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Proper Use of Aseptic Technique When Working with Bacterial and Fungal Cultures

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PI Name: Dr. Luisa A. Ikner, Bradley Schmitz, Dr. Charles P. Gerba, and Dr. Ian L. Pepper
Environmental Science Education Title: Proper Use of Aseptic Technique When Working with Bacterial and Fungal Cultures

Overview:

Aseptic technique is a fundamental skill widely practiced in the field of environmental microbiology that requires a balance of mindfulness and practice in the laboratory. Proper use of this technique reduces the likelihood of bacterial or fungal contamination of reagents, culture media, and environmental samples. Aseptic technique is also vital to ensure data integrity and maintain the purity of culture libraries that may be comprised of very rare and difficult to culture isolates. Sources of contamination in the laboratory environment include airborne microorganisms (including those adhered to dust and lint particles); microbes present on the laboratory bench workspace or on unsterilized glassware or equipment; and even microbes transferred from the body and hair of the technician. The use of aseptic technique is also a safety measure that lowers the potential for the transmission of microorganisms to laboratory technicians, which is particularly important when working with pathogens.

This video demonstrates several practices of aseptic technique to be conducted prior to, during, and after any procedures involving microorganisms on the benchtop. Media and reagents should be sterilized using either a pressurized steam autoclave or a filter sterilization apparatus employing a 0.22 µm filter to prevent contamination of test materials and samples. The work space should be disinfected, other materials should be sanitized as needed, and personal protective equipment (PPE) should be donned, including a lab coat, gloves, and safety goggles. In addition, this demonstration identifies common mistakes and errors that compromise aseptic technique, such as improper flaming and handling of culture inoculating loops and spread plate rods, and unnecessarily extended exposure of growth media to the air.

Procedure:

1. Fast-Track Portion

    1.1. Collect soil and/or water samples, and transport them to the laboratory for microbial analysis.

    1.2. Soil example: In the lab, weigh a 10 g sample of moist soil using an analytical balance; Water example: Pass a 100 ml sample of water through a membrane filter.

    1.3. Soil example: Dilute the sample (1:10) into 95 ml of phosphate-buffered saline (10 parts soil is equivalent to 5 parts of aqueous liquid), and vortex to mix; Water example: Aseptically place the membrane onto low-nutrient agarose media.

    1.4. Soil example: Perform subsequent 1:10 dilutions through at least 10^{-5} g soil

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per ml, and spread-plate selected dilutions in replicates of 2 or 3 onto a low nutrient-agarose medium; Water example: Perform 1:10 dilutions through at least the 10^{-3} dilution, which are spread-plated in replicates of 2 or 3 onto a low nutrient-agarose medium.

1.5. Soil and Water examples: Incubate the plates for 1 week at room temperature.

1.6. Soil and Water examples: Select colonies for isolation and streak onto fresh agarose medium.

1.7. Soil and Water examples: Incubate the streak plates for 1 to 2 days at room temperature and then evaluate for purity by plate analysis and Gram stain.

1.8. Soil and Water examples: Catalogue stock cryovials of environmental isolates and place into the -80 °C freezer for long-term storage.

1.9. Remove several of the cryovials from storage, and perform isolation streaks for each.

1.10. After incubation, the plates are visualized. Most growth plates display only one colony morphology and no additional contamination. 1 or 2 of the plates, however, display contamination (bacterial or fungal) of the growth medium or cultures.

2. Aseptic Technique Part I: Preparation for Performance of Lab Procedures

2.1. A key aspect of aseptic technique is the donning of PPE, the purpose of which is two-fold: 1) prevention of the transfer of microorganisms from the body and hair of the technician that would result in the contamination of samples and lab cultures, and 2) prevention of the transfer of potentially pathogenic microorganisms from environmental samples and known laboratory pathogenic isolates to the technician.

2.2. Obtain and apply the following PPE items: lab coat, latex or nitrile gloves (free from tears or holes), and safety goggles (**Figure 1**).

2.3. With regards to aseptic technique, as previously mentioned, and for safety in the event of using an open flame, tie back long hair.

2.4. A second important aspect of aseptic technique is the proper sterilization and storage of media/reagents to be used in the laboratory. Prepare liquid broth medium (*e.g.*, tryptic soy broth) and agar-based media (*e.g.*, R2A) by weighing the proper amount of dried base powder, which is added to the appropriate amount of de-ionized water.

2.5. For the broth medium, dissolve the powder on a hot plate with low heat applied, and dispense the liquid either in 100 ml volumes into glass screw-top flasks, or in 10 ml volumes into glass screw-top test tubes. Stir the agarose medium until dissolved on the hot plate.

2.6. Apply autoclave tape to the materials, and autoclave the media according to the manufacturer's instructions (*e.g.*, 20 min at 121 °C) [**Figures 2 and 3**; note that the color of stripes on the autoclave tape should change from white (pre-autoclave) to black (post-autoclave)].

2.6.1. Although the color change generally indicates that sterilization was successful, sterility checks using spore strip kits should be conducted at least monthly to verify the autoclaving process.

2.7. Cool the liquid broths to room temperature, and then store as such or under refrigeration temperatures of 4 °C.

2.8. Cool the agarose medium by placing the container into a water bath set to ~50 °C. Once cooled, the media can be poured into sterile Petri dishes. Allow the medium to cool and solidify, then consolidate for storage under temperatures specified by the manufacturer.

2.9. There are several varieties of culture media that cannot be autoclaved as the high temperatures degrade critical ingredients. Working with a liquid media that cannot be autoclaved requires filter-sterilization using a vacuum filtration system employing a 0.22 µm filter, followed by storage at the appropriate temperature.

2.10. Prior to placement of materials on the benchtop, disinfect the surface using an appropriate solution (*e.g.*, 500 ppm bleach).

2.10.1. Disinfection of the work surface is an important part of aseptic technique, as it lowers the risk of transferring contaminants from the working surface to cultures and sterile media.

