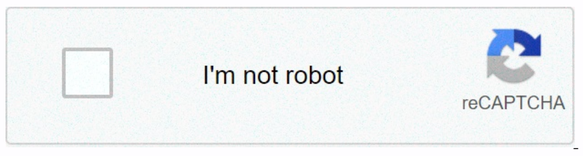

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Aseptic technique and culturing microbes lab report

Concepts for the study of nutrients in nutrient broth Agar Aseptic transfer Inoculating Microorganisms Isolation: Streak and application plate methods Introduction Microbiologists grow and keep microorganism cultures in the media. The substance can be in either liquid (broth) or solid (agar) form. Agar is a complex carbohydrate isolated from seaweed. It has many industrial, food and scientific uses. It is also a convenient solidifying agent for the media of microbiology, since very few microorganisms can metabolize it. Agar liquefies at 90-100 °C and solids at approximately 42 °C. Regardless of type, all media should be carefully prepared to contain the appropriate nutrients necessary for microbial growth and to prevent unwanted growth before the microbial sample is added. Sterile procedures Figure 1: The use of Agar has contributed to many advances in microbiology. It is also used in ice cream production. Most media are manufactured in a sterile procedure and sterilized after production. Media sterilization is usually carried out using autoclave, which uses pressure and steam to kill unwanted organisms, or membrane filtration at positive pressure to filter particles above a certain size. Since you do not have access to these sterilization techniques, the fixed media used in the experiments in this laboratory guide will be prepared sterilely for you. However, you need to digest the media and pour it into Petri dishes to grow bacteria. This process shall be carried out in a way that reduces the possibility of introducing an unwanted organism into the media before being used in the experiment. In addition, you need to inoflow (import a microorganism) of prepared media discs containing samples (or can be given to you) containing bacteria you collect from your surroundings and transfer bacteria from one plate to another or to other containers. These actions must be taken without contaminating the original plate, new plate, environment or yourself. This method is called aseptic technology and is used in almost all procedures for microbes. Growth media figure 2: Koch was the first scientist to isolate the anthracloth causative bacillus antracus using the potato slice method. There are many kinds of growth media. At the time, early microbiologists manufactured their own media from available materials, such as extracts from beef and vegetable slices. Robert Koch (1843–1910) was a German physician/scientist who pioneered many microbiological techniques that are still used. Koch was one of the first microbialologists to use a solid media format to cultivate microbes. He made slices of boiled potatoes and spread them on samples from sick animals. The potato served as a breeding ground for bacteria and with this technique Koch was able to isolate pure bacterial cultures from one Today, the media are available as a powder and is only liquefied. All full substrats must be provided for the different bacteria grown in them for nutrient needs. These nutrients must include a carbon source (which can also supply energy) and a nitrogen source. Both nutrients can be supplied from plant or animal sources. Other nutrients include phosphorus, sulfur, minerals and water. Media made from these sources are called unspecified or complex media because the exact makeup and amount of carbon and nitrogen is unknown. The specified material is the one in which each chemical component and its amount are accurately known and controlled to facilitate the growth of certain microbes. Different membranes are also used to enrich or select a certain type of bacterial growth; These are called selective media, which can be complex or defined. Aseptic technique Figure 3: Different microorganisms colonize in different forms and colors. The streak method (also called the coating method) can also affect the visual appearance of the colony. Aseptic technology is used to add bacterial samples to media discs and also to transfer bacterial colonies from one plate to another. As mentioned above, aseptic techniques minimize the likelihood of contaminating pure bacterial samples with unwanted microbes from the surrounding environment or those that normally live in our body. Aseptic techniques are also important to reduce the spread of cultivated bacteria (which may be potentially dangerous) into the environment, limiting or preventing exposure to pathogenic substances. In the microbiology laboratory, scientists can sterilize instruments with heat (Bunsen burner), autoclave (high pressure and temperature) or gas sterilization. During the tests in this laboratory kit, you will use sterile, disposable instruments or a candle (for sterilization) because you do not have access to laboratory-grade sterilization devices. You also need to use proper aseptic technique to ensure the cleanliness of your cultures. Below is a list of common aseptic techniques used in the microbiology laboratory: Reduce the environmental pollution potential by working in a clean, tidy space. Organize and read each exam carefully before starting. Collect and