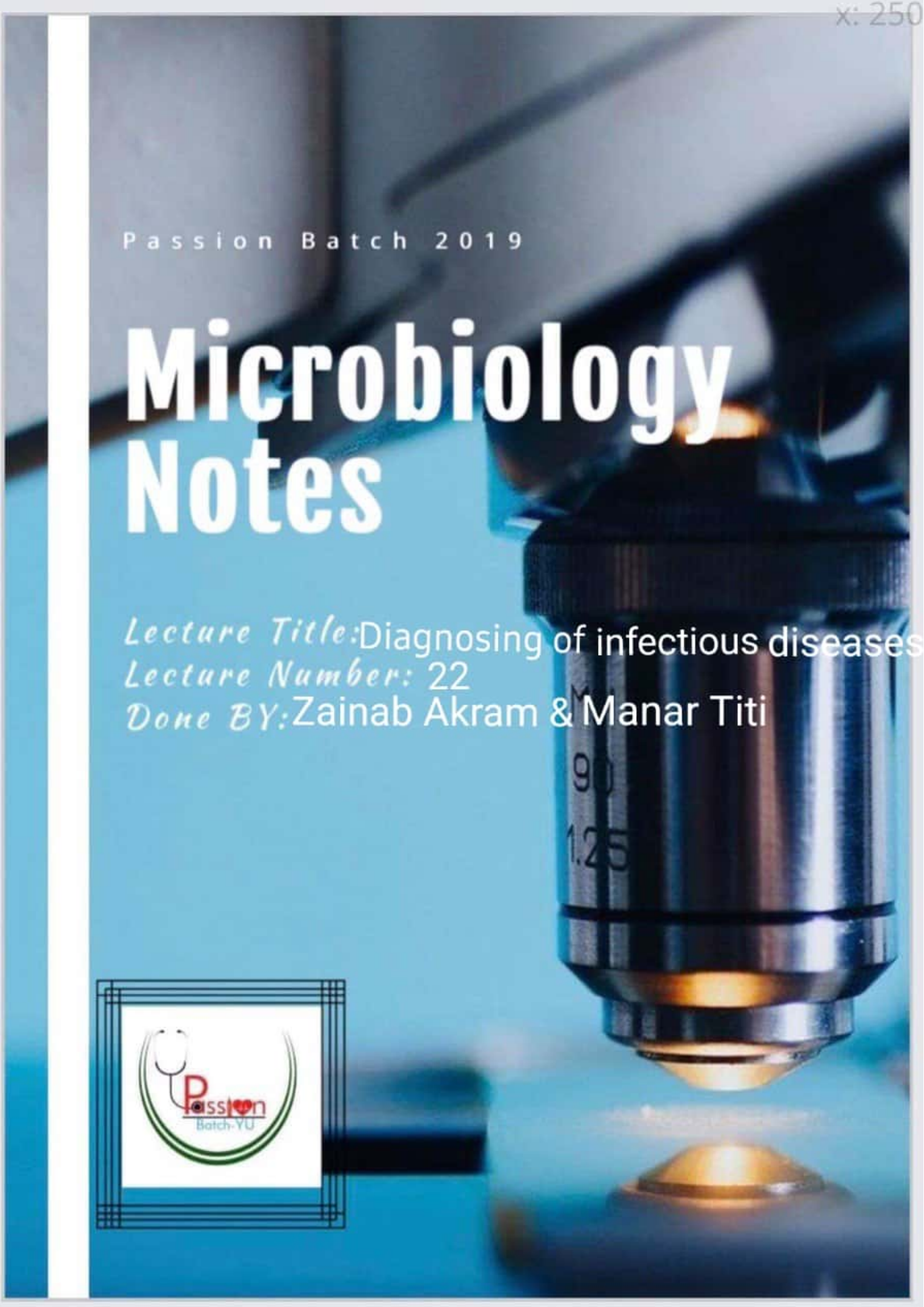

Diagnosing of infectious diseases

INTRODUCTION

Dr. Waleed Al Momani, MLT, PhD

Introduction

The most important role of specialists is the diagnosis of infectious diseases

Diagnosis of infectious diseases requires

Before we start the treatment we should diagnose the causative agent
We start diagnosis when the patient arrives to the hospital

1. Patient history

بنسأل المريض وين راح وين أجى خلال الفترة الماضية ومع مين عمل **contact** > كله عشان نعرف نحدد ال **causative agent** الصح
حتى هون أحنا لسا بنتوقع وما عندنا يقين تام

2. Physical examination

هون حتشوف حرارته وضغطه او مستوى السكرى وغيرة

3. Evaluating the patients signs and symptoms

هون مهم نعرف من متى بدأت تظهر هاى الأعراض ع المريض وهل كانت مستمرة او بتيجى وبتروح(**relapsing**)مثال عليها درجة
درجة حرارة المريض تعتمد على ال **toxic material**(**pyrogens**) يلى بتفرزها البكتيريا > منها بترفع درجة الحرارة كثير ومنها شوى

4. Implementing the proper selection, collection, transport and processing of appropriate clinical specimens

الان اجا دور العينة يلي حنطلبها من المريض >> من الأمثلة عليها (**urine,,blood,,CSF,,stool**)
بس لازم نعرف شغلة مهمة >> الطبيب الشاطر بكون **more precise** بإختيارة للفحوصات اللازمة فقط ،،
مش كل ما هب ودب

Clinical specimens

في بعض الأحيان (مثل حالات التسمم الغذائي) بنضطر ناخذ عينات غذائية ونفحصها مثل المايونيز #ولكن هذه العينات لا تعتبر clinical specimen

- All types of specimens collected from patients and used to diagnose or follow the progress of infectious diseases are called clinical specimens

ال clinical specimen جدا مهمة فى مساعدتنا معرفة تطور الحالة المرضية وهل فى استجابة للعلاج او لازم نغير الجرعة

- Role of healthcare professionals in the submission of clinical specimens

- Doctor, nurse, medical technologist

هذول ال٣ أشخاص مسؤولين مسؤولية تامة عن جمع high quality clinical specimen لأنه كل ما كانت ال specimen افضل كل ما كان عندنا diagnosis أفضل