2.11. Turn on a Bunsen burner. The flame type best suited for sterilizing the inoculating loop is an intense blue flame, with a definitive blue cone observed in the middle (**Figure 4**).

2.12. Slowly pass the inoculating loop through the hottest part of the flame (tip of the blue cone). It appears as red hot for the purpose of sterilization.

3. Bacterial Transfers, Demonstration 1: From Petri Plate to Petri Plate

One method of transferring bacteria is from one Petri plate displaying growth of a cultured isolate to another un-inoculated Petri plate containing an agar-based growth medium (**Figure 5**).

3.1. Slightly open the Petri plate containing the pure bacterial culture, and gently tap the hot inoculating loop onto the agar surface.

3.2. Retrieve one isolated colony from the surface of the plate using the cooled inoculating loop, and close the Petri plate.

3.3. Perform a streak for isolation using a fresh Petri plate containing the culture medium, with the lid slightly ajar.

3.4. For each portion of the isolation streak (3 total per plate), flame-sterilize the inoculating loop just prior to use. Also, flame-sterilize it just after the final streak is performed in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use or come into contact with the inoculating loops.

3.5. Place the streak(s) for isolation into an incubator for growth overnight.

3.6. Remove the plates the next day. One demonstrates an example of a successful aseptic streak for isolation. The other shows a plate contaminated with fungal or bacterial contamination.

4. Bacterial Transfers, Demonstration 2: From Broth Culture to Petri Plate Containing Growth Medium

4.1. A second method of transferring bacteria is from a broth culture exhibiting growth, as generally observed by turbidity, to an un-inoculated Petri plate containing growth medium (**Figure 6**).

4.2. Remove the cap from the test tube (or flask) containing the pure bacterial culture, and pass the opening of the container 2 to 3 times through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop).*

4.3. Carefully lower the inoculating loop into the tube/flask, and gently press against the side of the container to cool just prior to insertion into the broth culture.

4.4. Remove one loopful of broth culture (**Figure 7**), and immediately replace the cap.

4.5. Perform a streak for isolation using a fresh Petri plate containing the un-inoculated culture medium, with the lid slightly ajar.

4.6. For each portion of the streak (3 total per plate), flame-sterilize the inoculating loop just prior to use. Also, flame-sterilize just after the final streak is performed in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

4.7. Place the streak(s) for isolation into an incubator for growth overnight.

4.8. Remove the plates the next day. One demonstrates an example of a successful aseptic streak for isolation. The other shows a plate contaminated with fungal or bacterial contamination.

5. Bacterial Transfers, Demonstration 3: From Petri Plate Containing Growth to Sterile Liquid Growth Medium

5.1. A third method of transferring bacteria is from a Petri plate containing an isolated culture streak to an un-inoculated tube/flask containing sterile liquid growth medium (**Figure 8**).

5.2. Slightly open the Petri plate containing the pure bacterial culture, and cool the hot inoculating loop by tapping it gently onto the agar surface.

5.3. Retrieve one isolated colony from the surface of the plate using the cooled inoculating loop, and close the Petri plate.

5.4. Remove the cap from the test tube (or flask) containing the sterile liquid growth medium, and pass the opening of the container 2 to 3 times through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop)* (**Figure 9**).

5.5. Carefully lower the extracted colony into the liquid broth medium, and gently agitate the loop in order to release the bacteria. Immediately replace the cap.

5.6. Flame-sterilize the inoculating loop in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

5.7. Place the flask(s) into an incubator for growth overnight.

5.8. Remove the flasks from incubation the next day. Perform a dilution series in order to enumerate the culture.

5.9. Plate the dilutions from the series onto agarose culture media, and incubate the plates overnight.

5.10. Remove the plates the next day, and observe the contamination.

6. Bacterial Transfers, Demonstration 4: From Broth Culture to Sterile Liquid Growth Medium

6.1. A fourth method of transferring bacteria is from a broth culture exhibiting growth to an un-inoculated tube/flask containing sterile liquid growth medium (**Figure 10**).

6.2. Remove the cap from the test tube (or flask) containing the pure bacterial culture, and pass the opening of the container twice through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop).*

6.3. Carefully lower the inoculating loop into the tube/flask, and gently press against the side of the container to cool just prior to insertion into the broth culture.

6.4. Remove one loopful of broth culture, and immediately replace the cap.

6.5. Remove the cap from the test tube (or flask) containing the sterile liquid growth medium, and pass the opening of the container twice through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop.)*

6.6. Carefully lower the extracted loopful into the sterile liquid broth medium, and gently agitate the loop in order to release the bacteria. Immediately replace the cap.

6.7. Place the flask(s) into an incubator for growth overnight.

6.8. Remove the flasks from incubation the next day. Perform a dilution series in order to enumerate the culture.

6.9. Plate the dilutions from the series onto agarose culture media, and incubate the plates overnight.

6.10. Remove the plates the next day, and observe the contamination.

6.11. Flame-sterilize the inoculating loop in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

Representative Results:

The outcome of the procedure demonstrates proper aseptic technique and poor aseptic technique. **Figure 11** illustrates the contamination that can arise from poor aseptic technique when pouring agarose plates (top plate: sterile medium; bottom plates: contaminated media).

Applications:

Proper use of aseptic technique is vital for environmental microbiologists when sampling in the field and in the laboratory when working with media, reagents, and cultured isolates. Poor aseptic technique in the field can result in the transfer of microorganisms from the technician to critical environmental samples, as well as the cross-contamination of microbes from one sample to another. Such events are of importance, for example, in microbial ecology studies seeking to identify and compare bacterial and fungal populations that may be present in a given biome. Contamination of such samples can result in a loss of data integrity. Aseptic technique is also critical for the maintenance of laboratory culture isolates originating from field sampling or from well-established microbial and cell culture repositories. The time, labor, and financial costs that would be required of a lab in an effort to "clean-up" or replace contaminated cultures, particularly rare isolates from unique environments, could be very high and prohibitive, as some isolates may be irreplaceable.

