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Aim : To transfer a bacterial monoculture into a fresh medium using Aseptic Technique.

Requirements : Inoculation loop, inoculation needle, bunsen burner.

Procedure : Slowly turning the gas by igniting the burner. Flame can be adjusted by rotating the collar to control the flow of oxygen. Inoculation tools are sterilized by passing them through the hottest part of the flame. After being sterilised, the tools shouldn't set down on any surface. Thus after flaming a loop take the lid off the container of liquid culture & then passing the mouth through the flame, then dipping the loop into the liquid & again flame the mouth before replacing the lid. Lift the lid of the petri-plate and hold it over the base as a shield in order to prevent contaminants from falling onto the plate. Spread the bacteria over the surface of the plate. This was the way to transfer a bacteria from liquid culture to petri dish. Likewise, in order to transfer a bacteria from liquid to liquid culture, we need to sterilise the inoculating loop & then pass the mouth of the container through flask. Then dip the loop in the liquid & flame the mouth again before replacing the lid. Take the tube of liquid media & pass it through the flame, then dip the loop consisting bacteria into the tube flame. With similar way, we will transfer bacteria from petri plate to stab culture. Clear area of agar plate helps in cooling the needle. Rub the inoculation needle over a single colony and transfer bacteria to a culture tube with agar, then again after sterilised transfer bacteria from petri dish to liquid. Do the same

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procedure of cooling the needle and rub the loop over a single colony. Taking the liquid media remove the lid and the pass the mouth through flame. Then dip the loop with bacteria in that tube & similarly flame the mouth before putting the lid & again sterilise the needle.

Precautions :-

i) Handle the bunsen burner carefully
ii) sterilise the whole inoculation needle in the flame.
iii) Distribute the bacterial colony eventually over the plate.
iv) Flame the mouth of the tube both at the time of unlidding & putting the lid.

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Aim : To obtain single isolated pure colonies using Streak Plating.

Requirements : Sample of mixture of bacteria, petri plate containing solid medium, wire loop, bunsen burner.

Procedures : To cool the sterilized loop, touch it to a sterile agar plate, then dip the loop into a sample containing a mixture of bacteria to pick them up. Before continuing to streak the plate, the remaing bacteria on the loop are first killed in the flame, after cooling the sterilized loop, drag it through the previous path picking up a small number of bacteria and spreading them into a new area of the plate. After sterilizing and cooling the loop again, the procedure is repeated. With each new path, the loop picks up a smaller number of bacteria, and therefore can spread them farther and farther apart. During incubation, the bacteria multiply and produce colonies. Each colony consists of cells derived from a single parent bacterium. The isolated colonies found in the last streak represent isolated strains to ensure the strains purity. The streak plate procedure is usually repeated a few more times, using an isolated colony as the source of starting bacteria.

Precautions :

1) Label first, streak after that, help you avoid a big headache later.

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2) Make sure agar plates are free of droplets of condensed moisture. If moisture is present, leave the plate at room temp overnight.

3) Contact with the edge of the plate can introduce contaminate to the agar. Avoid touching the edge of the plate with the loop while streaking.

4) Only a small amount of inoculum is needed. Use isolated colonies if taking an inoculum from another plate or use only 5 to 10µl from a suspension.

5) Streak lightly in smooth, rapid movements to avoid gouging the agar plate. Gouged agar won't produce as many colonies.

6) Don't forget to sterilize the equipment which is a priority and open the plates at a safe distance from your face.

Result :-

- Streak plate technique is used to grow bacteria on a growth media surface so that individual bacterial colonies are isolated and sampled. Samples can be taken from the resulting isolated colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

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Aim :- To isolate or count the individual bacteria present in a diluted sample containing a mixture of different species.

Requirements :- Nutrient agar plate, Lazy Susan Turntable, L-shaped bent glass rod, 95% alcohol, Beaker.

Procedures :-

1) Prepare different dilutions of the sample.
2) Label the nutrient agar plate with wax marking pencil. Mention the organism name, type of agar, date and the plater's name.
3) Lift the plate's lid and use it as a shield to protect it from airborne contamination.
4) Take a clean and sterile pipete and pipette out 0.1 ml sample from the appropriate desired dilution series unto the center of the surface of an agar plate.
5) Replace the lid on the plate.
6) Properly dispose of the pipetting instruments used to inoculate the medium because it is contaminated.
7) Sterilize the L-shaped glass spreader by dipping this into 90 % alcohol and then flame the glass spreader.
8) Cool the rod for 10-15 seconds.
9) After forming the glass rod, lift the lid of the plate and use it as a shield from airborne contamination. Then touch the rod to the agar surface away from the inoculum to cool it.

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10) During spreading hold the plate lid with the base of your thumb and index fingers and use the tip of your thumb and middle fingers to rotate the base. At the same time move the rod in a back and forth motion across the agar surface. After a couple of turns, do one last turn with the rod next to the plate's edge. Alternatively, place the plate on a rotating platform and spread the inoculum.
11) Remove the rod from the plate and replace the lid.
12) Return the rod to the alcohol in preparation for the next inoculation. There is no need to flame it again.
13) Incubate the plate in an inverted position at the appropriate temperature for the assigned ~~type~~ time.

Observation and Result :-

After incubation observes the colonies on the agar plate. Some of the colonies will be free from each other. Select any colony from the plate and record the elevation, pigmentation and size.

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