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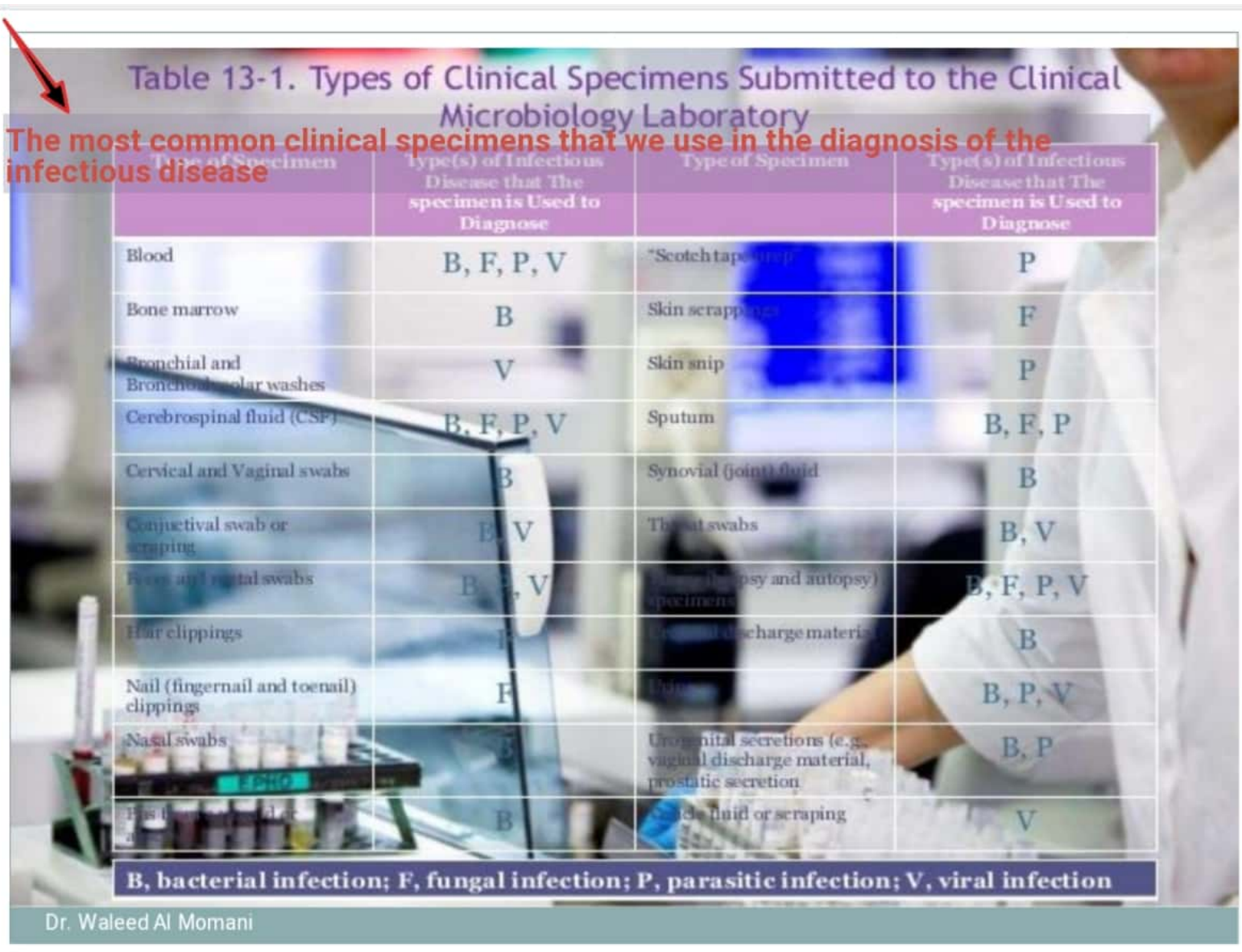

Table 13-1. Types of Clinical Specimens Submitted to the Clinical Microbiology Laboratory

The most common clinical specimens that we use in the diagnosis of the infectious disease

Type of Specimen	Type(s) of Infectious Disease that The specimen is Used to Diagnose	Type of Specimen	Type(s) of Infectious Disease that The specimen is Used to Diagnose
Blood	B, F, P, V	"Scotch tape" prep	P
Bone marrow	B	Skin scrapings	F
Bronchial and Bronchoalveolar washes	V	Skin snip	P
Cerebrospinal fluid (CSF)	B, F, P, V	Sputum	B, F, P
Cervical and Vaginal swabs	B	Synovial (joint) fluid	B
Conjuctival swab or scraping	B, V	Throat swabs	B, V
Fecal and rectal swabs	B, P, V	Tissue (biopsy and autopsy) specimens	B, F, P, V
Hair clippings	F	Urogenital discharge material	B
Nail (fingernail and toenail) clippings	F	Urine	B, P, V
Nasal swabs	B	Urogenital secretions (e.g., vaginal discharge material, prostatic secretion	B, P
Pus (from wound)	B	Vesicle fluid or scraping	V

B, bacterial infection; F, fungal infection; P, parasitic infection; V, viral infection

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Their Role in the Submission of Clinical Specimens