Legend:

Figure 1: A lab coat, latex gloves (free from tears or holes), and safety goggles.

Figure 2: Autoclave tape being applied to material.

Figure 3: Note the color change of stripes on autoclave tape from white (pre-autoclave) to black (post-autoclave).

Figure 4: The flame type best suited for sterilizing the inoculating loop – an intense blue flame, with a definitive blue cone observed in the middle.

Figure 5: A transfer of bacteria from one Petri plate displaying growth of a cultured isolate to another un-inoculated Petri plate containing an agar-based growth medium.

Figure 6: A transfer of bacteria from a broth culture exhibiting growth, as generally observed by turbidity, to an un-inoculated Petri plate containing growth medium.

Figure 7: One loopful of broth culture.

Figure 8: A transfer of bacteria from a Petri plate containing an isolated culture streak to an un-inoculated tube/flask containing sterile liquid growth medium.

Figure 9: After removing the cap from the test tube, pass the opening of the container 2 to 3 times through the hottest portion of the flame.

Figure 10: A transfer of bacteria from a broth culture exhibiting growth to an un-inoculated tube/flask containing sterile liquid growth medium.

Figure 11: Contamination that can arise from poor aseptic technique when pouring agarose plates (top plate: sterile medium; bottom plates: contaminated media).

PI Name: Dr. Luisa A. Ikner, Bradley Schmitz, Dr. Charles P. Gerba, and Dr. Ian L. Pepper

Environmental Science Education Title: Proper Use of Aseptic Technique When Working with Bacterial and Fungal Cultures

Overview: Aseptic technique is a fundamental skill widely practiced in the field of environmental microbiology that requires a balance of mindfulness and practice in the laboratory. Proper use of this technique reduces the likelihood of bacterial or fungal contamination of reagents, culture media, and environmental samples, and preserves stock and working cultures employed in laboratory procedures. Sources of contamination include airborne microorganisms (including those adhered to dust and lint particles), microbes present on the laboratory bench workspace or on unsterilized glassware or equipment, and even those transferred from the body and hair of the technician. The use of aseptic technique is also a safety measure that lowers the potential for transmission of microorganisms to laboratory technicians, which is of particular importance when working with pathogens.

In the "fast-track" portion, soil and/or water scientists collect samples, then process them in the lab by dilution and plating, and generate isolates for storage. Footage of preserved vials of cultured bacterial/fungal environmental isolates is shown. A scientist removes several vials for culture-based work in the laboratory. The scientist then analyzes various Petri dishes with isolates streaked from these laboratory stocks. One subset of plates will be pure culture isolates, thereby demonstrating sound aseptic technique. The second subset of plates will contain evidence of contamination (bacterial and/or fungal) resulting from poor aseptic technique. Aseptic technique is vital to maintain the integrity of data and culture libraries which may be comprised of very rare and difficult to culture isolates.

The "Procedure" portion lists several practices of aseptic technique that should be conducted prior to performing microbial transfers on the benchtop: proper media/reagent sterilization, preparation of the work space (i.e. disinfection of the benchtop and sanitization of other materials, as needed), and the donning of personal protective equipment (PPE) including a lab coat, gloves, and safety goggles. Four types of bacterial culture transfers will be demonstrated utilizing proper aseptic technique: from Petri plate to Petri plate; from broth culture to Petri plate; from Petri plate to sterile liquid media, and from broth culture to liquid media. For each type of transfer, all steps will be described. In addition, common mistakes, such as improper flaming and handling of the inoculating loop, and unnecessarily extended exposure of culture media to the air, will be identified.

Procedure:

1. Fast-Track Portion

1.1. Collect soil and/or water samples, and transport them to the laboratory for microbial analysis.

1.2. Soil example: In the lab, weigh a 10 g sample of moist soil using an analytical balance; Water example: pass a 100 ml sample of water through a membrane

filter.

1.3. Soil example: Dilute the sample (1:10) into 95 ml of phosphate-buffered saline (10 parts soil is equivalent to 5 parts of aqueous liquid), and vortex to mix; Water example: aseptically place the membrane onto low-nutrient agarose media.

1.4. Soil example: Perform subsequent 1:10 dilutions through at least 10^{-5} g soil per ml, and spread-plate selected dilutions in replicates of two or three onto a low nutrient-agarose medium; Water example: perform 1:10 dilutions through at least the 10^{-3} dilution, which are spread-plated in replicates of two or three onto a low nutrient-agarose medium.

1.5. Soil and Water examples: Incubate the plates for one week at room temperature.

1.6. Soil and Water examples: Select colonies for isolation and streak onto fresh agarose medium.

1.7. Soil and Water examples: Incubate the streak plates for one to two days at room temperature and then evaluate for purity by plate analysis and Gram stain.

1.8. Soil and Water examples: Catalogue stock cryovials of environmental isolates and place into the -80 °C freezer for long-term storage.

1.9. Remove several of the cryovials from storage, and perform isolation streaks for each.

1.10. After incubation, the plates are visualized. Most growth plates display only one colony morphology and no additional contamination. One or two of the plates, however, display contamination (bacterial or fungal) of the growth medium or cultures.

1.11. Possible sources of the contamination outlined include improper preparation of media/reagents, the potential transfer of microbes from the technician due to lack of PPE, and poor technique exercised during the bacterial transfers themselves.

2. Aseptic Technique Part I: Proper Use of Personal Protective Equipment (PPE)

2.1. A key aspect of aseptic technique is the donning of PPE, the purpose of which is two-fold: 1) prevention of the transfer of microorganisms from the body and hair of the technician that would result in the contamination of samples and lab cultures, and 2) prevention of the transfer of potentially pathogenic microorganisms from environmental samples and known laboratory

pathogenic isolates to the technician.

