

## **OPS CHALLENGE LABORATORY EVENT 2012**

**Event:** Perform all steps of an E. coli membrane filtration analysis using Hach products following all method requirements and aseptic techniques as outlined in the Hach m-ColiBlue24 method and using Standard Methods 9222B (18,19, & 20<sup>th</sup> ed), sections 1,3,5a,5b,& 6 as a reference for the filtration method.

### **General NOTES:**

1. Team Captain tells the Lead judge they are ready to begin and the Lead judge says “START” to signal the beginning of the event. The Lead judge and one other judge will be the timekeepers.
2. The event is complete when all tasks have been completed and the Team Captain hands in all of the work sheets to the Lead judge and states that the team is finished.
3. To ensure a fair contest and to avoid challenges, judges will not speak to contestants while the event is being performed.
4. The Event Coordinator will settle disputes with input from the event judges.
5. All team members must participate in the event, but are not limited to performing only one task.
6. After the event, the Event Coordinator may explain to the Team Captain what was done incorrectly, but will NOT reveal penalty points or total score.
7. Team members may ask judges questions before the beginning of the event, but the judge may choose not to answer the question, depending on the type of question asked.

**ALL STEPS OF THE PROCEDURE MUST BE PERFORMED FROM MEMORY. NO BOOKS OR PRINTED MATERIALS ARE ALLOWED IN THE LABORATORY COMPETITION AREA.**

## **Materials Required:**

*Teams must supply their own safety glasses (required) & calculators.  
Prescription glasses may be worn in lieu of safety glasses.*

Sterile Petri dishes / Sterile pads  
Sterile membrane filters with grids  
Forceps (2 per table)  
Small beaker for alcohol (100 ml)  
Alcohol Burner  
Membrane filtration apparatus (suction flask doubled, tubing, and suction pump)  
    Pump  
    Suction Flasks (x2)  
    Tubing  
    Rubber Stopper (x1)  
    Glass Tube or 10 ml pipet (Inserted through rubber stopper)  
Incubator  
    Petri dish rack for incubator  
Pen type colony counter  
Colony tally counter  
Sharpies  
m-ColiBlue24 ampules  
Funnel assembly  
Hach buffer dilution water pillows  
1 ml sterile pipettes (individually wrapped)  
10 ml sterile pipettes (individually wrapped)  
Safety bulbs (x3)  
100 ml bottles with sterile water (x2)  
Sterile 25 ml cylinders (x2)  
Sterile 100 ml Cylinders (x2)  
Autoclave bags with ties  
Bin for autoclave bags (Dish washing type bin)  
Autoclave tape  
QC sample tubes (x2)  
Sample tube rack  
Squeeze bottle with isopropyl alcohol (x1)  
1 liter squeeze bottle for dilution water. (x1)  
1 liter sterile water bottles (x2)  
Autoclave wrap  
Bottle of dilute “bleach” solution  
Paper towels  
Clippers (for opening pillows)  
Bottle containing sample (x1)

## **2 Minute Set Up:**

Prior to the start of the event, each team will be allowed 2 minutes to set up table items as they wish. Team members will not be allowed to move the incubator or the vacuum pump. Items inside of the taped off area must remain in the taped off area. The contents of the incubator may be verified by opening the door only. Do not remove samples from the incubator. No items (pipettes, bottles, filters) may be opened. Once the 2 minute set up has been completed, the teams will exit the area between the tables and the judges will place the required bench sheets face down on the tables. All teams will then start the event inside of the tables at the workstations.

## **Start:**

Anyone involved with the set up, preparation of samples and plates, funnel assembly, squeeze bottles etc... must clean hands once before touching anything by rubbing hands with isopropyl alcohol from the isopropyl squeeze bottle. **The team member wiping the designated work area does not have to clean their hands before wiping the designated work area, but must clean their hands *after* cleaning the designated work area unless they are only reading plates, calculating, or prepping for autoclaving.** Hands do not have to be cleaned for reading of plates, calculation of plates, or preparation for autoclaving. These items do not require aseptic technique and are to be done outside of the designated work area. All other work, unless specified, is to be done inside of the work area.

The vacuum pump is to be turned on ONCE at the beginning of the event (step 2 of "Blank Preparation") and off (Section XIII) at the end of the event. Use the knob on the pump to control the vacuum during the event. Turning this knob applies and releases the vacuum. There is no designated pump pressure for the event.