HEALTHCARE PROFESSIONALS

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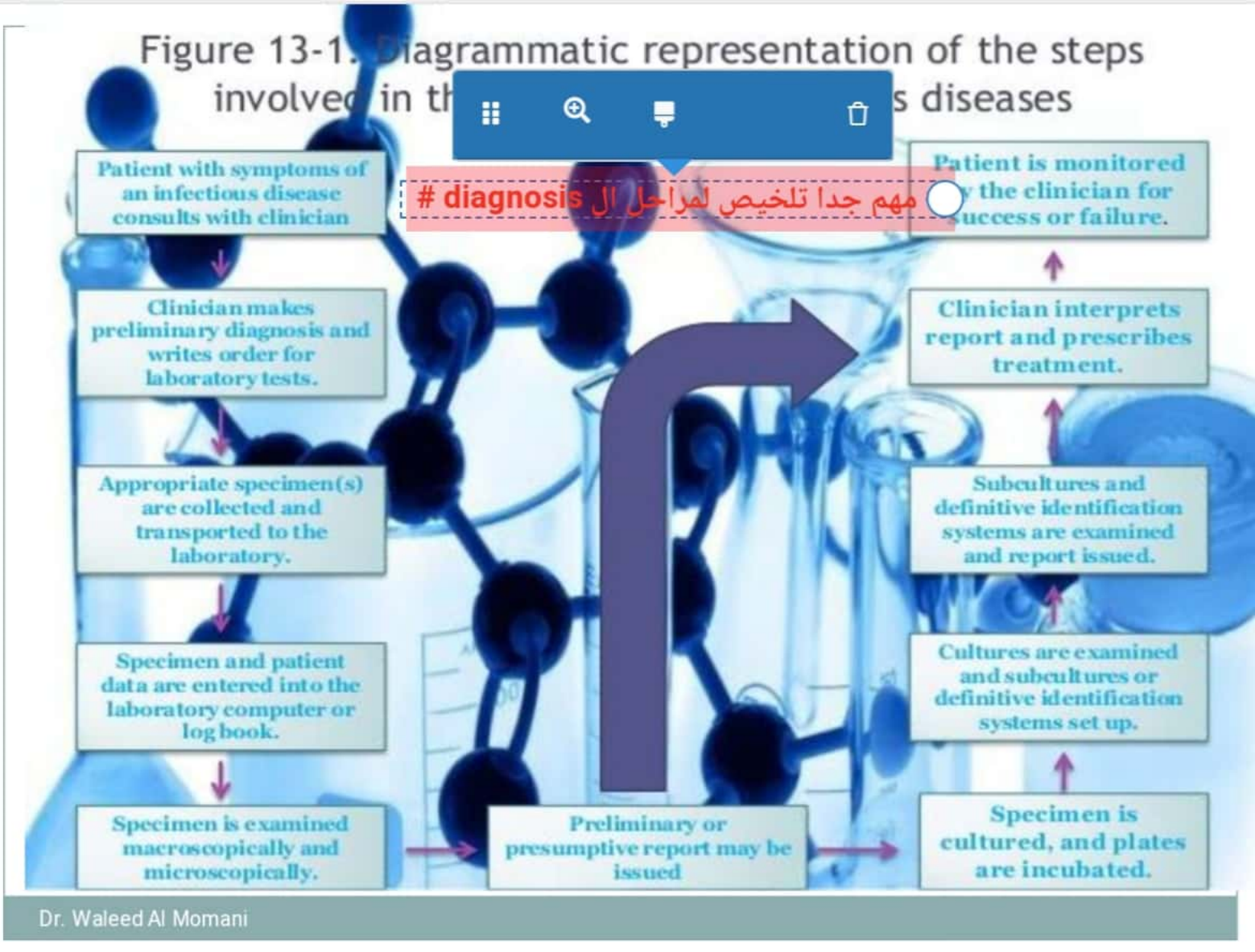

- Importance of high quality clinical specimen
- Clinically relevant laboratory results provide information about the patients infectious disease
- High quality specimen results in good results

 # High quality specimen >> must not have contamination

Proper selection, collection and transport of clinical specimen

- Specimen must be selected properly, the appropriate type of specimen
- Specimen must be collected in a manner that will minimize contamination with indigenous microflora

اذا كانت العينة **contaminated** وعملنالها **culturing** رح يكون عندي عدد كبير من ال **microorganisms** وبالتالي رح يسببلي **misleading in diagnosis**

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- The specimen should be collected from a site where the suspected pathogens is most likely to be found and where the least contamination is likely to occur

 # when you collect a specimen from a patient with tonsillitis you must collect from the scarp only >> because it contains the concentrated organism that we need in culturing (single organism) ... but if you collect from all over the tonsils >> your specimen will contain oral cavity normal flora and other organisms

- Specimen should be collected before antimicrobial therapy

 # نتفق على شغلة >> لما بدنا نجمع ال specimen >> ممنووووع وما بصيييير تعطي المريض ال antibiotic وبعدين تطلب جمع ال specimen
.... طيب ليش ؟؟ لانه انت عملت قتل للmicrobes وقللت عددهم (suppression) فما رح تلاقي microbes ورح تفكر انه ما في causative agent ولكن هذا ما يسمى بال false negative culturing

Proper selection, collection and transport of clinical specimen

- The acute stage the most appropriate time to collect most specimen # because every thing in this stage is max > the signs , symptoms and the viability of the organism is
max + the microorganism is in its active stage which is the proper conditions for culturing
- A sufficient quantity of the specimen should be collected
لازم من أول مرة بجمع فيها العينة اكون ماخذ عينة كافية لانه لو طلبت أكثر من مرة تاخذ عينة من المريض , ممكن يأثر ع الحالة النفسية اله
- All specimens should be placed into a sterile container
- Hazardous specimens must be handled with greater care
- Specimens should be collected and delivered to the lab as soon as possible

 # time is critical for the specimens >> some specimens must be processed in 30 minutes >> so if you leave it for more than 30 minutes it will not work so you have to collect it again

 # the maximum time for a specimen to be processed is 2 houres

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Types of clinical specimens usually required to diagnose infectious diseases

1. Blood # وصول البكتيريا للدم يعتبر خطر كبير لانه الدم بحالة حركة دائمة مما يزيد من احتمالية وصولة لمواقع كبيرة في الجسم

- Bacteremia: the presence of bacteria in blood, may occur following oral surgery, tooth extraction, and may occur during certain stages of many infectious diseases (meningitis, typhoid fever, other salmonella infections

there are 3 types of bacteremia :1- permanent > تواجدها دائم
2- transient > (as a stage of a disease) تعاملت مع الدم لـ ممر فقط عشان توصل لمكان ثاني > فقط
3- intermediate> لو كنت تفرشى اسنانك و جرحت اللثة هون حيكون فى بكتيريا بتدخل وبتطلع وما بتضر

مثلا typhoid fever يبدأ ظهور البكتيريا بالدم ع الأسبوع الثاني

- Septicemia: The presence of bacteria and their toxins in the bloodstream, most serious types are those caused by Gram negative bacteria due to endotoxin #septicemia is more dangerous than bacterimea

they work only when they are in the blood stream due to bacterial cell lysis

- To diagnose bacteremia and septicemia 3 blood cultures should be collected over 24 hr period # the best time for collecting blood sample is at the high fever state > bacteria is at its max vaiability