2.2. Obtain and apply the following PPE items: lab coat, latex or nitrile gloves (free from tears or holes), and safety goggles (Figure 1).

2.3. In regards to aseptic technique, as previously mentioned, and for safety in the event of using an open flame, tie back long hair.

3. Aseptic Technique Part II: Preparation of Media/Reagents

3.1. A second important aspect of aseptic technique is the proper sterilization and storage of media/reagents to be used in the laboratory.

3.2. Prepare liquid broth medium (e.g. tryptic soy broth) and agar-based media (e.g. R2A) by weighing the proper amount of dried base powder, which is added to the appropriate amount of de-ionized water.

3.3. For the broth medium, dissolve the powder on a hot plate with low heat applied, and dispense the liquid either in 100 ml volumes into glass screw-top flasks, or in 10 ml volumes into glass screw-top test tubes. Stir the agarose medium until dissolved on the hot plate.

3.4. Apply autoclave tape to the materials, and autoclave the media according to the manufacturer's instructions (e.g. 20 minutes at 121 °C) [Figures 2 and 3; note the color change of stripes on autoclave tape from white (pre-autoclave) to black (post-autoclave)].

3.4.1. Although the color change generally indicates that sterilization was successful, sterility checks using spore strip kits should be conducted at least monthly to verify the autoclaving process.

3.5. Cool the liquid broths to room temperature, and then store as such or under refrigeration temperatures of 4°C.

3.6. Cool the agarose medium to ~50 °C, and then pour into sterile Petri dishes. Allow the medium to cool and solidify, then consolidate for storage under temperatures specified by the manufacturer.

3.7. Working with a liquid media that cannot be autoclaved requires filter-sterilization using a vacuum filtration system employing a 0.22 µm filter, followed by storage at the appropriate temperature.

4. Bacterial Transfers, Demonstration 1: From Petri Plate to Petri Plate

4.1. One method of transferring bacteria is from one Petri plate displaying growth of a cultured isolate to another un-inoculated Petri plate containing

an agar-based growth medium (Figure 4).

4.2. Prior to placement of materials on the benchtop, disinfect the surface using an appropriate solution (e.g. 500 ppm bleach).

4.2.1. Disinfection of the work surface is an important part of aseptic technique, as it lowers the risk of transferring contaminants from the working surface to cultures and sterile media.

4.3. Turn on a Bunsen burner. The flame type best suited for sterilizing the inoculating loop is an intense blue flame, with a definitive blue cone observed in the middle (Figure 5).

4.4. Slowly pass the inoculating loop through the hottest part of the flame (tip of the blue cone). It appears as red hot for the purpose of sterilization.

4.5. Slightly open the Petri plate containing the pure bacterial culture, and gently tap the hot inoculating loop onto the agar surface.

4.6. Retrieve one isolated colony from the surface of the plate using the cooled inoculating loop, and close the Petri plate.

4.7. Perform a streak for isolation using a fresh Petri plate containing the culture medium, with the lid slightly ajar.

4.8. For each portion of the isolation streak (3 total per plate), flame-sterilize the inoculating loop just prior to use. Also, flame-sterilize it just after the final streak is performed in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use or come into contact with the inoculating loops.

5. Bacterial Transfers, Demonstration 2: From Broth Culture to Petri Plate Containing Growth Medium

5.1. A second method of transferring bacteria is from a broth culture exhibiting growth, as generally observed by turbidity, to an un-inoculated Petri plate containing growth medium (Figure 6).

5.2. Prior to placement of materials on the benchtop, disinfect the surface using an appropriate solution (e.g. 500 ppm bleach).

5.2.1. Disinfection of the work surface is an important part of aseptic technique, as it lowers the risk of transferring contaminants from the working surface to cultures and sterile media.

5.3. Turn on a Bunsen burner. The flame type best suited for sterilizing the inoculating loop is an intense blue flame, with a definitive blue cone observed in the middle (Figure 5).

5.4. Slowly pass the inoculating loop through the hottest part of the flame. It will appear as red hot for the purpose of sterilization.

5.5. Remove the cap from the test tube (or flask) containing the pure bacterial culture, and pass the opening of the container 2 to 3 times through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop).*

5.6. Carefully lower the inoculating loop into the tube/flask, and gently press against the side of the container to cool just prior to insertion into the broth culture.

5.7. Remove one loopful of broth culture (Figure 7), and immediately replace the cap.

5.8. Perform a streak for isolation using a fresh Petri plate containing the un-inoculated culture medium, with the lid slightly ajar.

5.9. For each portion of the streak (3 total per plate), flame-sterilize the inoculating loop just prior to use. Also, flame-sterilize just after the final streak is performed in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

6. Bacterial Transfers, Demonstration 3: From Petri Plate Containing Growth to Sterile Liquid Growth Medium

6.1. A third method of transferring bacteria is from a Petri plate containing an isolated culture streak to an un-inoculated tube/flask containing sterile liquid growth medium (Figure 8).

6.2. Prior to placement of materials on the benchtop, disinfect the surface using an appropriate solution (e.g. 500 ppm bleach).

6.2.1. Disinfection of the work surface lowers the risk of transferring contaminants from the working surface to cultures and sterile media.

6.3. Turn on a Bunsen burner. The flame type best suited for sterilizing the inoculating loop is an intense blue flame, with a definitive blue cone observed in the middle (Figure 5).

6.4. Slowly pass the inoculating loop through the hottest part of the flame. It will appear as red hot for the purpose of sterilization.

6.5. Slightly open the Petri plate containing the pure bacterial culture, and cool the hot inoculating loop by tapping it gently onto the agar surface.

6.6. Retrieve one isolated colony from the surface of the plate using the cooled inoculating loop, and close the Petri plate.

6.7. Remove the cap from the test tube (or flask) containing the sterile liquid growth medium, and pass the opening of the container 2 to 3 times through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop)* (Figure 9).