### **I. Preparing Petri Dishes**

Once the Team Captain signals the Start of the event, team members will clean their hands and then clean the designated work area (15" x 48", outlined with tape) by squirting a small amount of the dilute bleach solution onto the work area and then wiping area dry.

All dishes must be labeled legibly on the underside using a sharpie marker provided. Dish labels must be complete (no abbreviations) as outlined below. This must be done to all Petri dishes before any of the Petri dish lids are removed. Dishes may be labeled outside of the taped work area.

1. Label one dish as “**positive QC**”.
2. Label one dish as “**negative QC**”
3. Label one dish as “**Effluent 100 mL**”
4. Label one dish as “**Effluent 10 mL**”
5. Label one dish as “**Effluent 1 mL**”
6. Label one dish “**Blank**”

## **II. Use sterilized forceps to place a sterile absorbent pad in each Petri dish and replace lid.**

1. To sterilize forceps, dip them in the beaker labeled “alcohol” and hold over the alcohol burner for a count of two (one one-thousand, two one-thousand). **FOR SAFETY, BURNERS WILL NOT ACTUALLY BE Lit.**
2. Using the sterilized forceps, pick up an absorbent pad at the edge and place in a Petri dish.
3. Repeat steps 1 & 2 above for each dish.
4. Remove the lid immediately before placement of the pad, and replace the lid immediately after placing the pad. To minimize contamination, do not place the removed lid on the table.

## **III. Add m-ColiBlue24 media to each dish.**

1. Invert m-ColiBlue24 ampule 3 times (count 1, 2, 3), remove cap and pour 1 ampule evenly over the absorbent pad in each dish. **For mixing purposes, invert means to turn bottle/ampule upside down, and then return to the upright position. This motion counts as 1 inversion.**
2. Replace the Petri dish lid immediately after adding the ampule. To minimize contamination, do not place the removed lid on the table.

## **IV. Set up the membrane filter and funnel.**

1. Unwrap sterile funnel apparatus. **DO NOT TOUCH THE INSIDE OF THE FUNNEL OR THE BOTTOM FILTER SEAT.** These areas must remain “sterile”.
2. Place the bottom part of the funnel into the suction flask.
3. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).
4. Un-wrap a sterile filter. Avoid touching it with your hands.

5. Use the sterile forceps to grasp the filter at an edge, and lay the filter centered on the filter seat GRID SIDE UP.
6. Attach top part of funnel to bottom.

## V. Preparation of sterile water.

1. Prepare buffer solution by inverting each pillow 3 times (count 1, 2, 3). Add 1 potassium phosphate pillow & 1 magnesium chloride pillow to 1 liter sterile dilution water.
2. Cap bottle and repeat step one with a second 1 liter bottle of sterile water so that there are two liters prepared dilution water when completed. At this point the bottles with dilution water are normally autoclaved. Do to time constraints, we will pretend that this was done and henceforth treat the bottles as if they have been autoclaved.
3. Un-wrap sterilized 1 liter squeeze bottle. Invert 1 bottle of the “autoclaved” dilution water bottle 3 times (count 1, 2, 3) to mix. Without touching the lip of the bottle, the inside of the lid, or any part that goes into the squeeze bottle, aseptically pour sterile dilution water into the squeeze bottle. Replace lid on sterile water bottle, and attach lid of squeeze bottle. Do not allow anything to touch the nozzle tip of the squeeze bottle or the attached tube. This part may be held in your hand or set down on the inner surface of the squeeze bottle wrapper.

## VI. Blank preparation

1. Using sterile water from the squeeze bottle, add a small amount of sterile water to funnel.
2. **Turn vacuum pump on.** Using the valve on the pump to apply and release pressure, apply vacuum to funnel assembly. (This is where you check to see if the assembly is “tight” and that no water is seeping out between the top and bottom part of the assembly). Once the dilution water has passed through, release the vacuum.
3. For the dish labeled “Blank”: Mix a 1 liter bottle marked “Sterile Dilution Water” by inverting 3 times (count 1, 2, 3).
4. Using a sterile 100 mL cylinder, aseptically measure out 100 mLs of sterile water from the dilution water bottle and pour into the filter funnel.
5. Apply vacuum. Once water has passed through the filter, release the vacuum.
6. Remove top of filter assembly. Place on inside surface of the original sterile filter wrapper.
7. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).