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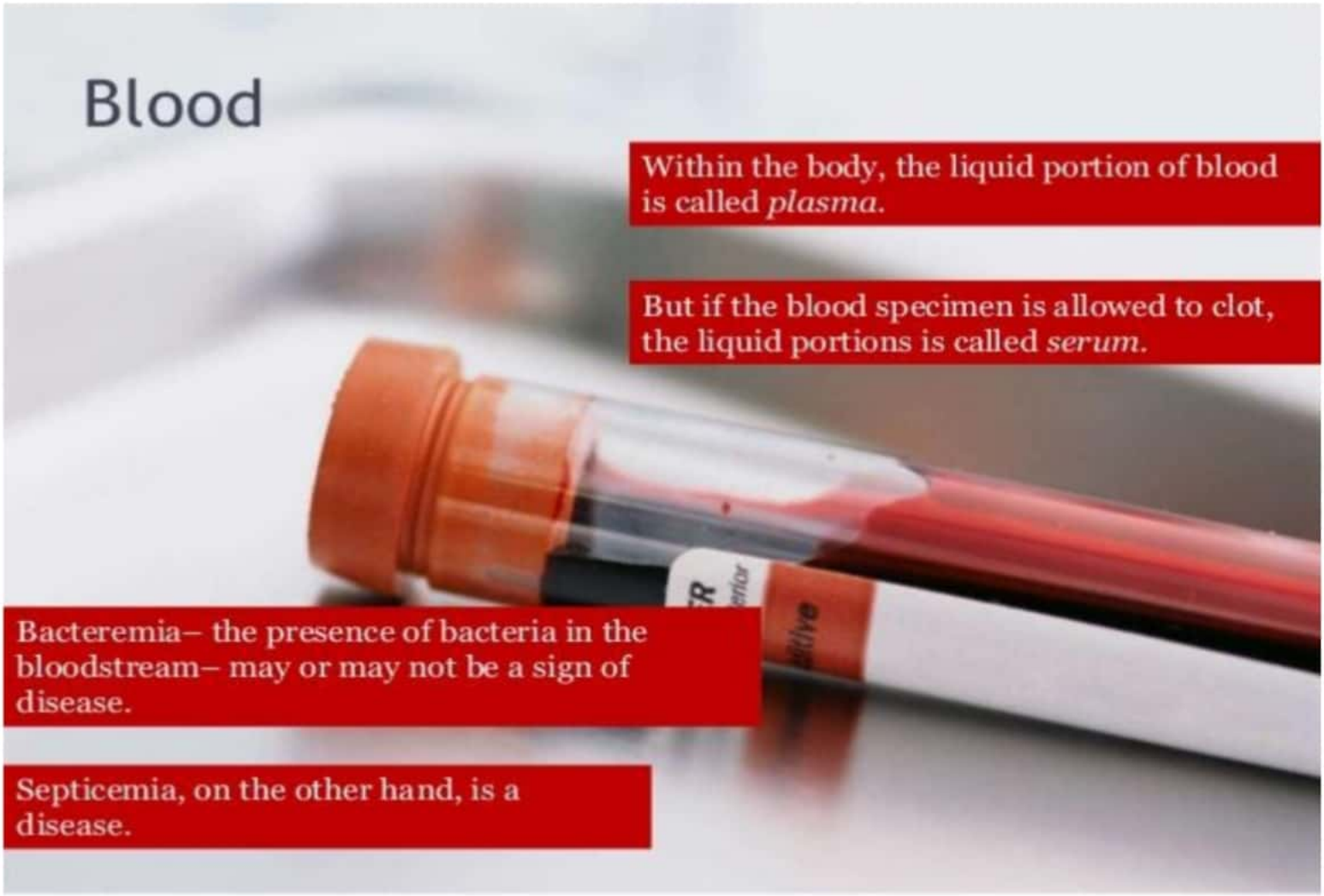

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2. Urine

we use it to detect the UTI .. and as we know > usually, most of the urinary tract is organism free except the urethra

- Mid-stream urine

UTI is frequent among females > because of the anatomical function of the urethra (short distance between urinary bladder and
the urethra increase UTI in females)

- Clean the area around the external opening of the urethra

- Urine specimen should be cultured in 30 min to prevent overgrowth

there are 2 wayes to be affected with UTI

1- ascending way .. urethra >urinary bladder >ureter > kidney

2-descending way.. kidney > ureter > urinary bladder> urethra

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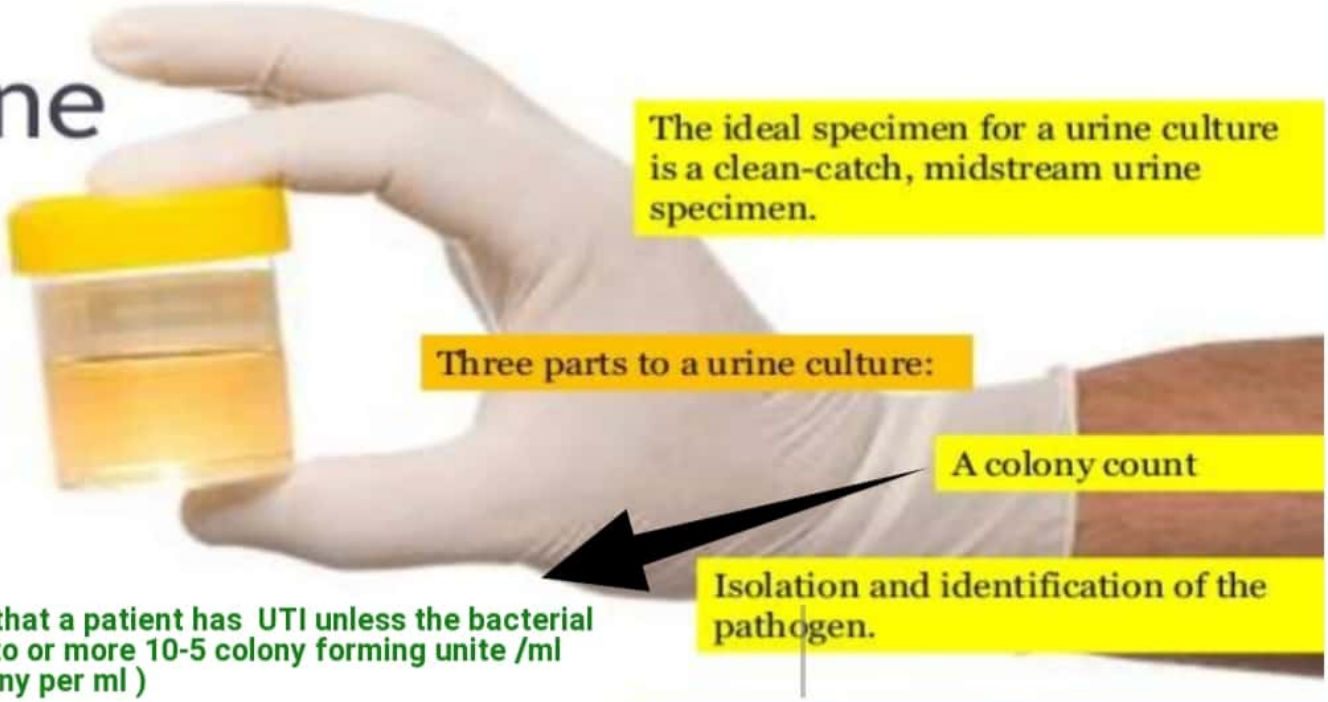
Urine



The ideal specimen for a urine culture is a clean-catch, midstream urine specimen.