6.8. Carefully lower the extracted colony into the liquid broth medium, and gently agitate the loop in order to release the bacteria. Immediately replace the cap.

6.9. Flame-sterilize the inoculating loop in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

7. Bacterial Transfers, Demonstration 4: From Broth Culture to Sterile Liquid Growth Medium

7.1. A fourth method of transferring bacteria is from a broth culture exhibiting growth to an un-inoculated tube/flask containing sterile liquid growth medium (Figure 10).

7.2. Prior to placement of materials on the benchtop, disinfect the surface using an appropriate solution (e.g. 500 ppm bleach).

7.2.1. Disinfection of the work surface lowers the risk of transferring contaminants from the working surface to cultures and sterile media.

7.3. Turn on a Bunsen burner. The flame type best suited for sterilizing the inoculating loop is an intense blue flame, with a definitive blue cone observed in the middle (Figure 5).

7.4. Slowly pass the inoculating loop through the hottest part of the flame. It will appear as red hot for the purpose of sterilization (Figure 11).

7.5. Remove the cap from the test tube (or flask) containing the pure bacterial culture, and pass the opening of the container twice through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop).*

7.6. Carefully lower the inoculating loop into the tube/flask, and gently press against the side of the container to cool just prior to insertion into the broth culture.

7.7. Remove one loopful of broth culture, and immediately replace the cap.

7.8. Remove the cap from the test tube (or flask) containing the sterile liquid growth medium, and pass the opening of the container twice through the hottest portion of the flame *(To prevent contamination, do not set the cap down onto the benchtop.)*

7.9. Carefully lower the extracted loopful into the sterile liquid broth medium, and gently agitate the loop in order to release the bacteria. Immediately replace the cap.

7.10. Flame-sterilize the inoculating loop in order to prevent contamination of the bench surface and as a consideration to others in the lab who may later use the inoculating loops.

Representative Result: The outcome of the procedure demonstrates proper aseptic technique and poor aseptic technique. Figure 12 illustrates the contamination that can arise from poor aseptic technique when pouring agarose plates (top plate: sterile medium; bottom plates: contaminated media).

Applications: Proper use of aseptic technique is vital for environmental microbiologists in the field when sampling and in the laboratory when working with media, reagents, and cultured isolates. Poor aseptic technique in the field can result in the transfer of microorganisms from the technician to critical environmental samples, as well as the cross-contamination of microbes from one sample to another. Such events are of importance, for example, in microbial ecology studies seeking to identify and compare bacterial and fungal populations that may be present in a given biome. Contamination of such samples can result in a loss of data integrity. Aseptic technique is also critical for the maintenance of laboratory culture isolates originating from field sampling or from well-established microbial and cell culture repositories. The time, labor, and financial costs that would be required of a lab in an effort to "clean-up" or replace contaminated cultures, particularly rare isolates from unique environments, could be very high and prohibitive, as some isolates may be irreplaceable.

Legend:

Figure 1: A lab coat, latex gloves (free from tears or holes), and safety goggles.

Figure 2: Autoclave tape being applied to material.

Figure 3: Note the color change of stripes on autoclave tape from white (pre-autoclave) to black (post-autoclave).

Figure 4: A transfer of bacteria from one Petri plate displaying growth of a cultured isolate to another un-inoculated Petri plate containing an agar-based growth medium.

Figure 5: The flame type best suited for sterilizing the inoculating loop – an intense blue flame, with a definitive blue cone observed in the middle.

Figure 6: A transfer of bacteria from a broth culture exhibiting growth, as generally observed by turbidity, to an un-inoculated Petri plate containing growth medium.

Figure 7: One loopful of broth culture.

Figure 8: A transfer of bacteria from a Petri plate containing an isolated culture streak to an un-inoculated tube/flask containing sterile liquid growth medium.

Figure 9: After removing the cap from the test tube, pass the opening of the container 2 to 3 times through the hottest portion of the flame.

Figure 10: A transfer of bacteria from a broth culture exhibiting growth to an un-inoculated tube/flask containing sterile liquid growth medium.

Figure 11: After passing the inoculating loop through the hottest part of the flame, it will appear as red hot for the purpose of sterilization.

Figure 12: Contamination that can arise from poor aseptic technique when pouring agarose plates (top plate: sterile medium; bottom plates: contaminated media).

