



# HEMATOPOIETIC & LYMPHATIC SYSTEM

-NACHAT BATCH-

SUBJECT : Microbiology lab 🧪

LEC NO. : One

DONE BY : Dana Khalaf 🧡🎀

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# HEMATOPOIETIC & LYMPHATIC SYSTEM

## Microbiology - Blood Culture Practical

Hashemite University  
Faculty of Medicine, 2<sup>nd</sup> year  
Hematopoietic and Lymphoid system  
Dr Mohammad Al-Tamimi, MD, PhD

### Objectives

- Definition, causes, types and course of bacteremia
- Clinical picture of bacteremia
- Blood culture: indications, contamination, venipuncture, volume, number, and timing
- Steps, laboratory processing and interpretation of blood culture
- Cases studies of bacteremia

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## Septicemia/Bactremia

بHLS infection في جزء مشترك وهو انو لازم يوصلو كلهم للblood ومنه يروحو lymph node, liver, ال spleen.

- **Bacteremia**: presence of viable bacteria circulating in blood. The blood is usually a sterile substance

- Types of bacteremia:

1. Extravascular via the lymphatic's
2. Intravascular via CVS infections

وجود organisms بالدم عادة مهم وخطير، وقد يكون موجود bacteremia او viremia او fungemia وهاد دلالة ع ضعف ال immune system، لما اعمل culture للدم لازم يطلع sterile ولو طلع معنا اي شي معناها في organism بالدم .

- Course of bacteremia:

1. **Transient**: Disruption of mucosal surfaces (dental or surgical procedures)

2. **Intermittent**: Associated with abscesses

3. **Continuous**: Infective endocarditis

- Intravenous catheter is a common causes of bacteremia in hospitals

مؤقتة، common، لو حد شال طاحونة ممكن بعض flora الموجودة بالفم تدخل وتوصل للدم وبما انو ال immune قوي ف بقتلها ويتختفي لما يكون في بكتيريا كتير يحاول يطلع للدم وجهاز المناعة بقتله في continuous supply of microorganism to the blood في بنحكي عن central venous catheter ف إذا ما ضلينا نغير الدم ب ال direct entry لل blood كل فترة وفترة بصير سهل ع microorganism تدخل

هدول بهمونا بالتشخيص لانو clinical picture , positive blood culture بتكون مختلفة حسبهم، إذا كان continuous فأني وقت بتاخذ فيه ال culture حتلاقي fever + positive blood culture.. لكن transient, intermittent مرات بكون positive ومرات negative لانها مو دائما موجود بالدم.

## Causes of Bacteremia

- *S. aureus*
- *S. pyogenes*
- *S. pneumoniae*
- *H. influenzae*
- *Enterobacteriaceae*
- *Bacteroides*
- *Pseudomonas aeruginosa*
- *Candida species* : Fungi

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## Bacteremia: Contaminants

- Contamination: Growth of organisms in the blood culture bottle that were not present in the patient's blood stream

الأصل انو الدم sterile ويناخذ عينة وبنزرعها، لو طلعت positive يكون في سببين: اما المريض عنده بكتيريا في ال-blood, او صار عنده contamination بأثناء واحنا بنعمل culture للعينة باللاب رحنا زرنا بكتيريا ما كانت أصلا بالدم ..

1. Coagulase (-) Staphylococci
2. Corynebacterium species
3. Bacillus species
4. Viridans Streptococci

هدول غالبا جزء منهم يكون contaminants

١- coagulase negative staphylococci : موجودين ع skin , ف وانا باخد العينة اخدت معها شوي من coagulase negative bacteremia, او وانا بزوع باللاب ايدي لمست ال culture

## Occurrence of False Positive Blood Cultures (Trash) Contamination

طلعت معنا positive بس هي عمليا false positive

	True (%)	Trash (%)
<i>S. aureus</i>	87	6
<i>Coag negative staph</i>	12	82
<i>Enterococcus</i>	70	16
<i>Diphtheroids</i>	2	96
<i>C. perfringens</i>	23	77

هدول غالبا contamination ويطلب اعادة الفحص مرة ثانية

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## Clinical Picture of Septicemia

- Looks very ill-septic
- <sup>Fever</sup> Hyperthermia / <sup>الوضع الممجب</sup> hypothermia
- Tachycardia
- Tachypnoea respiratory rate abnormal
- Septic shock-hypotension
- Multiorgan failure Late sign
- Fever of unknown origin
- Risk factors-intravenous catheter
- Clinical manifestation of source of infection:  
pneumonia, abscess, UTI, GE