Three parts to a urine culture:

- A colony count
- Isolation and identification of the pathogen.
- Antimicrobial susceptibility counting

we cant say that a patient has UTI unless the bacterial count equal to or more 10-5 colony forming unite /ml (100000 colony per ml)

less than that he isn't suffering from UTI >their is no need for antibiotics

#in some cases we repeat the test if the count was 10-4 ,, and if it still 10-4 or 10-3 >> consider as no infection (stable)

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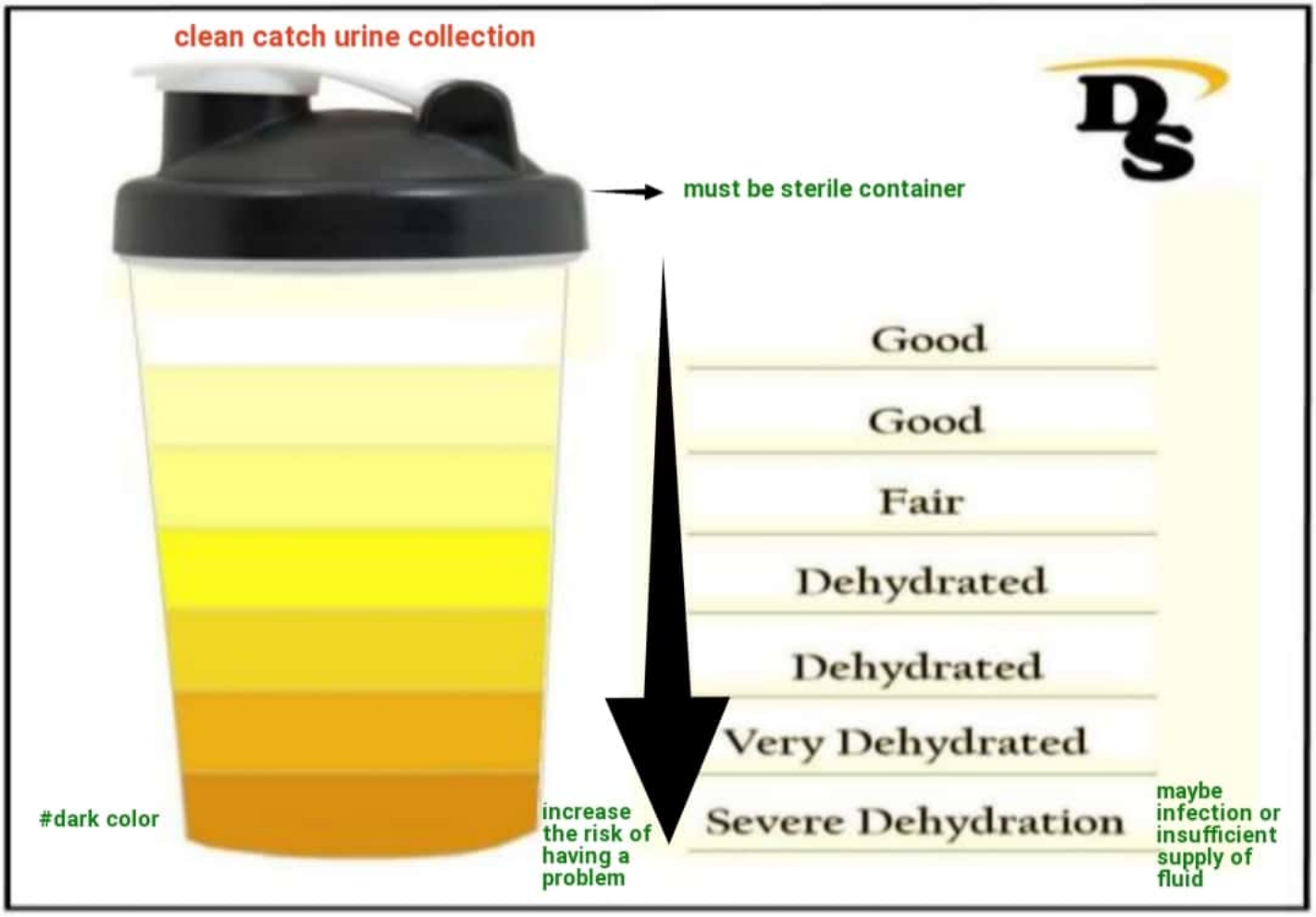

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Urine culture pass through 3 parts

- 1- colony count
- 2- Isolation and identification >> same as other bacterial infections
- 3- antimicrobial susceptibility
- colony counted using calibrated loop (0.01/0.001)/ml of urine # if there is a patient with no urine flow (obstruction due to chronic infection) we can collect urine by 2 ways :
  1- using catheter
  2- in blocked cases we use (suprapubic aspiration needle) > aspirate the urine directly from the bladder > in this procedure there is no contamination - in clean batch procedure , urine will be contaminated with normal flora from the urethra -
- CFU of 1x 105 /ml or higher is indicative of a UTI

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3. Cerebrospinal fluid

brain is the least portal of entry for bacteria , protozoa , fungus and viruses ... but in some cases they can reach CNS and cause 3 infectious diseases :
1- meningitis 2- encephalitis
3- meningoencephalitis

- CSF can be infected with bacteria, fungus, protozoa and viruses
- Meningitis is the inflammation of the membranes (meninges) that surround the brain and spinal column
- Encephalitis is the inflammation or infection of the brain

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- Meningoencephalitis is inflammation or infection of both the brain and meninges

- CSF must be collected in a sterile tube by a lumber puncture

- CSF must be rushed to the lab and must not be refrigerated **+ must be processed within 15 minute**

- Gram stain should be done as emergency and reported to the physician directly

لها الأولوية بال **processing** فور وصولها لل **lab** >> أولا كون عملية جمعها صعبة جدا تشكل خطورة الإصابة بالشلل في حال تم إصابة احد ال **nerves**

#العينة الوحيدة يلي ما بننتظر اوراق الفحص من اللاب حتى نعطي الدواء >> رنة التلفون كافية لتحديد مصير الإنسان خصوصا لو كان **bacterial infection**