manufacture all necessary reagents and instruments before the tests begin. Cover the discs immediately after pouring media into them. When you open a plate either to inoculate it or to transfer bacteria from it, use the lid as a shield to limit contamination from airborne sources. When using pipes containing substances or other liquids, hold the tube at an angle and immediately re-encapslate to limit contamination from airborne sources. Always use a new, clean, sterile, disposable loop or transferpipet when moving samples or bacterial colonies, unless otherwise specified in the test guidelines. Store the prepared, solidated agar plates (which have not yet been hijacked) upside down so that: that their forms do not fall to the surface of the media. Figure 4: Chlorine bleach is an approved sterilization fluid. Figure 5: Agar color may vary depending on the ingredients. It is often bright yellow or red. Disinfect all cultures at the end of each experiment (unless otherwise instructed) by adding 10% bleach to the culture plates and letting them sit at room temperature for 20 minutes. Household bleach contains 5.25% sodium hypochlorite and is corrosive, so the surfaces should be wiped with water after use of the bleach. A 10% solution of household bleach kills many organisms immediately, but can take up to 20 minutes for complete sterilization. When the plates are flooded, pour the bleach into the drain, close the plates in the Parafilm ™ dispose of them in the trash. Be sure to clean and disinfect the work area with disinfectant wipes or 70% ethanol after each test. Obtaining a pure bacterial culture is important for microbiologists because it greatly facilitates the identification of what kind of bacteria are present in the mixed population. Often, a dilution set is prepared from the sample in such a way that the initial amount of bacteria is reduced so that when the sample is coated on a substraus, individual bacteria are separated. These individually isolated bacterial cells then produce a colony of cells, all of which originate from stem bacteria. Colonies from different types of bacteria often show unique morphologies that can help identify the original bacteria. In the experiments outlined in this guide, always remember to notice the appearance of bacterial colonies growing on your plates (morphology, color, edges, structure, growth model). 1. Aseptically inocut one Trypticase Soy Broth tube, one Trypticase Soy Agar diagonal tube, one Trypticase Soy Agar plug and one Trypticase Soy Agar plate on B. subtilis plate. (See Figure 11) Be sure to mark all pipes with a wax mark. When drinking agar plates, use one of the patterns shown in Fig. 4 or Figure 5. This procedure is used as isolation and has a dilutive effect. The friction of the loop against the agar causes the organisms to fall out of the loop. At the end of the streak pattern, individual organisms are separated on the surface of the agar so far apart that they give birth to individual colonies after incubation. 2. Aseptically inocopulote one Trypticase Soy Broth tube, one Trypticase Soy Agar diagonal tube, one Trypticase Soy Agar plug and one Trypticase Soy Agar plate with E. coli. (See Figure 11) 3. Aseptically inook one Trypticase Soy Broth tube, one Trypticase Soy Agar diagonal tube, one Trypticase Soy Agar plug and one Trypticase Soy Agar plate with M. luteus. (See Figure 11) 4. Aseptically inook one Trypticase Soy Broth tube, one Trypticase Soy Agar diagonal tube, one Trypticase Soy Agar plug and one Trypticase Soy Agar plate with M. phlei. (See Figure 11) 5. Incubate all tubes and plates shown on the B. subtilis tube, coli, M. luteus and M. phlei at 37 °C. Place the pipes in the edidated test tube rack. Incubate the petri dishes upside down (lid on bottom) and stack in the petri plate holder on the shelf of the 37 °C incubator corresponding to the laboratory part. Incubation of plates upside down prevents condensation from falling into growing colonies and running together. (Store the test tube rack on the incubator shelf when not in use.) 6. To illustrate that microorganisms are all around us and to demonstrate the need for appropriate aseptic technique, contaminate three Tryptiase Soy Agar plates as follows: a. Remove the lid from the first agar plate and place the bare agar section in or out of the building for the duration of today's laboratory. Replace the lid, mark the plate as air and incubate it upside down at room temperature. Make this plate first. B. Split the second petri dish in half using the wax marker. You and your partner are both moistening a sterile cotton swagger in sterile water. Rub your swipe sample over the surface of the building or on yourself. Use this swipe sample to inoculate your half of another agar board. Mark the plate and incubate upside down at room temperature. c. Share a third petri dish to quarters and on the label as shown in Figure 12. First rub the fingers of one of your gloves over the glove quadrant. Remove the glove and rub your fingers over the quadrant of your fingers. Your partner does the same in his half of the plate. Mark the plate and incubate upside down in a petri plate holder at 37 °C. Make this plate last. Last.