ال microorganism بتوصل للدم ومن هناك ممكن تروح لل organs لكن  
abscess, UTI, pneumonia مرات بنشوف clinical picture تبعت ال source مثل ال

UTI, pneumonia

## Indications for Blood Cultures

- Presence of 2 or more of:
  1. Core Temperature  $<36$  or  $>38$  hyper  $\rightarrow$  hypo
  2. Respiratory Rate  $>20$  per min
  3. WBC  $>12$  or  $<4 \times 10^9$   $\uparrow$  WBCs  $\rightarrow$   $\downarrow$  WBCs
  4. Pulse  $>90$  bpm
  5. Altered mental state

# القاعدة العامة إنو إذا في تنتين من هذول لازم اعمل blood culture لكن عمليا هو بعتمد ع المريض ...  
مثلا لو اجا شخص حرارته ٣٨ وال pulse ١٠٠ وقادر يمشي وعنده اعراض strep A وعاملته tonsillitis هون  
مافي داعي اطلبه blood culture, اما لو حرارة الشخص كانت ٣٨.٥ وال pulse ١٠٠ وموجود ب ICU هو يطلب  
blood culture

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## Blood Culture

- A microbiological culture of the blood to detect infection with a micro organism, specifically bacteria or candida
- The primary means for establishing a diagnosis of sepsis is by **blood culture positive**
- A sample of the patient's blood is obtained and cultured, growth is detected, and the organisms are isolated, identified, and tested for antimicrobial susceptibility **species**

ال blood culture بتعطيني مجموعة من المعلومات بتساعدني اعرف ال prognosis منها : بتعطيني species ال specific لل bacteremia وبتقلي شو ال antibiotics الشغالين واللي مو شغالين

### 1. Venipuncture

- The skin over the vein must be carefully disinfected to **reduce the probability of contamination** حتى امنع contamination بعمل proper
- Although it is not possible to "sterilize" the skin, quantitative counts can be markedly reduced with a combination of **70% alcohol and an iodine-based antiseptic** بحط 70% كحول ويفكرها ويس تنشف بحط iodine based antiseptic حتى اقتل معظم normal flora من مكان ما بدي اخذ العينة
- Blood is drawn **directly into a blood culture bottle or a sterile blood collection vacuum tube containing an [anticoagulant] free of antimicrobial properties** الللي اله antimicrobial ما يستخدمه زي sodium citrate
- Sodium polyanethol sulfonate is currently preferred; **citrate and EDTA** have antibacterial activity ما بقدر استخدمهم
- Blood should not be drawn through indwelling venous or arterial catheters unless it cannot be obtained by venipuncture

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## 2. Volume

- The number of organisms present in blood is often low (1 organism/mL)
- Small samples yield fewer positive cultures than larger ones. For example, as the volume sampled increases from 2 to 20 mL, the diagnosis increases by 30 to 50%
- Samples of at least 10 mL should be collected from adult patients كل ما زادت حجم العينة اللي أخذتها احتمال تطلع culture positive

## 3. Number

- If the volume is adequate, it is rarely necessary to collect more than two or three blood cultures to achieve a positive result
- In intravascular infections (eg, infective endocarditis), a single blood culture is positive in more than 95% of cases [باخذ أكثر من عينة بأوقات مختلفة] يكونوا أقل in transient + intermittent
- Studies of sequential blood cultures from bacteremic patients without endocarditis have yielded 80 to 90% positive results on the first culture, more than 90 to 95% with two cultures, and 99% in at least one of a series of three cultures كل ما زدنا عدد cultures بتكون النسبة احسن وطبعا بين كل عينة وعينة في تقريبا 6-8 ساعات

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## 4. Timing

- The best timing schedule for a series of two or three blood cultures is dependent on the bacteremic pattern of the underlying infection and the clinical urgency of initiating antimicrobial therapy
  1. **Transient** bacteremia is usually not detected, because organisms are cleared before the appearance of any clinical findings suggesting sepsis صعب تحصل على positive blood culture لانو جهاز المناعة دايركت بشيلهم
  2. The **continuous** bacteremia of infective endocarditis is usually readily detected, and **timing is not critical** بأي وقت بتكون positive
  3. **Intermittent** bacteremia presents the greatest challenge because fever spikes generally occur after, rather than during, the bacteremia. Bacteremia is more likely to be present and sustained in the early acute stages of infection بناخذ العينة قبل ما توصل لل maximum fever ، اما لو أخذتها بدرجة حرارة