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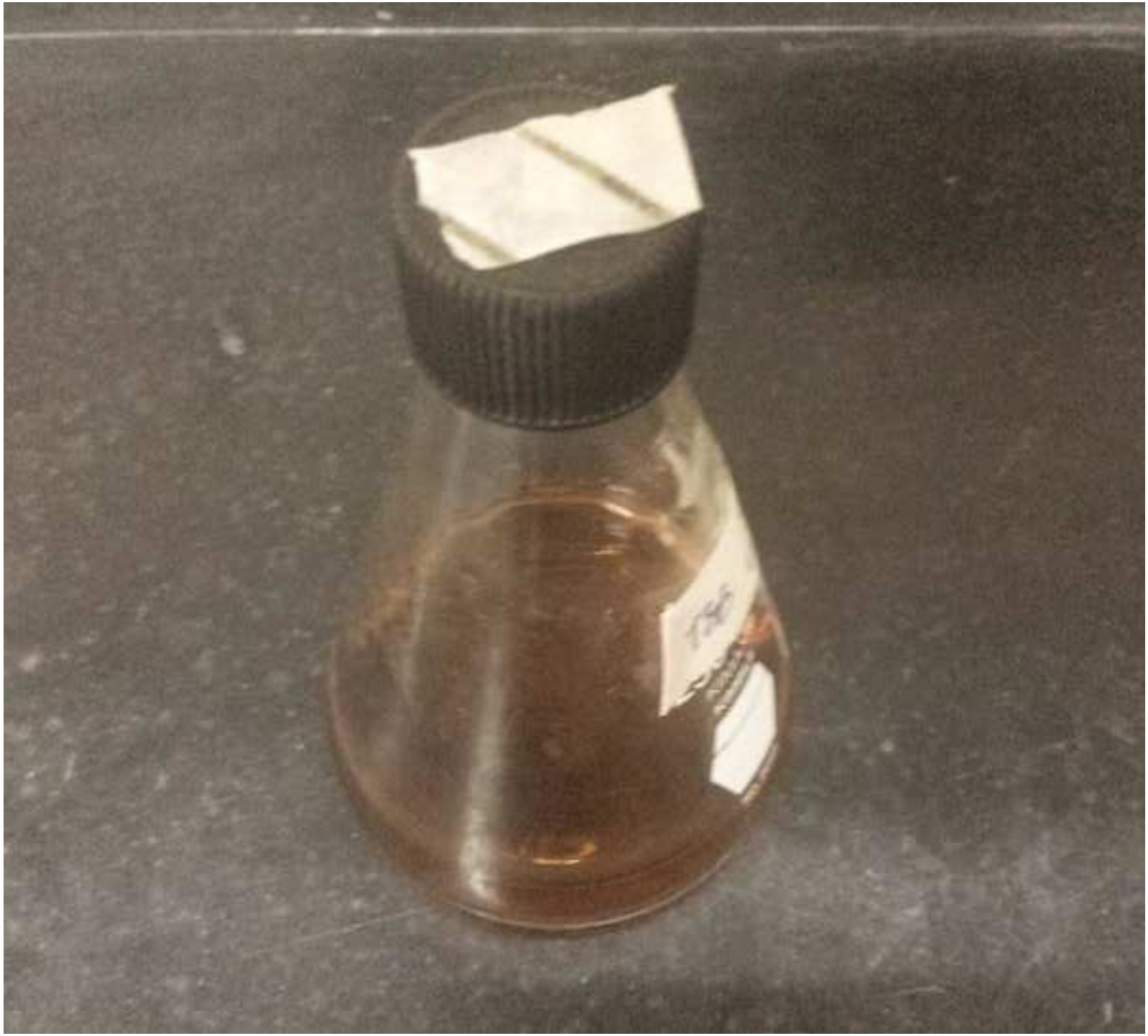

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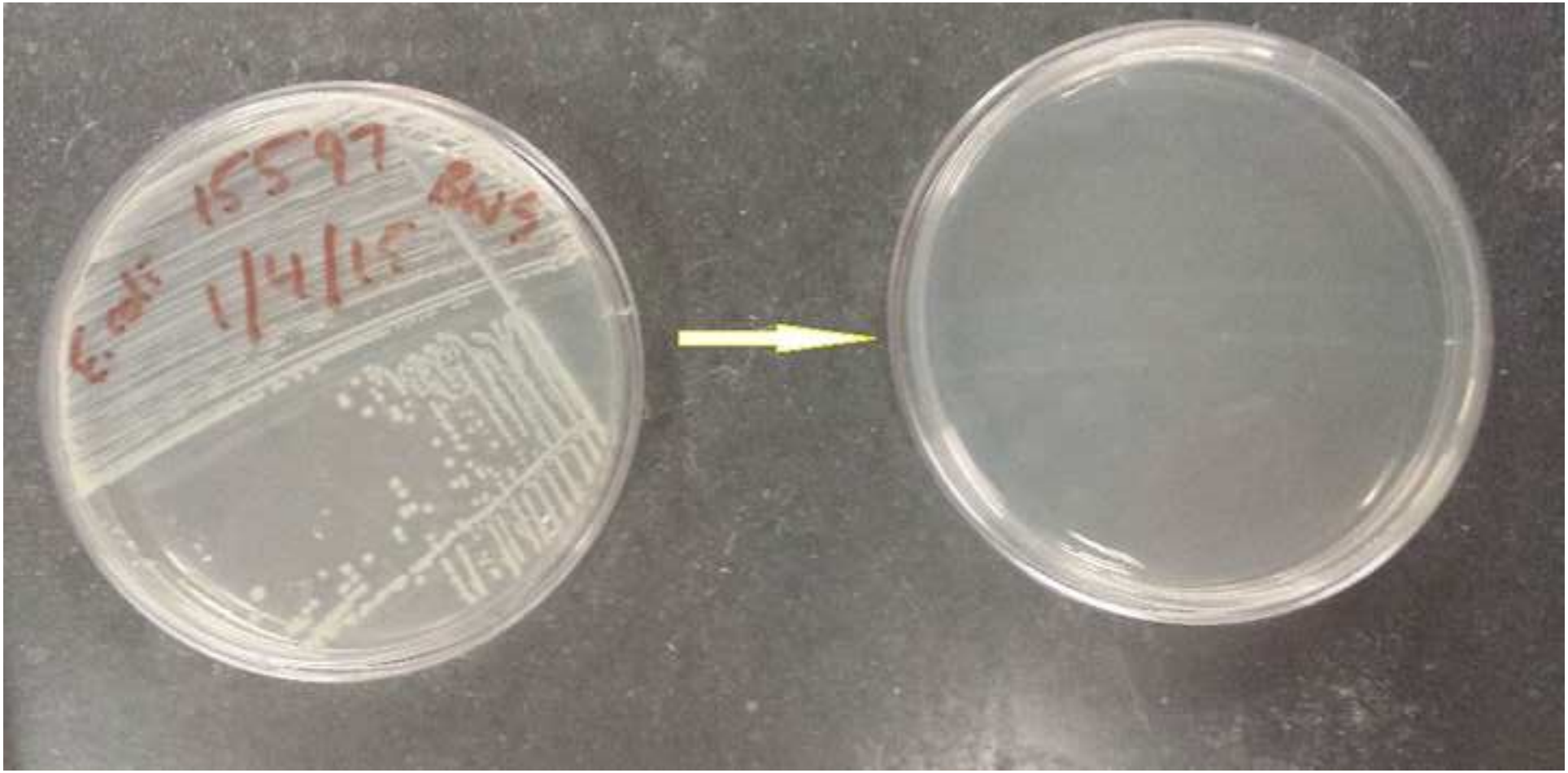