#لو كان **viral infection** ما بنقدر نعمل اشى

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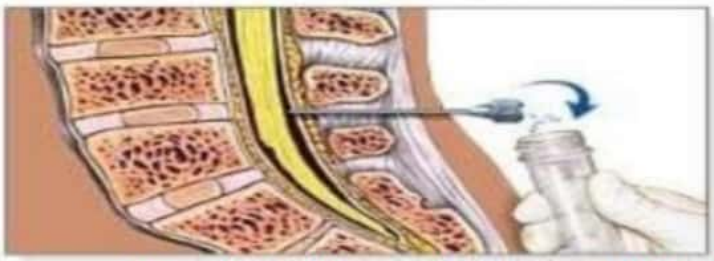
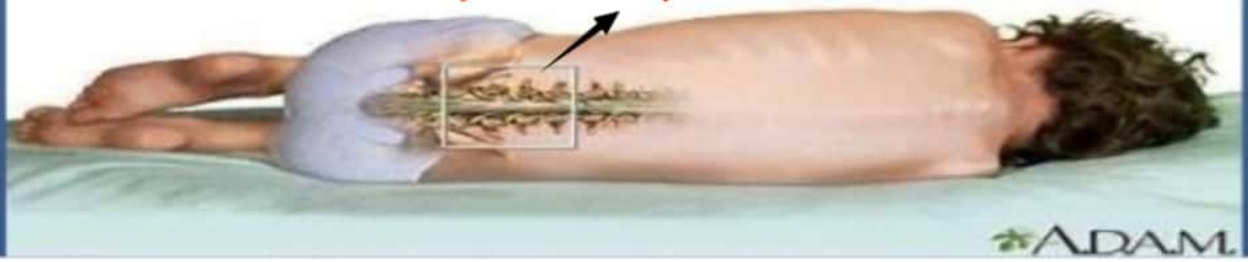
Cerebrospinal Fluid

Cerebrospinal fluid specimens are treated as STAT (emergency) specimens in the CML, where workup of the specimens is initiated immediately upon receipt.



Spinal fluid is collected for testing

it is collected by anesthesia specialist between lumber 4-5



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4. Sputum

سو لما أطلب من المريض يعملي deep couph فال sputum رح يمر بكل طريق ال normal flora (trachea ,mouth)

- It is pus that accumulates deep in the lungs of a patient with pneumonia, tuberculosis or other LRT infections # bacterial influenza also can be collected in the same way

  # it is very difficult to collect sputum without contamination >> so the first step after collecting is decontamination >>by washing with disinfectant >> then smear

- Handling should be with extreme care because one could easily be infected with the pathogens

  #أول مشتبه به بنفكر فيه هو ال acid fast bacteria يلى بتسبب ال TB

- Better specimen may be collected by bronchial aspiration through a bronchoscope or by transtracheal aspiration

  # نفس فكرة ال suprapubic aspiration needle الخاصة بال urine

suprapubic aspiration needle + bronchoscope or transtracheal aspiration >> mostly used in unconscious patients

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Sputum

Laboratory workup of a good quality sputum specimen can provide important information about a patient's lower respiratory infection, whereas workup of a patient's saliva cannot.

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5. Throat swab >> mainly used for tonsillitis

- To detect strep throat caused by streptococcus
  group A
- If other pathogens are suspected (N. gonorrheae, C. diphtheriae) it should be noted on the request to use special media

Sexually transmission disease
throat ولكن بهاى الحالات بكون متواجد بأماكن غريبة مثل ال

use culture media other than blood

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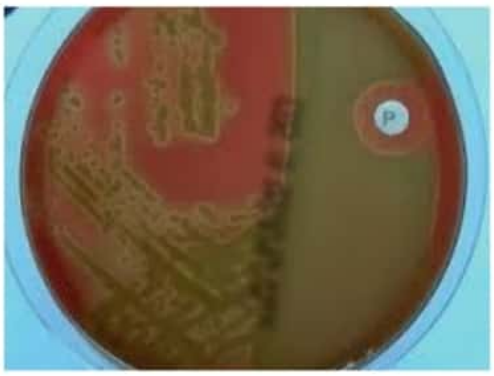

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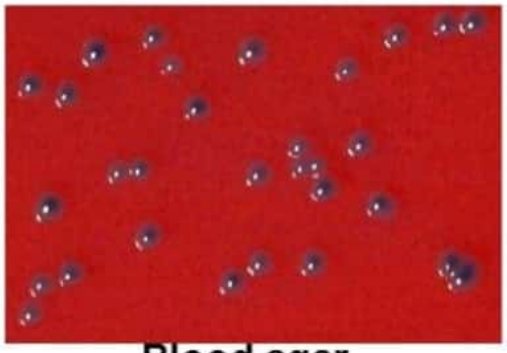
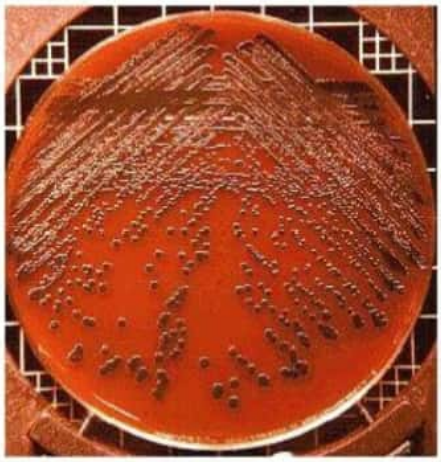
Growth of diphtheria bacilli