- Closely spaced samples are less likely to detect the organism than those **spaced an hour or more apart** بفالي بين العينات وقت
- In urgent situations, when antimicrobial therapy must be initiated, two or three samples should be collected at brief intervals and therapy begun as soon as possible
- It is generally not useful to collect blood cultures while the patient is receiving antimicrobics unless none were collected before therapy or **there is a change in the clinical course suggesting superinfection** ممكن يكون في organism تاني

يفضل اخذ عينة الدم قبل ما ابلش intravenous antibiotics لانو ممكن تصير ال culture negative

لو المريض وضعو عادي باخذ كل ساعه عينة اما لو خطير باخذ كل نص ساعه عينة حتى الحق أعطيه

antibiotics

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## How to Take Blood Culture

بجهاز كل الأدوات اللي بحتاجها وبتبع الإجراءات اللي اتفقنا عليها

### • Equipment and materials:

1. Sharps bin
2. Gloves (non-sterile)
3. Needle and Syringe or Safety Blood Collection system
4. Alcohol/iodine disinfectant
5. Dressing as required
6. Blood Culture bottles
7. Blood culture sticker for notes

اسم المريض + وقت دخوله

## Steps

اهم شيء ما يمس Contamination

- Taking a blood culture is clinically indicated for this patient
- Decontaminate blood culture bottle tops
- Carry out hand hygiene before touching the patient
- Apply alcohol based skin antiseptic and leave to dry
- Don't touch critical parts
- Inoculate blood culture bottles first
- Document rationale, date and time of blood culture and operator

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## Laboratory Processing

يستخدم *general media* حتى أعطي كل أنواع *microorganisms*

- The basic blood culture procedure of incubating blood in an enriched broth is quite simple, but considerable effort must be expended to ensure detection of the broadest range of organisms in the least possible time
- Daily examination of cultures for 1 week or more and a routine schedule of stains and/ or subcultures of apparently negative cultures are required to detect organisms such as *H. influenzae* or *N. meningitidis*, which usually do not produce visual changes in the broth
- Automated blood culture systems detect metabolic activity (primarily **CO<sub>2</sub> generation**) in broth culture for initial detection in place of the conventional visual and staining examinations. These systems detect growth sooner than conventional methods but still require subculture for confirmation, identification, and susceptibility testing
- Because the blood is **normally sterile**, the interpretation of **blood cultures growing a pathogenic organism is seldom a problem** *Contamination / Bacteremia*
- The major problem is the differentiation of agents causing **transient** bacteremia and skin contamination from those opportunists associated with an intravascular or extravascular infection

بدي اشخص المريض بأسرع وقت ممكن ف يستخدم جهاز بقيسلي لل metabolites اللي بتصير

موجودة لما البكتيريا تنمو بالدم

مثلا بقيس co2 اذا طلع او نزل ف بقلي انو في organism عم يستخدم co2 والنتيجة تبع

هالجهاز بتطلع خلال 6-8 ساعات

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- Despite skin disinfection, 2 to 4% of venipunctures result in contamination of the culture with small numbers of cutaneous flora such as *S. epidermidis*, corynebacteria (diphtheroids), and propionibacteria
- The presence of these organisms in blood cultures can be considered a result of skin contamination unless
  1. Quantitative procedures indicate large numbers (5 organisms/mL)
  2. Timing of growth- very rapid growth within 24 hours
  3. Repeated cultures are positive for the same organism
  4. Multiple puncture sites are positive for the same organism

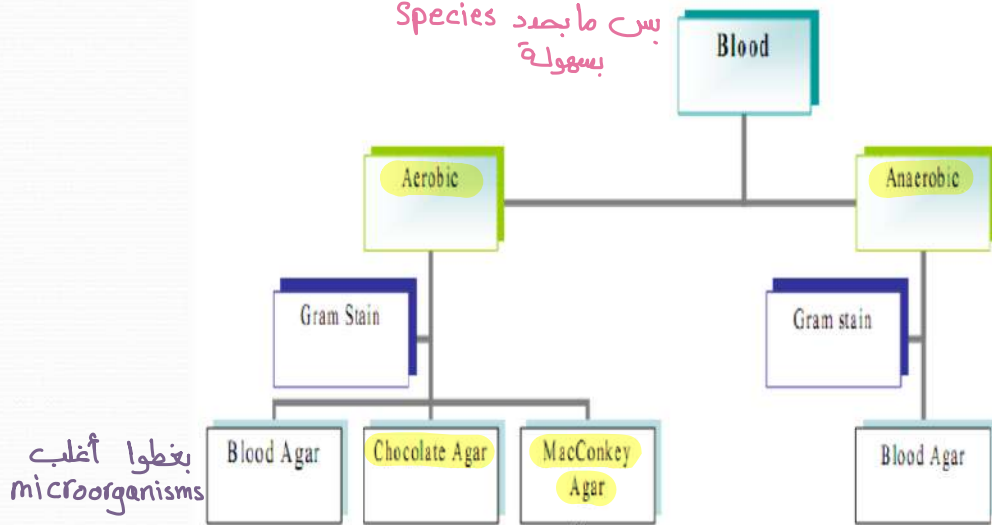
مثلا طلعت staph epidermidis حتى اتأكد انها true بطلب عينة ثانية من مكان ثاني بعد كم ساعة إذا طلعت نفسها برجع بطلب عينة تالته من مكان ثالث بعد كم ساعة برضو إذا طلعت نفسها هون بتأكد انها true مو contamination