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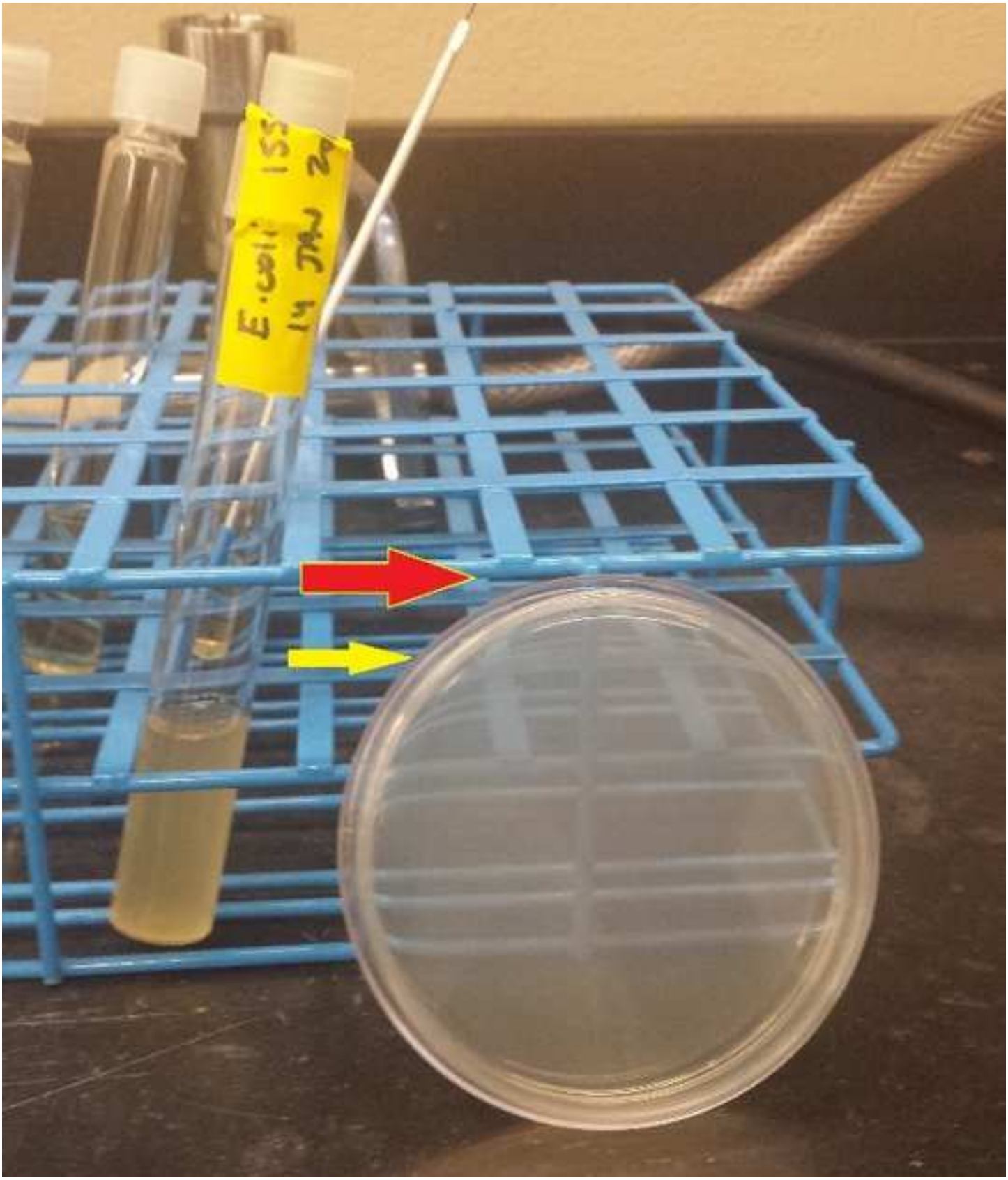

