Microbiology -Lab

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✓ Blood is known to be a sterile body fluid , meaning that it doesn't normally have any bacteria or other MO in it .

✓ To reveal presence of bacteria or other MO in the blood , we tend to perform a test called Blood Culture .

✓ When it is performed , diagnosis of :

- ❖ Septicemia .
- ❖ Endocarditis .
- ❖ Bacteremia 2° to several localized or systemic infections .

✓ We generally tend to draw blood as 10ml from each hand , with a time interval of 20-30mins approx. ; that's mainly to exclude the probability of Transient bacteremia .

✓ Commonly used anticoagulant is :SPS , others are used to a lesser extent as they have some effects on bacterial growth which in turn affect the result we get .

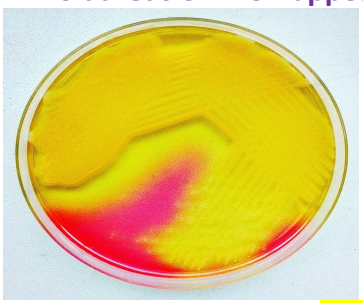
✓ After the sample in the incubator , what signs may appear indicating bacterial growth ,thus a positive test result ?

I. Turbidity .
II. Color .
III. pH .
IV. Bubbles .
V. Hemolysis of blood .
VI. Clotting .

✓ What are the commonest cultural media used ?

I. Blood agar (Strep.)
II. Chocolate agar (H.influenza and N.meningitidis).
III. Macconkey agar (G-).
IV. Sabouraud Dextrose agar or SDA (Fungi).
V. Mannitol Salt agar or MSA .

- ✓ **Positive result of the test doesn't necessitate that it's bacteremia as the + result may stem from a contamination has occurred during the preparation .**
- ✓ **Negative result of the test doesn't exclude the probability of bacteremia ; as it may be caused by fastidious bacteria that need special types of cultural media in order to be diagnosed , and you didn't use them thus you get a negative result despite the presence of the bacteria in the blood .**
- ✓ **The result considered to be clinically significant if it's revealed presence of one of the following (gave a + result for the following):**
  - I. **Anaerobes .**
  - II. **Fungi .**
  - III. **Gram negatives .**
- ✓ **You're a lab technician , blood sample has been sent to you (20ml) for a patient of suspected bacteremia , after performing proper instructions for culture , which of the following results you consider as clinically significant to report for the physician , indicating positivity for bacteremia :**
  1. **1st bottle : S.epidermidis , 2nd bottle : no S.epidermidis .**
     (You probably exclude bacteremia , as such result usually stems from a contamination rather than a true infection , remember that S.epideremidis is NF).
  2. **1st bottle : S.epidermidis , 2nd bottle : S.epidermidis .**
     (Once the 2 bottles contain the same MO , it can't be a coincidence to have contamination in both bottles as there is a spacing time interval in between).
- ✓ **Staph Vs. Strep :**
  - **Staph appear as clusters ,whereas strep have a chain-like appearance .**
  - **S.aureus on MSA appear like :**



(1)MSA contains NaCl making it selective for G+ .
(2)It also contains Mannitol and the indicator phenol red ,which is a pH indicator .
(3)S.aureus is known to produce yellow colonies , but how do you explain the change in agar color from red into yellow ?
(4)If the cultured organism is able to ferment the mannitol , an acidic byproduct formed causes the phenol red in the agar to turn yellow ,so that you get the appearance shown .

  - **Differential test is : Catalase Test , where the Staph is +ve and strep is -ve .**

(See this figure ,helping you to remember the real manifestation of the test)

- ✧ **H_2O_2 ------> H_2O + O_2 .**
- ✧ **O2 bubbles : indicator of a +ve result .**
- ✧ **You put few drops of catalase reagent (H2O2) on the plate , Containing Strep. and Staph. , you will notice the O2 bubbles on Staph. plate whereas no bubbles will be seen on the one containing Strep.**

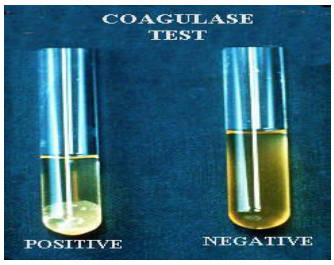
✓ **Staph. (S.aureus , S.epidermidis and S.saprophyticus)**

