

Testing Procedures for E. coli

Storage of Plates:

Refrigerate unopened packages upon receiving them. Make sure you use the film before the expiration date that is on the package.

Once opened, make sure you seal the package carefully by folding the end of the package over and taping it shut to make sure the plates aren't left exposed to the air. Opened packages should be kept at room temperature and at less than 50% relative humidity. NOTE: Do NOT refrigerate opened packages. Once the package is opened you must use the plates within one month. Open the package only when you are ready to begin your testing.



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Handle the film with care, opening the plates themselves only when necessary.

Sterilization:

Before the students go and collect their water samples to be tested, there are some simple sterilization that needs to take place to insure that there is no contamination before samples are collected.

Disinfect the students work area by using either a disinfectant or by wiping the countertop with ethanol (or other type of alcohol) both before and after use.

For the beaker tongs and forceps used in the sterilization process, dip the end of the beaker tongs and forceps into ethanol and then pass through a flame. Allow the tongs and forceps to cool briefly before handling anything else or placing on the paper towel. After sterilization make sure to only handle when needed, and place on a piece of paper towel where human hands haven't touched.

For the plastic sample bottles used in the collection of the water sources, using the sterile beaker tongs, submerge the plastic container in a beaker that contains boiling distilled water. Hold under the boiling water for a couple of minutes. Remove from the boiling water, shake all excess water out of bottle. Place open end of container down on the paper towel to allow complete drying.

Again, using the beaker tongs, place the cap from the sample bottle into the boiling water for a couple of minutes. Remove from water, shake off excess water, place on paper towel until dry.

When the sampling bottle and cap are dry, handling only the outside edge of the cap, carefully screw on the sterilized cap to the sterilized bottle. Do not take the lid off until you are ready to collect the water sample.

For the 1.0 mL glass pipets used to place water sample on the Petrifilm EC, place them in a small tray so they can lay flat. Pour boiling distilled water over the pipet until it is covered. Allow to soak in the boiling water for a couple of minutes. Using the sterilized forceps, remove from the tray making sure the water flows out of the pipet. Place on the paper towel to cool and dry. Do not handle until ready to use. A 1.0 mL graduated pipet will work, carefully open the package and remove just one pipet, use the graduated markings on the pipet for the 1.0 mL of water to be tested, and then discard the pipet after use.

NOTE: All of the equipment can be sterilized if you have an autoclave. Set the autoclave to 121 °C and insert the equipment for a minimum of 12 minutes. If you are using a plastic graduated pipet, do not try to autoclave.

Collection Techniques.

River Techniques:

Walk up stream a little bit being careful not to stir up the sediment on the river bottom. Holding the container mouth pointing upstream, submerge the container so it is under the surface of the water. Carefully unscrew the top of the cap, making sure not to touch the inside of the cap or the collection bottle. When the bottle is full, screw the cap onto the container while still submerged.

Lake Techniques:

Walk carefully out into the lake until the water is just below your waste. Be careful not to stir up sediment from the bottom. Holding the container mouth facing the center of the lake, submerge the container so it is under the surface. Carefully unscrew the top of the cap, making sure not to touch the inside of the cap or the collection bottle. When the bottle is full, screw the cap onto the container while still submerged.

If you are taking the sample back to the lab to do the testing, make sure to put the collected sample into a cooler until reaching the lab. If you are waiting even longer than your immediate return, place the sample into the refrigerator until actual testing takes place.

Testing Procedures:

1. If the Petrifilm package has been stored in the refrigerator, let the package come to room temperature before you open it. You don't want any condensation on the package when you open it.
2. Place the Petrifilm plate on a level surface with the gridded side down. Carefully lift the top of the film as shown in the figure below.

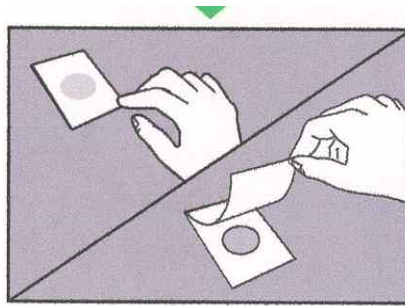


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3. Using the sterile pipet, transfer 1.0 mL from the water sample collected into the pipet. Care must be taken not to allow any of the water to go up into the bulb when taking it from the sample. Holding the pipet (with the sample of water in it) perpendicular to the Petrifilm plate, place 1.0 mL of the sample onto the center of the bottom film.