Blood agar

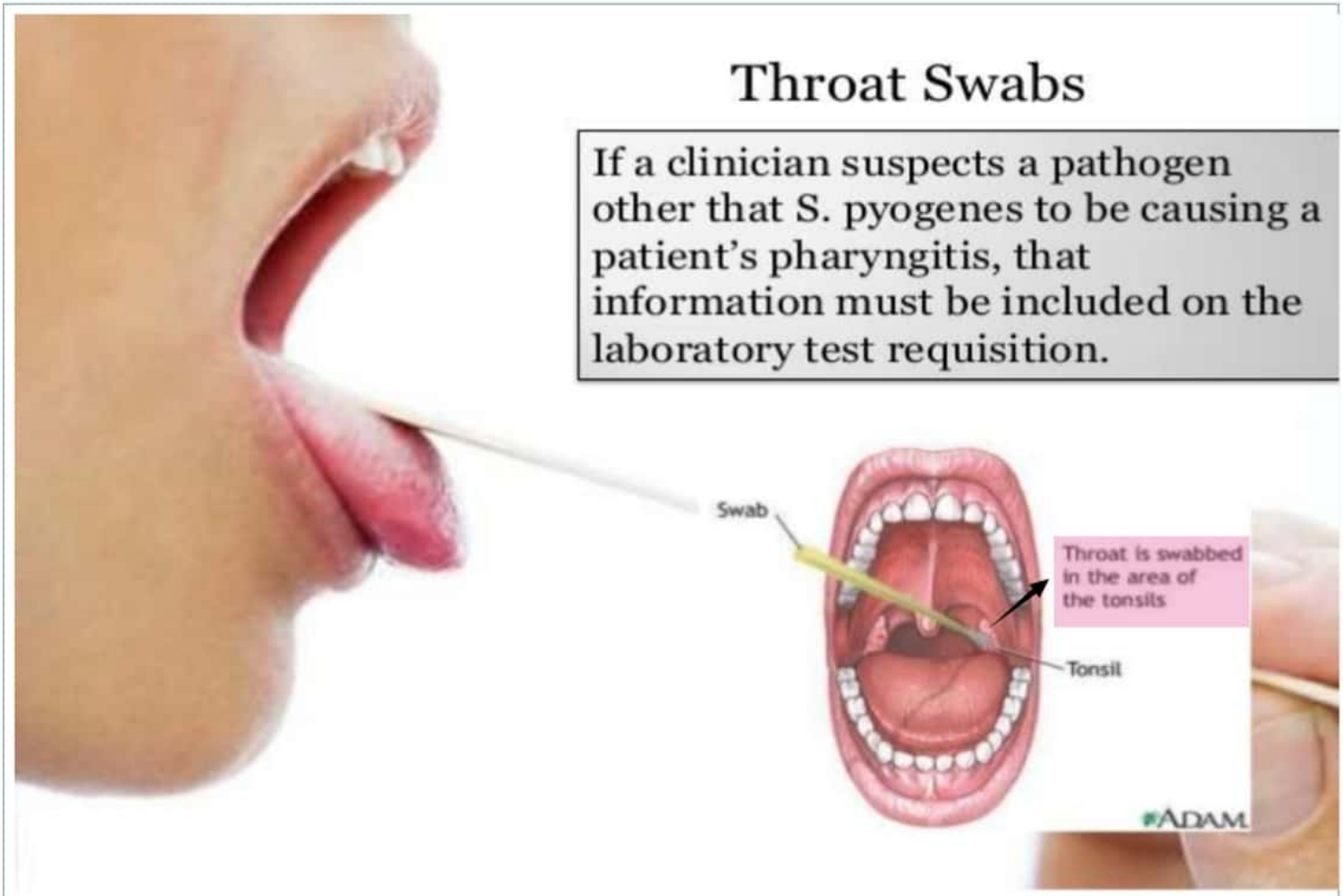


Tellurite blood agar

Loeffler's serum slope

Throat Swabs

If a clinician suspects a pathogen other that S. pyogenes to be causing a patient's pharyngitis, that information must be included on the laboratory test requisition.



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6. Wound specimen

- The type of wound (dog bite, burn, postsurgical) should always be indicated

لازم احدد ايش نوع ال **infection** عشان اعرف مصدر ال **normal flora** من وين جاى و ايش هيه احتمالية

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Wound swab

The laboratory test requisition that accompanies a wound specimen must indicate the type of wound and its anatomical location.

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Gonococcus

7. GC Cultures

to detect the infection in the reproductive system either male(urethritis) or female(vaginitests or cervicitis)

- GC is a fastidious bacterium, it is microaerophilic, capnophilic
- Nontoxic swabs should be used to collect specimens (vaginal, urethral, throat, rectal cervical) for GC culture
- Should be inoculated directly in a special media (Thayer-Marten) and incubated in CO_2 incubator with 5-10% O_2

GC is very sensitive to the cotton swap >> so we use special swap called (charcoal treated swaps)

we can diagnose Neisseria gonorrhoeae by only gram stain

gram negative deblococci

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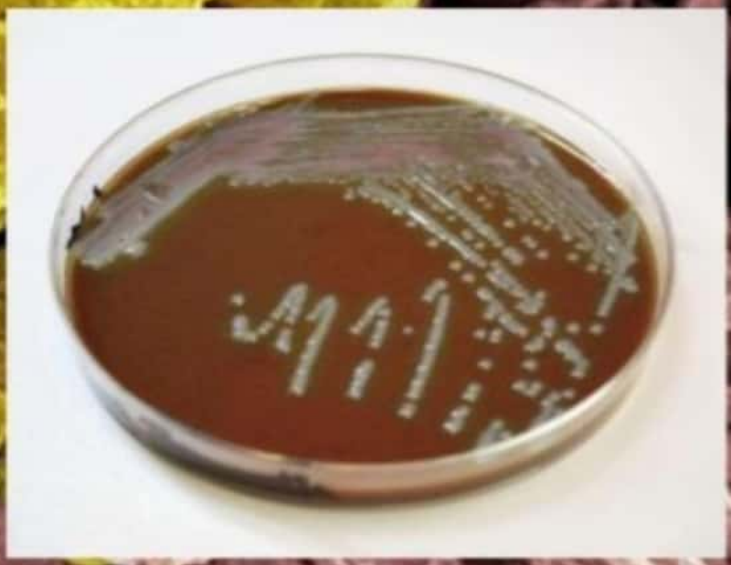
GC Culture

When attempting to culture Neisseria gonorrhoeae, one should rember that it is a fastidious, microaerophilic, and capnophilic organisms.



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7. Fecal specimen

- Fecal specimen should be collected at the lab and processed immediately to prevent the decrease in temperature and pH which will lead to the death of Salmonella and Shigella

هذول متعودين يعيشوا بجسم المريض ع درجة حرارة 37 و PH محددة فلما تنزل درجة الحرارة لل room temperature فورا بموتوا
+هذول أول المتهمين بتواجدهم بال fecal specimen

- The colon is anaerobic, fecal bacteria are obligate, aertolerant and facultative anaerobes

ولكن في بعض الحالات بتساعدنا ال patient history لتوقع وجود ميكروبات ثانية >> مثل لو كان المريض ماكل وجبة سمك وكان بشكى من watery rice diarrhea فالمسبب بكون vibrio cholera يلى وصلته من السمك

- The fecal specimen is cultured anaerobically whenever food poisoning caused by Clostridium spp. is suspected

لو كان ماكل برجر وكان معه food poisoning فالمسبب بكون ال E-coli.