- **Coagulase test : differential test for S.aureus .**

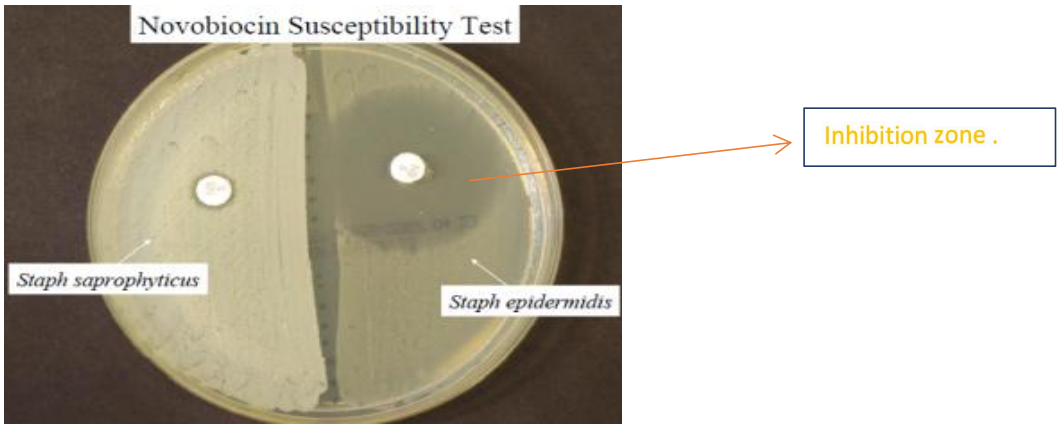
(Has to ways to be done ; slide test or tube test , preferably using the slide test)

Dr's question : Why do *we use plasma not serum* of the blood sample we have (++ we get the serum and plasma separated by centrifugation) ?
Ans : Serum is the part of blood which is similar to plasma in composition but exclude clotting factors , so that fibrinogen which is the target of enzyme coagulase is found in the plasma not in the serum . Remember , coagulase enzyme enables the conversion of fibrinogen to fibrin .

If you performed the slide test and got a -ve result for coagulase (No clots appear) , you then have to perform the tube test in order to assure your result ,why?
That's because we have 2 types of coagulase enzyme which are Bound coagulase and Free coagulase , slide test is only for bound one whereas , tube test reveals both .



- **Novobiocin test : differential test for S.epidermidis (+Ve : S.epidermidis , -ve :S.saprophyticus).**
- **Notice this figure , showing you the inhibition zone formation which is a +ve result of Novobiocin test , indicating the presence of S.epidermidis :**



✓ **Rheumatic fever diagnostic tests :**

1. **Throat swap .**
2. **Serological test .**

- **Strep. is catalase -ve , often cultured on blood agar because of its hemolytic activity , further classified depending on C-carbohydrate (lancefield classification).**
- **Throat swap -diagnosis :**

✧ **1st :we add one drop of extraction enzyme (إنزيم الاستخلاص) on bacterial colonies .**
✧ **2nd : we add a specific antibody - remember that lancefield classification based on antigen-antibody rxn- suppose you add A-Antibody.**

- ✧ **3rd : Mixing up for 1-2 mins .**
- ✧ **4th : If agglutination observed , then it's group A strep . If not , you move up to use another antibody ,repeating the previous steps .**

■ **Serological- based diagnosis :**

- ✧ **Streptococcus pyogens produce 2 types of streptolysin (SLO : antigen and SLS: non-antigen).**
- ✧ **Test : ASOT.**
- ✧ **Steps :**

In latex agglutination test , the sample to be tested is prepared to be mixed with latex beads coated with a specific antigen , in our case here , coated with SLO

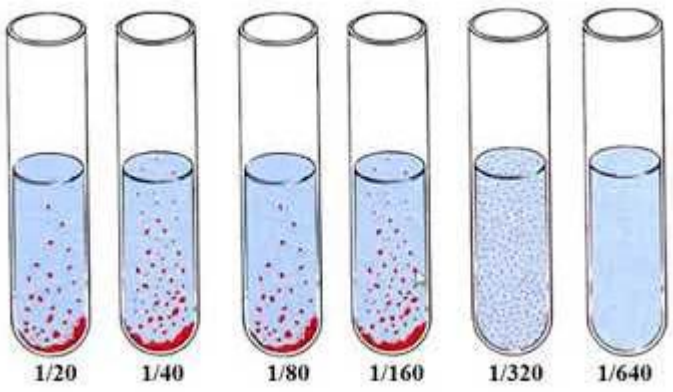
1. **Qualitative :**
   - ◆ **Put a drop of the serum sample you have on a slide , a patient that have S.pyogen infection , is supposed to have ASO in the serum .**
   - ◆ **Add one drop of latex .**
   - ◆ **Mixing them up , if agglutination is observed it is +ve result , otherwise it is -ve .**

Sensitivity of ASO latex is : 200 IU/ml
Sensitivity of the latex reagent has been adjusted to yield agglutination when the level of ASO is greater than 200 IU/ml .

2. **Quantitative (Titer test):**

In serological tests , we use 2-fold dilution .

Serum = 50µL



The 1st tube : serum .
The rest : NaCl .

$$D = \frac{X}{X+D_1}$$

⬇ Concentration ,⬆ Dilution .

- **How to prepare a tube with dilution of (1/20) ?**
  D = 1/20
  X = 50
  D_1= ?
  From the formula written :
  D_1 is equal to --> 950µl of NaCl .

- Volume in the 1st tube = 1000µl , how much to add NaCl to the other tubes : 1000/2 =500µl .
- The previous can be done on slides alternative to tubes .
- Titer : آخر تخفيف يعطي نتيجة ايجابيّة
- Approximate concentration of Abs = Reciprocal of titer * sensitivity of the kit
  - ■ Suppose the titer is :1/16 , the approx. [ASO] is : 16*200 =3200 IU/ml .
- Increasing titer indicates current infection , constant titer indicates previous ,past infection .