

NAME: _____ PERIOD: _____

Laboratory Experiment: MICROBE BIO-BOTS IN THE NCS MIDDLE SCHOOL

Mr. Galloway File = LAB-EXP-MICROBE-PETRI-CULTURE-Packet-GALLOWAY-BLANKS

Adult Supervision Level: High **REMEMBER LAB SAFETY RULES – NO FOOLISHNESS!!**



MATERIALS: Bottled-Agar Hot-Bottle-Hand-Protection Sterile-Plastic-Petri-Dishes Sterile-Distilled-Water Sterile-Cotton-Swabs (BEWARE Q-tip swabs are clean but NOT sterile).

Keep sterile Petri dishes closed until ready to pour agar in them. Microbes in the air can easily infect an open Petri dish.

SCIENTIFIC & BIBLICAL BACKGROUND:

Agar is pronounced Auger, sounding like jogger with no J in front. Agar is a jelly forming substance extracted from red seaweed. Microscopic organisms (bacteria, fungi, protists) called microbes are tiny BIO-BOTS (biological robots) designed by God to serve/function as HELPERS, CLEANERS and RECYCLERS in creation. They live inside us, all over us, and everywhere on earth. Almost all microbes are good and cause not harm. God originally did NOT create them to cause diseases. All that He made was VERY GOOD (Gen. 1:31). The few microbe types that are NOW harmful have degenerated due to accumulated mutations (genetic entropy) so that they dysfunction and cause disease and death. SIN caused SEPARATION from GOD who originally held all things together in perfect order. That SEPARATION started a process of DEVOLUTION so that over time all things in the universe are gradually falling apart. From supernovas and black-holes in outer space to the mutation meltdown in every group of creatures, this old infected creation is dying. In Genesis 2 God warned Adam and Eve that if they separated from Him, they and creation would die. Literally the Hebrew says, “dying-dying”, meaning a progressive process of escalating disorder, dysfunction, disease, and death.

LAB TABLE PREP:

1. Clear all unnecessary things off your lab table.
2. Wind can cause unwanted microbes to enter your dishes when opened, so keep air movement to a minimum. Turn off fans, AC, and try not to breathe into your dish – or wear a lab mask over your face.

PREPARING THE AGAR AND PETRI DISHES:

- DAY ONE:**
1. Melt the agar inside the bottle is with a microwave. Start at a low setting and only a few seconds at a time, since some microwaves heat too quickly. You do not want to boil the liquid in the bottle. DO NOT REMOVE CAP, but only loosen it before heating. If you remove it, then unwanted microbes could get inside and infect (contaminate) the agar.
 2. When the agar begins to for a few bubbles, take it out and swirl it around gently to mix it. Repeat this a many times (can be 5-15 times) until ALL the agar is melted into a liquid. It is best to do this until all lumps are melted, but lumps won't affect your experiment.
 3. Put the empty Petri dishes flat on the table, near the edge. It'll make it easier to pour the agar in it.
 4. Take the cap off and don't breathe into it.
 5. Open the Petri dish lid just a little bit so you can pour the agar from the bottle into it. Hold your breath or use a mask so you don't infect the agar.
 6. Gradually pour agar until the bottom of the dish is covered to ABOUT 1/8 inch thick. There is enough agar in each bottle to prepare about 10 dishes.
 7. IMMEDIATELY replace the Petri dish lid.
 8. Repeat this process with each Petri dish.
 9. Keep Petri dishes covered and let them sit on the table or counter for **OVERNIGHT** until they are completely cooled and solid gel.

DAY TWO: 10. Now they are called AGAR PLATES.

11. Hold lids on the plate and carefully turn the whole plate with its lid upside down. The agar plate must stay upside down during the whole experiment. This keeps water condensation from forming on the surface of the agar gel. It is OK if the LID that is now on the bottom gets condensation on it.

* If the plate were right-side-up and condensation collected on the agar, then separate colonies of microbes growing on the agar could more easily spread and contaminate each other.

**SCOTTY waiting (worrying) →
at his Doctor check up!**

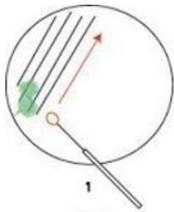


EXPERIMENT SETUP:

PREPARING THE PLATES FOR INOCULATION WITH MICROBES:

1. Keep the plates at room temperature.
2. Use a marker to divide the BOTTOM of each Petri dish into FOUR equal pie sections. Then use a piece of tape on the bottom so you can label each one with your class period and group number.
3. You will use the SWAB to collect a sample from a location your group chooses. Pour sterile water on the swab. DO NOT let the swab touch any surface other than the collection area you choose and then LATER only ONE QUARTER PIE SECTION of the agar.

FIRST INOCULATION OF ONLY A ¼ PIE SECTION OF THE PLATE

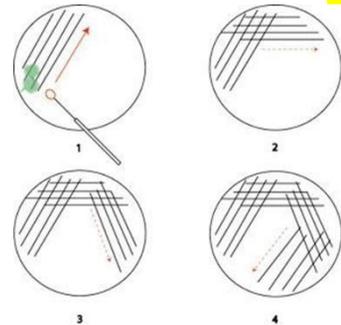


1. When you're ready to inoculate the agar with your sample, HOLD-UP the bottom part of the Petri dish with the agar about 3 inches high (keep it upside down). FIRST you will ONLY be inoculating ONE QUARTER PIE SECTION of the agar with the swab.
2. Don't push the swab hard on that ONE QUARTER section of agar. Just let it glide over the surface of that ¼ pie section. Make a zig-zag pattern of inoculation lines back and forth IN THAT ¼ PIE SECTION ONLY.
3. Put the plate back DOWN onto the already upside-down lid immediately.