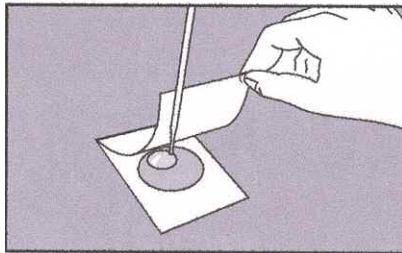


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4. Carefully roll the film onto the bottom portion of the plate. Do NOT drop the top film down.

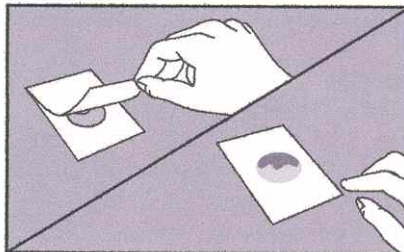


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5. With the flat side down (not the side with the circular ridge), place the spreader on the top film over the inoculum.

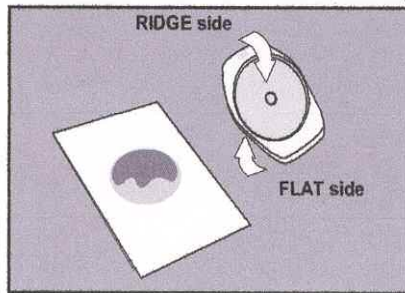


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6. GENTLY apply pressure on the spreader to distribute the inoculum over a circular area. Do not twist or slide the spreader.

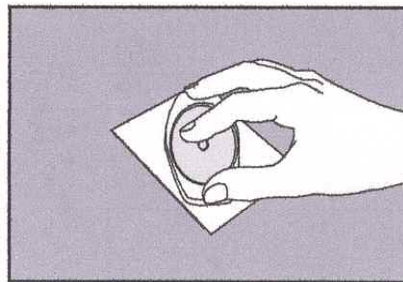


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7. Carefully lift the spreader up and then wait for at least one minute before handling the plate to allow the gel in the plate to solidify.

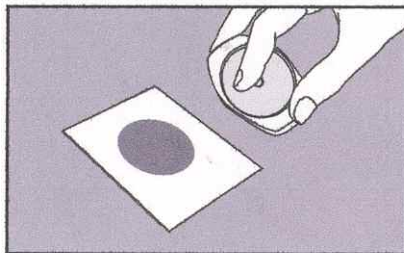


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- After the plates have sat for at least a minute, you are then ready to start the incubation period. You may stack up the Petrifilm plates up to 20 high for the incubation period if so desired. If you have access to an incubator, place the plates in the incubator with the settings at $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $48\text{ hours} \pm 2\text{ hours}$. If you do not have access to an incubator, place the plates in a warm, dry area for 48 hours and then read.*

Interpretation of Results

- You've followed all the necessary steps, and when you take the plate out to interpret the results, you get something that looks like the illustration to the right. Please pay careful attention to the following instructions, many of the mistakes made from interpretation can be avoided if you're careful and follow the protocol.

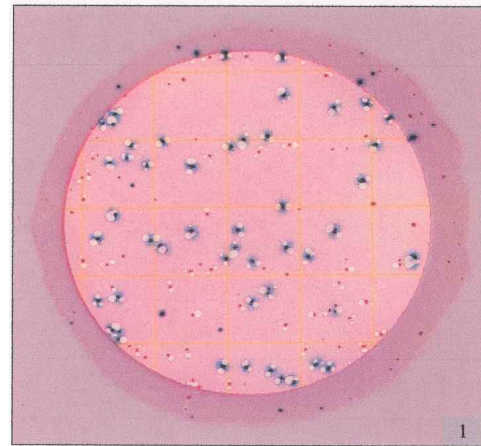


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- You should notice a change in the gel color on the plates when you do your testing. As the *E. coli* or coliform count increases, the color of the gel turns a darker red or purple-blue. Background bubbles are a characteristic of the gel and are not a result of bacterial growth. Looking at the plate below, notice the difference between 1, 2, and 3. The bubbles on three are not from bacteria.

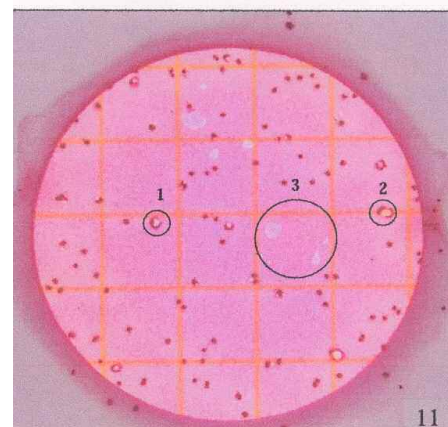


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The picture to the right shows various bubble patterns that are associated with gas producing colonies and should all be enumerated.