#لو كان ماكل canned food مثل الفول فالمسبب بكون clostridium botulinum وبتعمل عند المريض flaccid paralysis or diarrhea or constipation

E-coli , vibrio cholera ,salmonella and shigella >>> facultative anaerobes
clostridium botulinum >> anaerobic

 # من هالمعلومتين لازم نعرف انه ال stool specimen لازم نعمللها زراعة ب anaerobic condition

The Pathology Department ("The Lab")

من هون لنهاية المحاضرة مطالبين فيه وممكن ييجي عليه أسئلة

- The CML is an integral part of the Pathology Department (which is frequently referred to simply as "the lab").
- The Pathology Department is under the direction of a pathologist (a physician who has had extensive, specialized training in pathology

Anatomical Pathology

Most pathologists work in Anatomical Pathology, where they perform autopsies in the morgue and examine diseased organs, stained tissue sections, and cytology specimens

Anatomical Pathology include:

1. Histopathology Laboratory

2. the Cytology Laboratory

3. the Cytogenetics Laboratory

Clinical Pathology consists of:

1. CML
2. Clinical Chemistry Laboratory
3. Hematology Laboratory
4. Blood Bank
5. Immunology Laboratory

The Clinical Microbiology Laboratory

CML maybe under the direction of a pathologist, a microbiologist or, in smaller hospitals, an MT who has had many years of experience working in microbiology

Responsibilities

The primary mission of the CML is to assist clinicians in the diagnosis and treatment of infectious diseases

The four major responsibilities of the CML are to

(a) Process clinical specimens,
(b) Isolate pathogens
(c) identify pathogens
(d) perform antimicrobial susceptibility testing when appropriate to do so.

In general, the processing of clinical specimens in the CML includes

(a) examining the specimen macroscopically
(b) Examining the specimen microscopically
(c) inoculating the specimen to appropriate culture media.

A less frequent responsibility of the CML is to process environmental samples whenever there is an outbreak or epidemic within the hospital.

Isolation and Identification of Pathogens

To isolate bacteria and fungi from clinical specimens, specimens are inoculated into liquid culture media or onto solid culture media.

Bacteriology Section

The responsibility of the Bacteriology Section of the CML is to assist clinicians in the diagnosis of bacterial diseases.

CML professionals gather “clues” (phenotypic characteristics) about a pathogen until they have sufficient information to identify it.

The various phenotypic characteristics

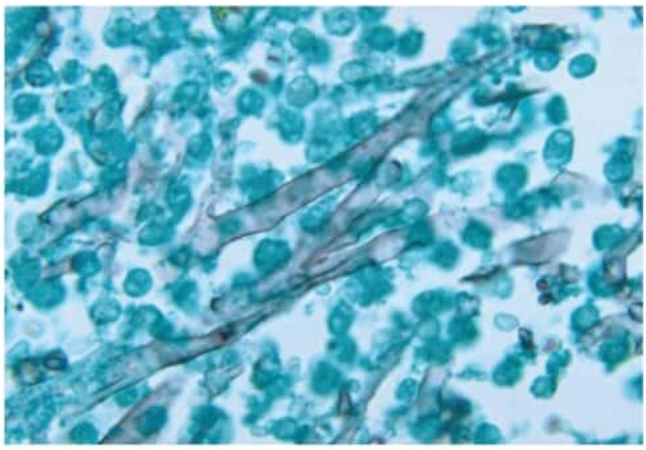
- Gram reaction (i.e., Gram-positive or Gram-negative)
- Cell shape
- Morphologic arrangement of cells
- Growth or no growth on various types media
- Colony morphology
- Presence or absence of a capsule
- Motility
- Number and location of flagella
- Ability to sporulate

- Location of spores (terminal or subterminal)
- Presence or absence of various enzymes
- Ability to catabolize various carbohydrates and amino acids
- Ability to reduce nitrate
- Ability to produce indole from tryptophan
- Atmospheric requirements
- Type of hemolysis produced

Mycology Section

The overall responsibility of the Mycology Section of the CML is to assist clinicians in the diagnosis of fungal infections (mycoses).

When isolated from clinical specimens, yeasts are identified using various biochemical tests, primarily based on their ability to catabolize various carbohydrates.



Fungal hyphae

A colony (mycelium) of an *Aspergillus*

Parasitology Section

The overall responsibility of the Parasitology Section of the CML is to assist clinicians in the diagnosis of parasitic diseases

infections caused by endoparasites (parasites that live within the body), such as parasitic protozoa and helminths (parasitic worms).

In general, parasitic infections are diagnosed by observing and recognizing various parasite life cycle stages (e.g., trophozoites and cysts of protozoa; microfilariae, eggs, and larvae of helminths) in clinical specimens.

Parasites are identified primarily by the characteristic appearance (e.g., size, shape, internal details) of the various life cycle stages that are seen in clinical specimens. Sometimes, whole worms or segments of worms are observed in fecal specimens

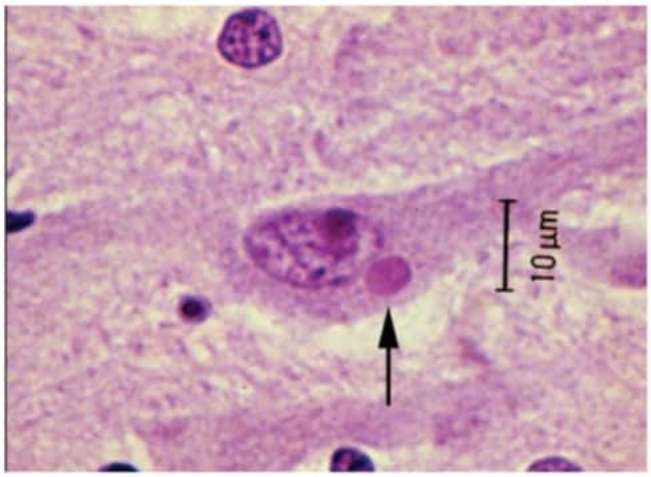
Virology Section

The overall responsibility of the Virology Section of the CML is to assist clinicians in the diagnosis of viral diseases.

Many viral diseases are diagnosed using immunodiagnostic procedures

Techniques used to identify viral pathogens

1. Observation of intracytoplasmic or intranuclear viral inclusion bodies in specimens by cytologic or histologic examination



2. Observation of viruses in specimens using electron microscopy

3. Molecular techniques such as nucleic acid probes and polymerase chain reaction assays

4. Virus isolation by use of cell cultures; viruses are identified primarily by the type(s) of cell lines that they are able to infect and the physical changes (called cytopathic effect or CPE) that they cause in the infected cells

Mycobacteriology Section

- The primary responsibility of the Mycobacteriology Section (or "TB Lab," as it is often called) of the CML is to assist clinicians in the diagnosis of tuberculosis

- Various types of specimens (primarily sputum specimens) are processed

- *Mycobacterium* spp. are identified using a combination of growth characteristics and various biochemical tests