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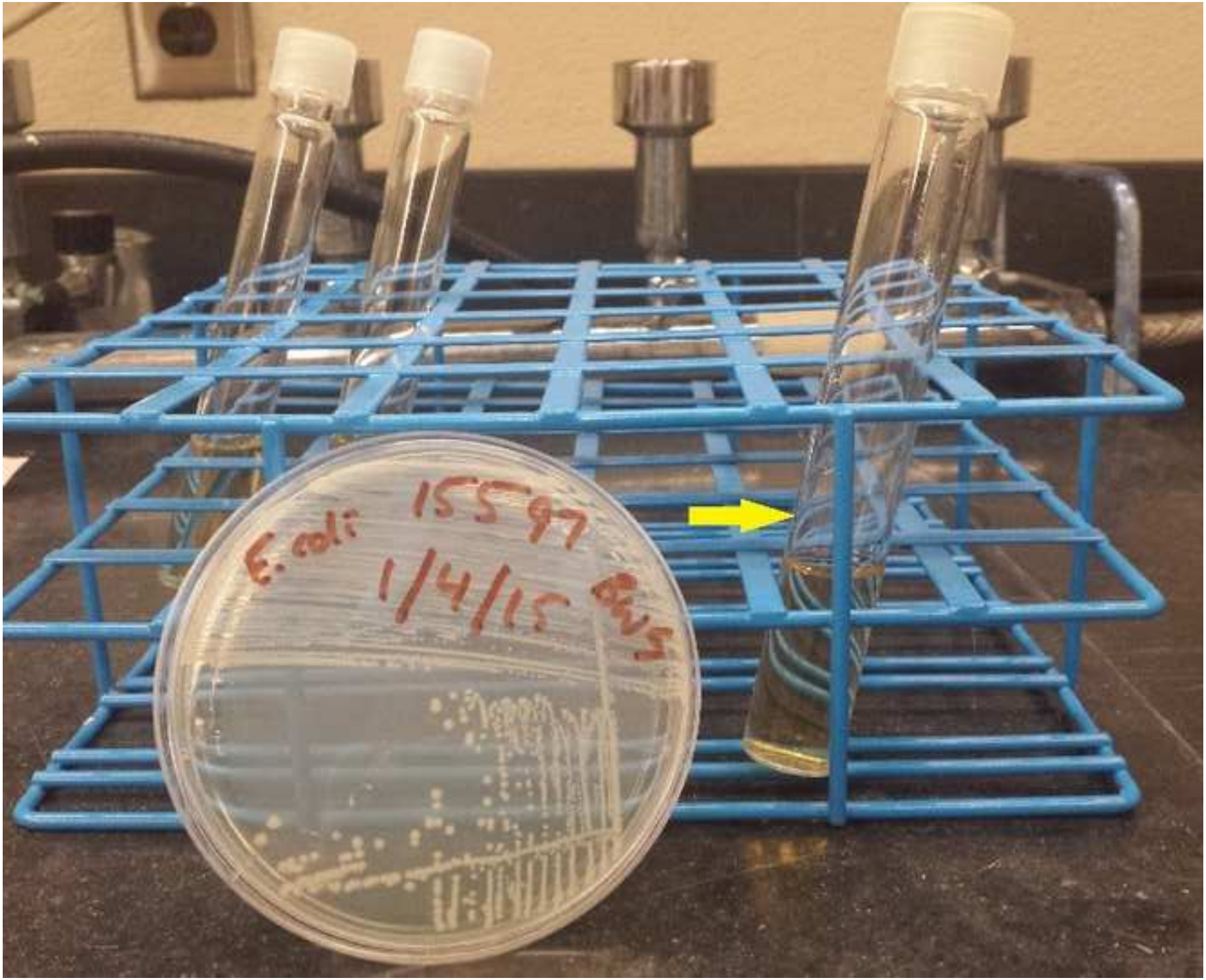

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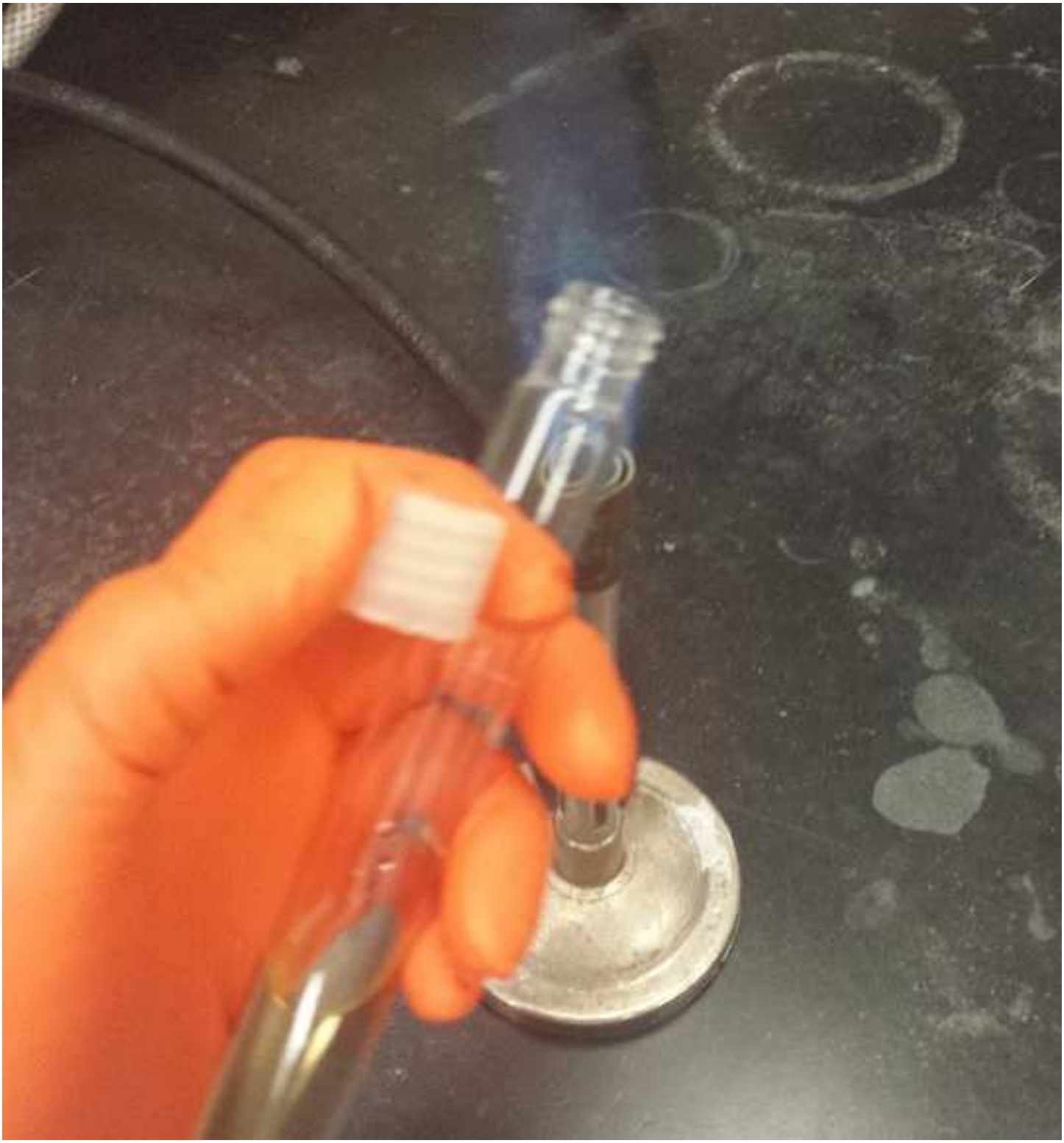

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