Blood culture

الجهاز اللي حكيانه فوق  
بس ما بصد species  
بسهولة

Blood culture  
bottle



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# HEMATOPOIETIC & LYMPHATIC SYSTEM

#امتحان اللاب سيكون case بصورة واحنا نوصل للتشخيص، فرکزوا

## Case 1

### History:

31 years old female complained from **fever, malaise and sore throat**. Her doctor obtained a throat swab and prescribed her **amoxicillin** antibiotics. **2** days later, she had a **maculopapular skin rash** all over her body, **left upper abdominal pain and swelling**. Throat culture was **negative**.

ممكن strep A tonsillitis  
غالبا viral  
مافى بكتيريا بالدم  
بدى احمود طبيعته

### Physical Examination:

V/S T: **38.6** (high) P: **95/min** BP: 120/84 mmHg RR: 17/min  
مو بكتيريا خطيرة لانه الوبع عادي

Generalized lymphadenopathy and splenomegally → **HLIS infection**

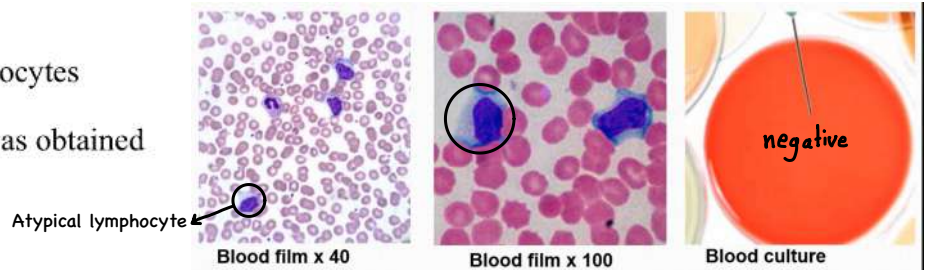
Throat was inflamed and red with enlarged tonsils

### Investigations:

CBC: Hb: 12.9 g/dl **Leukocytosis** WBC 12.07/mm<sup>3</sup> with **lymphocytosis** Platelets: normal  
RBCs: normal

Blood film: atypical lymphocytes

Blood sample for culture was obtained



### Tasks:

1. List differential diagnosis in order?
2. What investigations you would like to order?
3. Treatment?

## Treatment and Prevention

- Treatment of infectious mononucleosis is largely supportive. More than 95% of patients recover uneventfully
- In a small percentage of patients, splenic rupture may occur; restriction of contact sports or heavy lifting during the acute illness is recommended

مافى دايء ل antiviral غالبا

## التشخيص: EBV (infectious mononucleosis)

حتى أتأكد ممكن اطلب serum for EBV (Igm+IgG)

إذا طلعت + Igm - , IgG معناها التشخيص صح

ولو بدى اتأكد اكثر بطلب PCR لل saliva و blood إذا طلع + هيك مية بالمية صح

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## Case 2

### History:

26 years old male accountant returned recently from **India** after attending a workshop on accounting software's. He complained from progressively **increasing fever, fatigue, headache, associated with watery diarrhea (3 times per day)** for the last 3 days.  
*GIT infection*

### Physical Examination:

V/S T: **39.4** (خطيرة (بكتيريا) **P: 75/min** (نازل) BP: **100/79** mmHg (normal) RR: **14/min**

\*\*Splenomegally

### Investigations:

CBC: Hb: 14.9 g/dl WBC: **neutropenia** Platelets: normal RBCs: normal

Blood samples for blood culture were obtained

### Tasks:

1. List differential diagnosis in order?
2. What investigations you would like to order?
3. Treatment?

### Enteric fever:

- Antibiotics (chloramphenicol, ceftriaxone, ciprofloxacin)