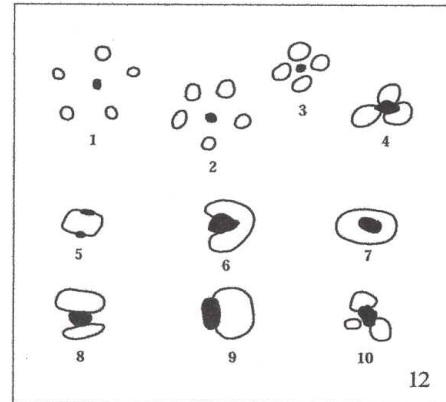


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11. In the plate to the right, notice there are not red colonies with gas around them nor are there any blue/purple colonies with gas around them, this would be given a reading of 0 for both coliform and E. coli. The box labeled 1 is to show the color changes in the gel.

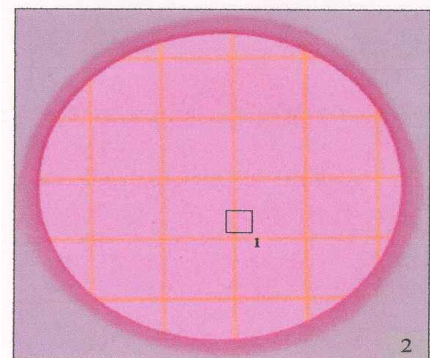


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12. When counting colonies, do not count any colonies that appear on the foam barrier as these have been removed from the selective influence of the medium. See circle 1 on the plate.

Counting the number of dots you see with gas associated with them, you should come up with 28 colonies.

Total coliform count = 28

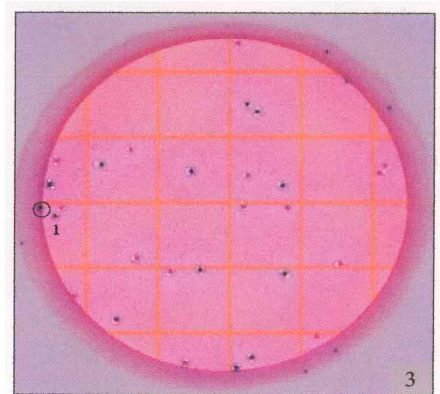


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13. When counting colonies you may find that either back lighting or front lighting may help you see the colonies better. Any blue in a colony (blue to red-blue) indicates the presence of *E. coli*. Circle one shows a red/blue colony using back lighting. Circle two shows the same colony with front lighting.

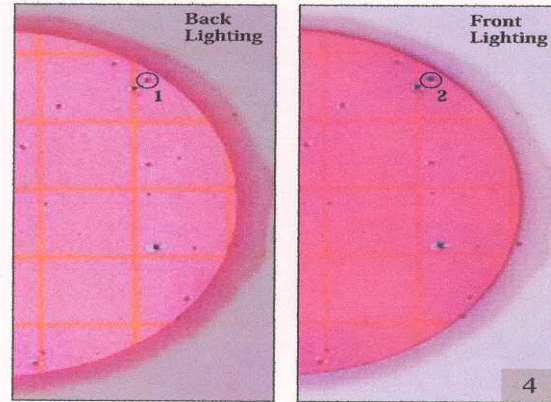


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14. Let's use the plate to the right to make sure that you are counting the number of *E. coli* colonies correctly. Please take a look at the plate and count. The answer will be in the reading of procedural step #15.