8. Grasp the filter on the edge. Remove the lid to the dish labeled “Blank”. Place the filter into the dish by inserting the filter against the inside edge of the dish against the pad, grid side up and use a rolling motion to exclude any air from between the filter and pad until the filter is laying flat. Make sure that the filter touches the entire pad. Replace Petri dish lid.
9. Invert Petri dish.

**VII. Sample preparation.** Shake the sample bottle 20 times prior to each filtration. A shake consists of one full down and up motion of the bottle, or one full side to side motion, or one full inversion and back motion.

1. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).
2. Use the sterile forceps to grasp a new filter at the edge, and lay the filter centered on the filter seat **GRID SIDE UP**.
3. Attach top part of funnel to bottom.
4. Using sterile water from the squeeze bottle, add a small amount of sterile water to funnel.
5. Apply vacuum to seat filter. Once the dilution water has passed through, release the vacuum.
6. For the dish labeled “1 mL”: Mix the 1 liter bottle marked “Sterile Dilution Water” by inverting 3 times (count 1, 2, 3).
7. Using a sterile 10 mL pipette, add 10 mLs of the dilution water from the dilution water bottle to the filter funnel.
8. Shake sample 20 times. Using a sterile 1 mL pipette, add 1 mL of sample to the dilution water in funnel.
9. Apply vacuum to funnel only after sample has been added to the dilution water.
10. Once sample has passed through filter, Use a sterile cylinder to add 20 mLs of sterile dilution water to the funnel.
11. Once the 20 mLs has passed through the filter, add another 20 mLs of dilution water. After that passes, add a third volume of 20 mLs of dilution water to the filter.
12. Once the third volume has passed, release the vacuum.

**VIII. Transfer the filter to a sterile Petri dish.**

1. Remove top of filter assembly. Place on inside surface of the original sterile filter wrapper.
2. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).

3. Grasp the filter on the edge. Remove the lid to the dish labeled "1 mL". Place the filter into the dish by inserting the filter against the inside edge of the dish against the pad, grid side up and use a rolling motion to exclude any air from between the filter and pad until the filter is laying flat. Make sure that the filter touches the entire pad. Replace Petri dish lid.
4. Invert Petri dish.

### **IX. Rinse filter assembly.**

1. Replace the funnel on the filter seat.
2. Apply vacuum and using the squeeze bottle rinse the inside walls of the filter funnel for a count of 3 (count one one-thousand, two one-thousand, three one-thousand).
3. Release the vacuum.

### **X. Sections VII, VIII are repeated for the 10 mL sample:**

1. Sterilize forceps, dip them in the beaker labeled "alcohol" and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).
2. Use the sterile forceps to grasp a new filter at the edge, and lay the filter centered on the filter seat GRID SIDE UP.
3. Attach top part of funnel to bottom.
4. Using sterile water from the squeeze bottle, add a small amount of sterile water to funnel.
5. Apply vacuum to seat filter. Once the dilution water has passed through, release the vacuum.
6. For the dish labeled "10 mL": Mix the 1 liter bottle marked "Sterile Dilution Water" by inverting 3 times (count 1, 2, 3).
7. Using a sterile 10 mL pipette, add 10 mLs of the dilution water from the dilution water bottle to the filter funnel.
8. Shake sample 20 times. Using a sterile 10 mL pipette, add 10 mL of sample to the dilution water in funnel.
9. Apply vacuum to funnel only after sample has been added to the dilution water.
10. Once the sample has passed through the filter, use a sterile cylinder to add 20 mLs of sterile dilution water to the funnel.
11. Once the 20 mLs has passed through the filter, add another 20 mLs of dilution water. After that passes, add a third volume of 20 mLs of dilution water to the filter.
12. Once the third volume has passed, release the vacuum.
13. Remove top of filter assembly. Place on inside surface of the original sterile filter wrapper.

14. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).
15. Grasp the filter on the edge. Remove the lid to the dish labeled “10 mL”. Place the filter into the dish by inserting the filter against the inside edge of the dish against the pad, grid side up and use a rolling motion to exclude any air from between the filter and pad until the filter is laying flat. Make sure that the filter touches the entire pad. Replace Petri dish lid.
16. Invert Petri dish.