**التشخيص: salmonella**

حتى اتأكد أكثر ممكن اعمل mortality test or antibodies  
Kidney function, liver: investigations التانية اللي ممكن اعملها  
abdomin function, ultrasound

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## Case 3

### History:

HPI: 76 years old male known to have chronic leukemia on chemotherapy. Was admitted 3 days ago for 2 sessions of chemotherapy and discharged home with mild nausea. 2 days ago he had mild fever, productive cough stained with blood, and lateral chest pain. He was seen by his doctor and prescribed antibiotics for chest infection. Today the patient reported shortness of breath, high grade fever, being very tired and lost ability to get out of bed, with no urination for the last 24 hours.

PMH: DM, HTN, CML Chronic myeloid leukemia  
Hypertension

Current medications: Predneslone, Insulin SC, Atacand plus

FH: DM, CAD

بقا immunity

### Physical Examination:

V/S T 40.1 P:115/min BP:145/90 mmHg RR:24/min كلشي عالي

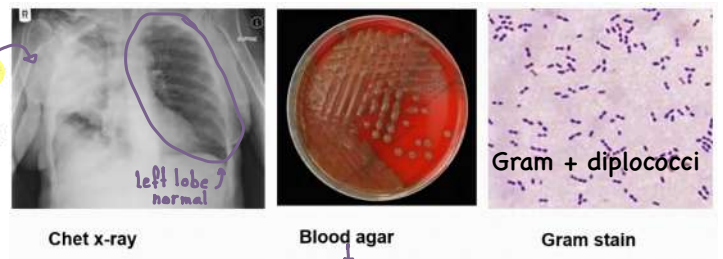
Respiratory: right side bronchial breathing and crepitations

### Investigations:

CBC: Hb: 14.9 g/dl WBC 100/mm<sup>3</sup> with neutrophilia Platelets: normal  
RBCs: normal

Chest X ray: opacification of middle right lobe

Blood samples were obtained for blood culture



Chest x-ray

Blood agar

Gram stain

Positive for alpha hemolysis

### Tasks:

1. List differential diagnosis in order?
2. What investigations you would like to order?
3. Treatment?

التشخيص: bacteremia عامله strep pneumonia

ال investigation:

chest x ray , liver function test , اضل أتابع , Blood film ,

لازم أتابعه منيح واعطيه antibiotics وادخله ال ICU

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علينا ...

## Case 4

### History:

54 years old male was admitted to the hospital ICU with severe central chest pain. After multiple femoral vein catheterization trials, complete obstruction of left coronary artery was observed and the patient was treated accordingly using central jugular vein line. 3 days later the patient reported no further chest pain yet he complained from fever, shivering and generalized weakness and asked to remove the central line tube as it become painful to him.

### Physical Examination:

V/S T 39.5 P:125/min BP:125/90 mmHg RR:19/min

Hyperemia and swelling around central line

### Investigations:

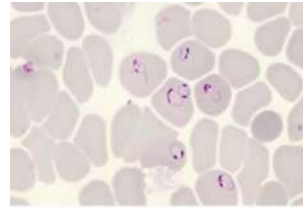
CBC: Hb: 14.9g/dl WBC 18.7/mm<sup>3</sup> with neutrophila Platelets: normal RBCs: normal

Echocardiography indicated cardiac enlargement, inflammation and vegetations

Blood sample for microbiological culture was obtained

### Tasks:

1. List differential diagnosis in order?
2. What investigations you would like to order?
3. Treatment?



Blood film x 40



Blood culture

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## Case 5

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### History:

HPI: 33 years old male patient working at the military forces has returned recently from his service in Ethiopia for 6 months. He was seen at the emergency department for high grade fever with shivering was given voltaren intramuscular injection and sent home. Next day at around the same time he had the same fever with shivering and sweating was given the same injection and sent home.

### Physical Examination:

V/S T 39.5 P:168/min BP:125/90 mmHg RR:20/min

### Investigations:



CBC: Hb: 10.0 g/dl WBC 6.7 /mm<sup>3</sup> Platelets: normal RBCs: low

### Tasks:

1. List differential diagnosis in order?
2. What investigations you would like to order?
3. Treatment?

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كل عام وانتوا بألف خير حياة   
لا تنسوا تستغلو رمضان بالدعاء  
ولا تنسوا اهلنا بغزة 

بالتوفيق... 

dana