**كلية الرشيد الجامعة**

**قسم تقنيات المختبرات الطبية**

**المرحلة الثانية**

**Microbiology practical**

**Lab 4**

**Methods of culture**

**Presented by**

**M.Sc Hawraa D. Salman**

***Lab -4- Methods of Culturing***

***Methods of culturing used to:***

***1-Isolation of M.O. from sample.***

***2-For culturing M.O. in the sample.***

***3-Obtain pure culture.***

***The methods of culturing are :-***

***1-Pipetting by pipette (in broth medium)***

***2-Inoculation by loop (in broth medium)***

***3-Streaking by loop (on solid medium)***

***4-Stabbing by needle (on solid or semi-solid medium)***

***5-Swabbing by swab (on solid medium)***

***6-Pouring plate method (on solid medium)***

***7-Spreading by spreader (on solid medium)***

***1-Pipetting by pipette or micropipette***

***This method was suitable in the dilution to extinction***

 ***laboratory, the purpose from this method is to count***

 ***the M.O, (viable plate count).***

***2-Inoculation by loop***

***This method was used to increase the number of M.O.***

 ***in liquid media***

***Method***

***1-Hold the tube that contain the broth culture***

 ***in your hand .***

***2-Mix the tube that contain the culture to spread***

 ***the bacteria through the broth***

***3-Hold the other tube that contain the sterile broth***

 ***in the same hand that carrying the broth culture .***

***4-Sterile the loop.***

***5-Remove the two cotton plugs of the tubes by the***

***hand that carrying the loop.***

***6-Sterile the upper of the tubes by flame.***

***7-By the sterile loop , transfer drop from broth***

*** culture to sterile broth.***

***8-sterile the upper of the tubes and close them .***

***9- Sterile the contaminated***

***loop with flame.***

***10-Put the new cultured tube***

 ***in the incubator***

 ***at 37C˚ for 24 hr.***

***3-Streaking by loop***

***Streaking on agar plate in order to isolate bacteria , this will enable you to isolate pure cultures of bacteria by obtaining isolated pure colony.***

***A-Streaking on plates***

***-ABCD method:***

***1-Transfer by sterile loop colony from solid medium or drop from broth medium to solid medium in petri dish near the edge , spread it to become point A***

***2-Sterile the loop and cool it in the edge of solid medium.***

***3-Rotate the dish , then touch the A area by***

***sterile loop ,vertically draw several parallel lines***

 ***to form in their end point B .***

***4-Repeat step 2.***

***5- Rotate the dish in the same direction, and touch***

***the B point by sterile loop , vertically draw several***

 ***parallel lines to form in their end point C .***

***6-Repeat step 2.***

***7- Rotate the dish in the same direction,***

 ***and touch the C point by sterile loop ,***

***vertically draw several parallel lines to***

 ***form in their end point D .***

***8- From D draw one zigzag line to the***

 ***middle of dish.***

***9-Repeat step 2.***

***10-Put petri dish in the incubator***

***at 37 C˚ for 24hr.***

***-Continuous streaking***

***1- Sterile the loop and cool it in the edge of solid medium.***

***2-transfer the culture to the solid media***

***3-spraed the culture as continuous parallel***

***lines to the end of the dish.***

***4-Sterile the loop.***

***5-Incubate at 37C˚ for 24 hr as inverted dish.***

***B-Streaking on slant***

***1-By sterile loop , take a colony from***

***solid medium or drop from cultured***

 ***broth medium.***

***2-Remove the cover of slant tube***

 ***and sterile the upper part.***

***3-cultivate on the slant medium by***

 ***streaking , then sterile the tube and***

***close it.***

***4-sterile the loop by flame and then put the slant in the incubator at 37C˚ for 24 hr.***

***4-Stabbing by needle***

***This method is used to study the ability of bacteria to:-***

***1-Grow with presence O2 or not.***

***2-Motility.***

***3-Production of some chemical***

***compounds during the metabolism.***

***Method***

***1-Sterile the loop by flame .***

***2-Hold one colony or part from bacterial***

 ***growth from solid medium by needle.***

***3-Open the tube that contain sterile semisolid***

 ***medium and sterile the upper of tube.***

***4-Stab the needle that contain bacteria in the***

***medium vertically ,then hold it directly.***

***5-Sterile the upper of the tube again and close it.***

***6-Sterile the needle by flame.***

***7-Incubate the tube at 37C˚ for 24 hr***

***5-Swabbing by swab***

***Used to obtain heavy growth for some biochemical tests such as sensitivity test and for colony counting***

***Method***

***1-Submerge the sterile cotton swab in bacterial***

***broth culture.***

***2-Swab on the sterile solid medium parallel***

***lines over the medium.***

***3-Rotate the plate and repeat step 2***

***4-Put the swab on alcohol (used for one time).***

***5-Incubate the tube at 37C˚ for 24 hr***

***6-Pouring plate method***

***This method is used for counting the colonies***

***Method***

***1-Prepare serial dilutions from 10-1 to 10-5 of sample***

***2-Transfer by sterile pipette 0.1 ml of each dilution to sterile petri-plate***

***3-Prepare sterile solid culture medium and cooled it to 45-50 C˚ in water bath***

***4-Aseptically pour agar into each petri-plate , swirl the plate to mix the sample with the agar.***

***5-Allow the agar to cool and solidify.***

***6-Incubate the inverted plate at 37C˚ for 24 hr***

***7-Spreading by spreader***

***Used to spread the bacteria on the surface of the solid medium for colony counting***

***Method***

***1-Prepare 3 dilutions (10-1 , 10-2 , 10-3) from sample.***

***2-Prepare nutrient agar plates and label them.***

***3-Using sterile pipette , transfer 0.1 ml from each of dilution tubes and the original sample to agar plates***

***4-Sterilize the glass spreader by putting it in alcohol and burn it by burner.***

***5-After the cooling of spreader , spread the sample over the surface of the plate.***

***6-Incubate inverted plates at 37C˚ for 24 hr***

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