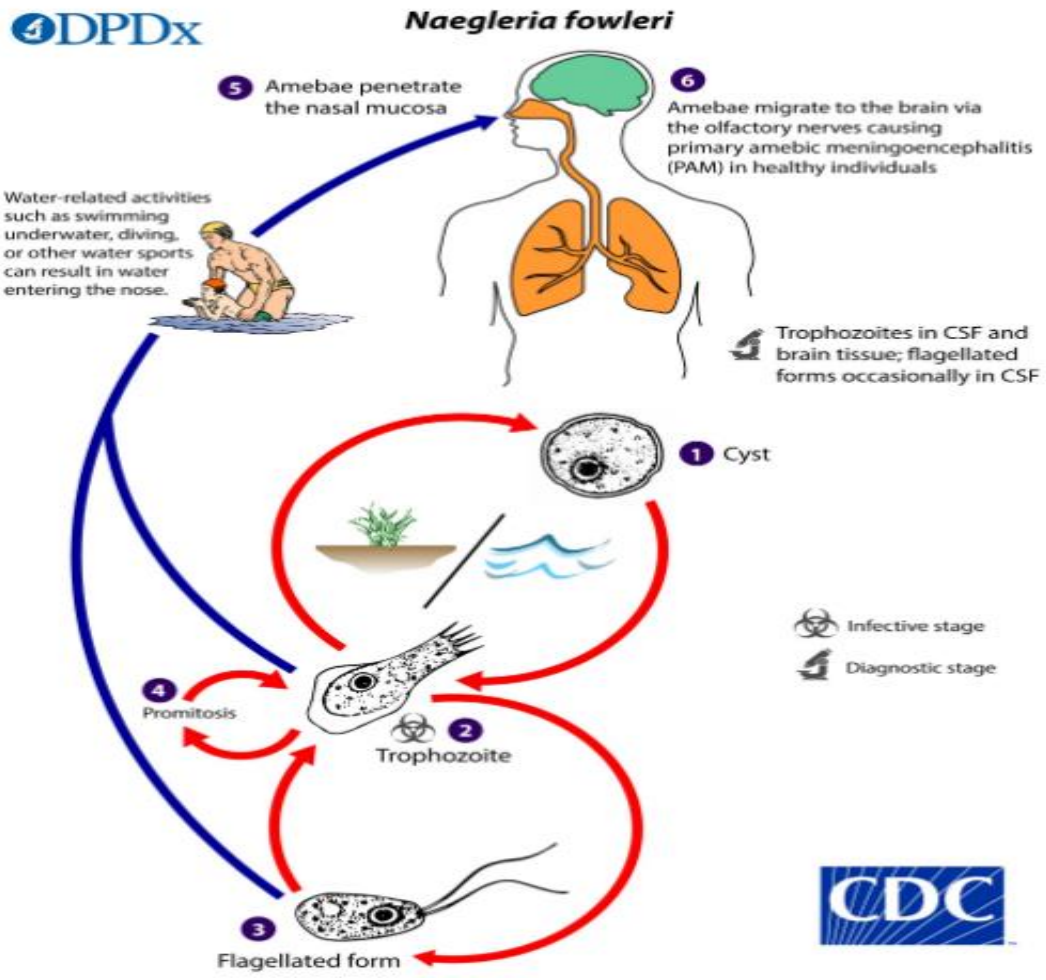
— yeasts.

If the yeast-like fungus *Cryptococcus neoformans*is suspected, mix a loopful ofthe sediment with a loopful of India ink on a slide, place a coverslip on top,and examine microscopically for the typical, encapsulated, spherical, buddingyeast forms.





A rare and generally fatal type of meningitis is caused by free-living amoebae found in water (***Naegleria fowleri*)** which enter through the nose and penetrate the central nervous system. They may be seen in the direct wet preparation as active motile amoebae**.**



Gram-stained smears

As the causative agent of bacterial meningitis may often be observed in a Gram-stained smear, this examination is extremely important.

Acid-fast stain (Ziehl–Neelsen)

Examination of an acid-fast-stained preparation of the sediment or of the fibrin web is indicated when tuberculous meningitis is suspected by the physician.

Measure protein (Lower limit15mg/dL and Upper limit40–45mg/dL) **Measure glucose** (Lower limit50mg/dL and Upper limit 80 mg/dL)

Culture

- The CSF cultures are performed by streaking a loopful on Blood agar, chocolate agar and MacConkey agar, then incubated at 35 -37 C in an atmosphere enriched with carbon dioxide. All media should be incubated for 3 days, with daily inspections.
- When tuberculous meningitis is suspected by *Mycobacterium tuberculosis*, at least three tubes of Löwenstein– Jensen medium should be inoculated with a drop of the sediment and incubated for 6 weeks.

When *Cryptococcus neoformans*is suspected, either from the India ink preparation or on clinical grounds, the sediment should be inoculated on two tubes of Sabouraud dextrose agar, and incubated at 35 C for up to 1 month. *C. neoformans* also grows on the blood agar plate, which should be incubated at 35C for 1 week.

VITEK 2 System for Rapid Identification of Clinical Isolates

The fully automated VITEK 2 system (bioMérieux) can provide **identification** results for microbial identification (bacteria and yeast identification) rapidly, accurately and reliable species-level identification in a few hours. It improved microbial identification and **antibiotic susceptibility testing** (AST) for all microbial isolates which isolated from different clinical specimens (blood, CSF, urine, stool, wound, burns, and others…).

The VITEK 2 system can:

- Reduce time to microbial identification and antibiotic susceptibility testing results
- Reduce waste with a miniaturized card-format that measures 10 cm x 6 cm x 0.5 cm and weighs only 16 grams
- Meet the needs of any size laboratory
- Offer an extensive identification and susceptibility menu.

bioMérieux Customer:

Microbiology Chart Report

Printed October 2(2021 7:25:09 PM CDT

Patient Name: 33
Location:
Lab ID: 28B

Patient ID: 28B
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: BLOOD

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.20 hours	**Status:**	Final
Selected Organism	99% Probability	**Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber:	2607734651564010		
ID Analysis Messages				

Susceptibility Information	**Analysis Time:**	9.08 hours	**Status:**	Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Meropenem	>= 16	R
Ampicillin	>= 32	R	Amikacin	>= 64	R
Amoxicillin/Clavulanic Acid	>= 32	R	Gentamicin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Ciprofloxacin	>= 4	R
Cefotaxime	>= 64	R	Norfloxacin	>= 16	R
Ceftazidime	>= 64	R	Fosfomycin	<= 16	S
Cefepime	>= 64	R	Nitrofurantoin	256	R
Imipenem	>= 16	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

AES Findings

Confidence:	Consistent