## **XI. Preparation of the 100 mL sample.**

Section IX and section VII 1-5 are repeated:

1. Replace the funnel on the filter seat.
2. Apply vacuum and using the squeeze bottle rinse the inside walls of the filter funnel for a count of 3 (count one one-thousand, two one-thousand, three one-thousand). Release the vacuum.
3. Sterilize forceps, dip them in the beaker labeled “alcohol” and hold over alcohol burner for a count of two (one one-thousand, two one-thousand).
4. Use the sterile forceps to grasp a new filter at the edge, and lay the filter centered on the filter seat **GRID SIDE UP**.
5. Attach top part of funnel to bottom.
6. Using sterile water from the squeeze bottle, add a small amount of sterile water to funnel.
7. Apply vacuum to seat filter. Once the dilution water has passed through, release the vacuum.
8. For the dish labeled “100 mL”, shake sample 20 times and pour out 100 mLs of sample into an unused sterile cylinder.
9. Pour the 100 mLs of sample into the filter assembly.
10. Apply vacuum to funnel only after sample has been added to the filter assembly. Rinse the graduated cylinder 3x with sterile water and pour through filter.
11. Once sample has passed through filter, Use the sterile cylinder to add 20 mLs of sterile dilution water to the funnel.
12. Once the 20 mLs has passed through the filter, add another 20 mLs of dilution water. After that passes, add a third volume of 20 mLs of dilution water to the filter.
13. Once the third volume has passed, release the vacuum.

## **XII. Follow section VIII to place filter in dish labeled 100 mL.**

**XIII. Repeat section XI** for 100 mLs of the *E. coli* (positive) QC which can be poured straight from the bottle into the funnel. See preparation of QC samples below.

Repeat section XI for 100 mLs of the *P. aeruginosa* (negative) QC which can be poured straight from the bottle into the funnel. *See preparation of QC samples below.*

**Once all samples have been filtered, turn the vacuum pump completely off.**

## **XIV. Preparation of QC samples.**

1. Label the 2 small bottles of sterile water provided. Label one as “*E. coli*” (positive QC), and the other “*P. aeruginosa*” (negative QC).
2. Remove rehydrated bacteria tubes from incubator.
3. Invert tubes 3 times (count 1, 2, 3).
4. Open tube and aseptically transfer rehydrated *E. coli* to bottle marked *E. coli*.
5. Re-cap bottle and invert bottle 3 times (count 1, 2, 3).
6. Open tube and aseptically transfer rehydrated *P. aeruginosa* to bottle marked *P. aeruginosa*.
7. Re-cap bottle and invert bottle 3 times (count 1, 2, 3).

### **Once all samples have been filtered:**

Place the inverted Petri dishes in the incubator. Fill in the *Blank* Bench Sheet. Include Sample/Set up Date, analyst initials, time in incubator, sample I.D., sample volumes, and appropriate incubator temperature. There will be a clock positioned on the table next to the incubator to record times. This is done outside of the work area.

## **XV. Reading plates**

1. Remove the 6 samples from the incubator which have been incubating for 24 hours.
2. On the *Preset* bench sheet, record sample read date, analyst (readers) initials, time out, and incubator temperature.

3. Count the number of blue colonies on each plate and record the count in the appropriate box on the bench sheet that includes the previous days set up information.
4. Calculate the colony count for each sample according to section XVI and record in the appropriate box on the bench sheet.
5. Record the final calculation in the Colonies/100 mls box.
6. Record the results for the two QC samples and the blank as either “Positive Growth” or “Negative Growth” on the bench sheet.
7. Place dishes in an autoclave bag, seal bag, and mark with a piece of autoclave tape. Place in the “to be autoclaved” bin.

**Dispose of all trash (used pipettes, wrappers, empty dilution water pillows) in the container marked “Trash” which will be located on the table near the vacuum pump.**

#### **XVI. Calculations:**

1. From the 3 sample filters (1, 10, & 100 ml), select filters with 20-80 **BLUE** colonies. Total number of blue colonies on the plate cannot be greater than 200. *If total number of **BLUE** colonies is greater than 200, record as TNTC (Too Numerous to Count) in the appropriate box.*
2. If only one filter meets the requirement of 20-80 **BLUE** colonies, use the following equation:

$$\frac{\text{\# of BLUE colonies}}{\text{Total Volume of Sample (s)}} \times 100 = \text{E coli / 100 mL}$$

3. If more than one filter meets the requirements of 20-80 **BLUE** colonies, sum the number of colonies and sample volumes for the plates which meet the 20-80 requirement, and use the above equation. **OMIT ANY PLATES OUTSIDE OF THE 20-80 RANGE.**

**XVII.** A 10 question multiple choice quiz will be included. The quiz may cover E. coli holding times, incubation temperatures, and short definitions related to microbiology work.