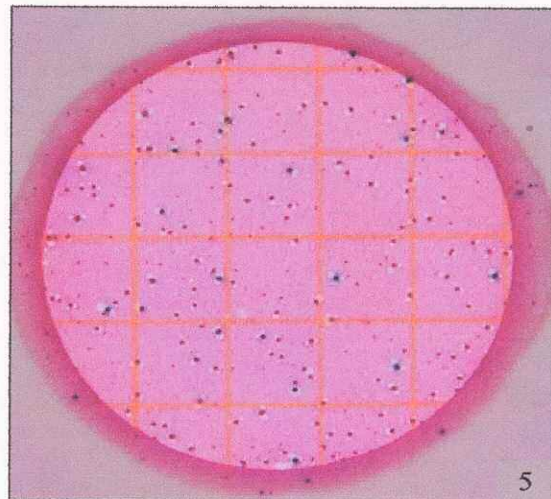


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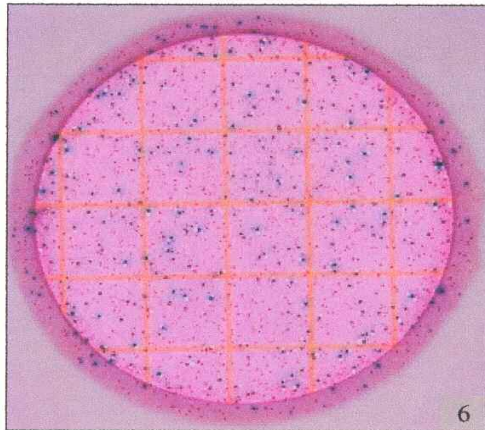
15. In counting the number of coliform colonies (from the plate shown in procedural step #14), notice that there are a whole lot of red dots on this plate. As the circular growth area is 20 cm^2 , estimates can be made on plates by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate. Don't forget, *E. coli* are also coliforms and should be counted in the total.

Estimated total coliform count = 150

E. coli count = 17

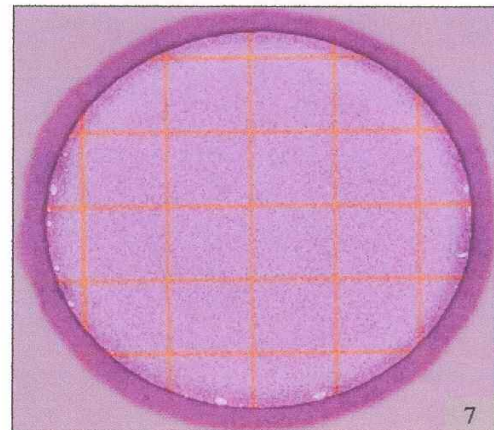
Below are some examples when coliform's are too numerous to count. If in your sampling, you get any results that are similar to the picture, follow the directions as given by 3M.

TNTC (Too Numerous to Count) *To obtain a more accurate count, dilute the sample further.*



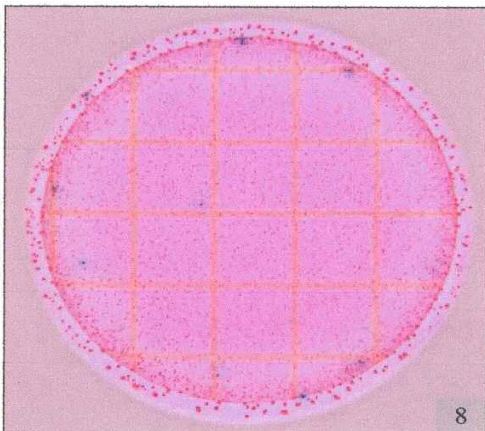
Actual count ~ 10^6

Petrifilm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color from red to purple-blue.



Actual count ~ 10^8

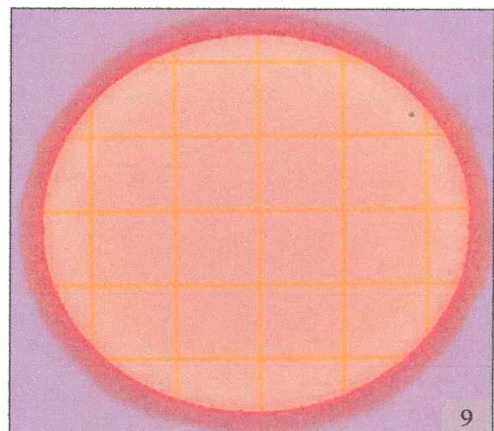
High concentrations of *E. coli* may cause the growth area to turn purple-blue.



Presumptive *E. coli* count ~ 8

Estimated total coliform count ~ 10^8

When high levels of coliforms are present ($>10^8$), some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli*. Pick blue colonies without gas and confirm if necessary.



Actual count ~ 10^8

When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

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Please note, all pictures used in the Testing procedure portion are used by permission and were provided by 3M's Petrifilm E. coli/Coliform Count Plate Interpretation Guide and were downloaded from:

http://solutions.3m.com/wps/portal/_s.155/84505/_s.155/92829

and clicking on:

E. coli/Coliform Count Plate Interpretation Guide.

Clean Up Activities:

When you are done interpreting the plates, do not lift the film up (unless you are going to isolate a particular colony for further study or add to additional cultures, if so, lift the top film and pick the colony from the gel. The medium will stick to the top film).

You will need to disinfect before disposing of the plates. To disinfect, submerge the plates in simple household bleach for about an hour. After removing from the bleach, they are safe to dispose of in the trash.

NOTE: If you have a scanner on your computer, as long as you don't open the plates, you can place them on the scanner and scan the plates for a photo record of the samples.