FIRST INCUBATION: (APPROXIMATELY DAY 2 TO DAY 6)

1. The best environment for incubation is a biological lab incubator, but cultures are easily grown without one as long as they are in a fairly warm environment.
2. Keep the inoculated agar plate (top & bottom) upside down. Place it inside a zip-bag to prevent the agar from drying out and to control odors. It's OK if condensation forms inside the Petri dish.
3. Open zip bags daily to let fresh air in for the microbe colonies. Do not open the Petri dishes yet. Keep agar plates upside down and in a warm environment. Normal room temperature is good. But a bit warmer than room temp helps the colonies grow faster. It provides more energy for chemical reactions like osmosis and metabolism.
4. Colonies should begin to grow visible in a day or two in a warm environment and 2-4 days if cooler.

** (APPROXIMATELY DAY 5, 6 or 7)



MAKING A STREAK-PLATE with the OTHER 3 SECTIONS Using the METAL LOOP:

1. Take the agar plate out of the zip bag, but keep it upside down on the table. (In step 3, you will sterilize the metal loop and use it to carefully streak (drag) a sample of microbes from ONLY one colony onto the other 3 quarter pie sections.)

2. Use a flame to sterilize the loop before each streak inoculation. Hold the loop handle with a heat-protection pad and heat the end of the wire in a flame until it turns bright red. Let the loop cool about 10 seconds before touching the agar ¼ section that has colonies growing on it.

3. Have a partner lift the agar plate up a few inches enough so you can streak the plate. To streak the plate, you gently take the sterile loop and touch ONLY ONE COLONY that is growing. Then gently use the loop to DRAG some of the microbes in that colony onto a SECOND SECTION of the agar pie sections that is next to the initial section where you grew colonies.

4. Next you will use the loop to gently streak (drag) THIS TIME from the SECOND agar pie section to a THIRD SECTION.
5. Next you will use the loop to gently streak (drag) THIS TIME from the THIRD agar pie section to the FOURTH SECTION.
6. CLOSE the petri dish by placing the agar plate bottom back down onto the upside-down lid.

** (APPROXIMATELY DAY 6 to DAY 8)

SECOND INCUBATION:

1. Put it back in the bag to prevent the agar. It's OK if condensation forms inside the Petri dish.
3. Open bags daily to let fresh air in for the microbe colonies. Do not open the Petri dishes yet. Keep agar plates upside down and in a warm environment. Normal room temperature is good. But a bit warmer than room temp helps the colonies grow faster. It provides more energy for chemical reactions like osmosis and metabolism.
4. Colonies should begin to grow visible in a day or two in a warm environment and 2-4 days if cooler.

** (APPROXIMATELY DAY 6, 7, OR 8)

OBSERVATION AND PHOTOGRAPHING RESULTS:

1. Hopefully your plate will grow a few new colonies on at least 3 of the 4 agar pie quarters. When it does, take pictures of the plate to preserve the results for later consideration. Take pictures above, below, and from a side angle of the agar surface.
2. Now before disposing of the plates (see below how to do it safely) examine the colonies carefully and record your observations. What are the colony numbers, sizes (diameter, height), shapes, colors, consistencies, etc.

DISPOSAL (BY AN ADULT): Use rubber gloves and eye protection. Put the plates into a sink. Pour bleach onto the colonies in the plates. Put them into a plastic trash bag and toss them into an OUTSIDE trash container.

LAB REPORT: Complete the following and submit online to Turn-it-In.

RESEARCH QUESTION: Where in the Middle School are the most microbes?

HYPOTHESIS (testable prediction stated in an "IF, THEN" Format):

EXPERIMENTAL DESIGN VARIABLES:

Controlled Variables (what should be the same in each group's experiment):

Independent variable:

Dependent variable:

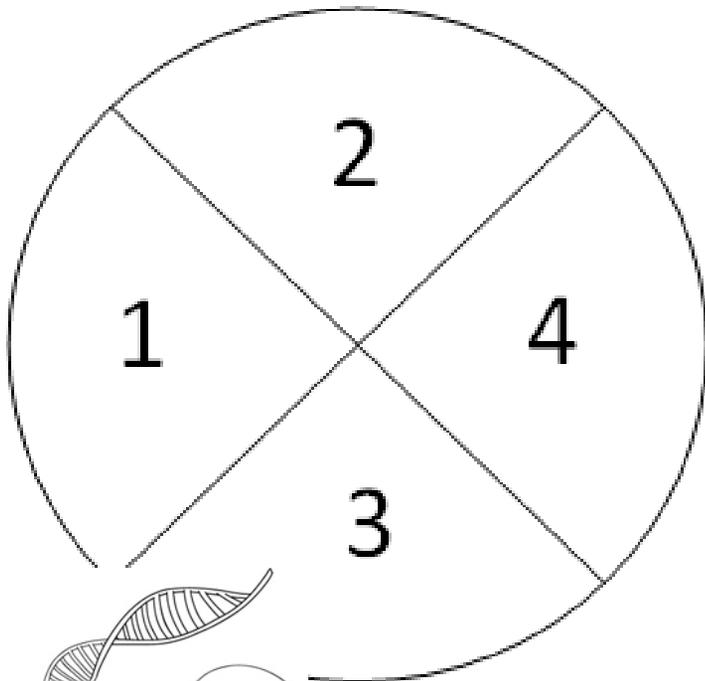
RESULTS OBSERVED:

General description of colonies' growth time, shapes, colors, sizes, etc.:

Specific details like measured colony sizes, etc.:

CHART-DIAGRAM of your plate and colonies:

CONCLUSIONS:



APPLICATIONS TO REAL LIFE:

