

Environmental microbiology lab report assignment



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Materials Distilled water Test tube 6 Unopened packages of 1 sterile cotton swab 2 sterile nutrient agar Petri dishes 1 sterile blood agar Petri dish Incubator Refrigerator Bunsen burner Gas connection Plastic tubing Inoculating loop 12 sterile glass slides Wax pencil Igniter Crystal violet dye Gram's iodine Ethyl alcohol Seafaring dye Paper towels Wire rack Sink Brightened compound microscope Lens paper Immersion oil Pen and paper

Methods I.

Collecting the environmental specimens: 1. Place some distilled water into the test tube. 2. Open one package of sterile cotton swabs by peeling apart the packaging at the top. Do not peel the package apart completely, just at the top. 3. Take out the cotton swab, dip it into the test tube of distilled water, and place it back into the original package with the cotton tip facing down. 4. Repeat the previous two steps for three more unopened packages of sterile cotton swabs. 5. Choose four locations from which environmental samples can be taken. For example, use the bottom of a shoe, a light switch, or a sink handle). Write these locations down on a piece of paper and number them E1 -E4 for later reference. (For example, E1 is the sink handle). 6. At each location, take out one of the wetted sterile cotton swabs and rub it against a small area of the surface being sampled. Make sure to turn the swab as well to ensure that the sample is on all sides of the cotton swab. After swabbing, place each swab back into its original package, with the cotton tip facing downwards. Keep track of which swabs were used for which samples.

Label them if necessary. II. Collecting the throat and nose samples: steps for one more unopened package with a sterile cotton swab. 5. Take one of the

wetted sterile cotton swabs and rub it against the inside of one's nose. Make sure to turn the swab as well to ensure that the sample is on all sides of the cotton swab. After swabbing, place the swab back into its original package, with the cotton tip facing downwards. 6. Repeat the previous step, but instead take a sample from the back of the throat. III. Aseptic transfer of the environmental specimens: 1.

Take the two nutrient agar Petri dishes and turn them bottom side up. Then, using a wax pencil, draw a line down the middle of each dish. Label each of these four sections EI, EH, EH, EH so that the origin of each sample is known for later. 2. Take out one cotton swab that was used on an environmental surface out of its packaging. Keep this swab in one hand. . With the other hand, open the lid of one sterile nutrient agar Petri dish slightly at an angle. 4. Take the cotton swab in the other hand and gently swab it against the appropriately labeled half of the nutrient agar's surface evenly. . Close the lid of the nutrient agar Petri dish and place the cotton swab back into its packaging. Dispose of the cotton swab and package in the appropriate container. 6. Repeat the previous four steps for the other three cotton swabs used on the environmental surfaces. Make sure to use the appropriate swab for the appropriately labeled section of the Petri dish. 7. Place the two inoculated nutrient agar plates into the incubator in an inverted position, or with the lid facing downwards, to prevent condensation on the agar's surface. IV.

Aseptic transfer of the nose and throat specimens: 1. Take the one blood agar Petri dish and turn it bottom side up. Then, using a wax pencil, draw a line down the middle of the dish. Label each of these two sections N and T so
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that the origin of each sample is known for later. 2. Take out the cotton swab that was used on the inside of the nose out of its packaging. Keep this swab in one hand. 3. With the other hand, open the lid of the sterile blood agar Petri dish slightly at an angle. 4. Take the cotton swab in the other hand and gently swab it against the appropriate half 5.

Close the lid of the blood agar Petri dish and place the cotton swab back into its packaging. Dispose of the cotton swab and package in the appropriate container. 6. Repeat the previous four steps for the other cotton swab used on the back of the throat. 7. Place the inoculated blood agar plate into the incubator in an inverted position, or with the lid facing downwards to prevent condensation on the agar's surface. V. Making heat fixed bacterial smears of all the samples: . Take the twelve sterile glass slides and label their corners using the wax pencil.

Use the igniter to ignite the Bunsen burner flame. 6. With one hand, take the inoculating loop and pass it through the flame until it is red 7. With the other hand, open one of the Petri dishes slightly. 8. Take the sterilized inoculating loop and lightly touch it to one of the colonies on the agar's surface. 9. Close the Petri dish lid and take the inoculating loop and gently smear it in the drop of water on the appropriately labeled slide so that it coincides with the sample you took from. Smear from side to side to create a thin film. Let this slide air-dry. 10. Pass the inoculating loop through the flame again until it is red-hot. 1. Repeat the previous eight steps for the rest of the samples and slides. Remember to take two samples from each of the six locations, each from a different colony. Also remember to place the colony samples on the appropriately labeled slide. 12. Once the twelve slides have dried, pass each

one through the Bunsen burner flame once or twice. Do not hold the slide in the flame, as this will cause the sample on the slide to burn. 13. If the nutrient agar and blood agar Petri dishes are going to be used again, place them in the refrigerator, if not, place them in the appropriate container. VI.

Gram staining all of the samples: 1. Separate the twelve heat fixed slides into three groups of four. This makes it easier to apply the dyes to the slides for the appropriate amount of time. 2. Take one of the three groups of heat fixed slides and place them on the wire rack on top of the sink. 3. Take the crystal violet dye and apply it to the slides on the rack generously, making sure to cover the entire slide. Leave the crystal violet dye on the slides for thirty seconds. 4. Rinse the crystal violet dye off of the slides with distilled water, and place the slides back onto the wire rack. 5.

Place the Gram's iodine generously onto only one of the slides and let it sit for ten seconds. Rinse the slide immediately with distilled water and return it to the wire rack. Repeat this step for the other three slides, making sure to do each slide individually to ensure that the Gram's iodine does not stay on the slide for more than ten seconds. 6. Take one slide and hold it at an angle over the sink. Take the ethyl alcohol and carefully place ten drops of it onto the slide, allowing it to slide off quickly. Immediately rinse the slide with distilled water and place it back on the wire rack.

Repeat this step for the other three slides, making sure to do each slide individually to ensure that the ethyl alcohol does not stay on the slide for too long. 7. Take the safranin dye and apply it to the slides on the rack generously, making sure to cover the entire slide. Leave the safranin dye

on the slides for thirty seconds. 8. Rinse the saffranin dye off of the slides with distilled water, and place the slides onto a paper towel to dry. The excess water on the slides can be blotted off gently with another paper towel. . Repeat the previous seven steps for the other two groups of four slides.

VII. Determining the morphology and the gram stain results of the samples:

- 1 . Take out the brightened compound microscope, plug it into an outlet, and turn the power switch on.
2. Use the lens paper to wipe off the objective and ocular lenses.
3. Take one of the gram stained slides and place it onto the stage of the microscope. Use the stage clips to keep the slide in place.
4. Focus using the xx low power objective lens by first using the coarse adjustment knob to bring the lens as close to the slide as possible. Focus by moving the coarse adjustment knob to move the lens away from the slide. . Use the knobs on the stage to move the slide up and down, and side to side to find a portion of the slide with a good amount of sample on it.
6. Get the immersion oil and place a small drop of it onto the slide where the light is shining through it.
7. Switch into the xx oil immersion objective lens and focus using only the fine adjustment knob.
8. If necessary, use the light source and the condenser to alter the illumination of the slide.
9. Observe the color shown on the slide and determine if it is pink or purple. If it is purple, it is gram positive, if it is pink, it is gram negative.