

# LABORATORY PRACTICAL AND SCHEDULE

## MICROBIOLOGICAL SAMPLING AND ANALYSIS

April 11, 2008

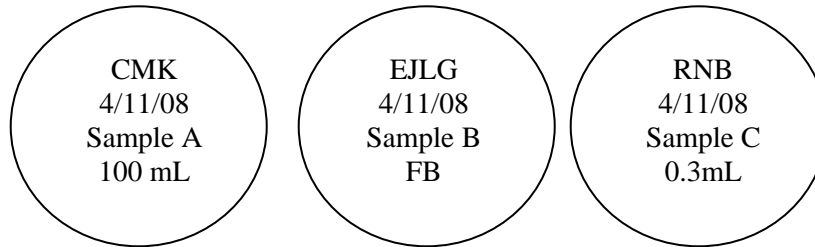
**Experimental Setup:** The class is divided into teams of two for this laboratory exercise. Students will plate water samples using the membrane filtration technique (modified mTEC and mEI methods) and analyze water samples using enzyme substrate tests (Colilert and Enterolert Quantitray-2000 methods).

**Table 1.** Outline of laboratory activities

Time	Activity	Teams
8:00 – 9:45	Presentations in lecture room	
9:45 – 10:30 am	<b>Demonstration</b> —membrane filtration (in lecture room), tour of lab, equipment, and QA/QC	Split into three groups
10:30 – 10:45	<b>Break</b>	
10:45 – 11:45 am	<p><b>Membrane filtration method</b> in main lab</p> <ul style="list-style-type: none"> <li>• Each student prepares a 1:100 sample dilution 1 mL of sample into 99 mL of buffer water</li> <li>• Students membrane filter sample volumes One student plates on mEI, the other on mod. mTEC</li> <li>• Students incubate plates</li> <li>• Clean up</li> <li>• Students count demonstration plates and calculate results</li> </ul>	Group A (2 people per team)
	<p><b>Enzyme substrate test</b> in side lab</p> <ul style="list-style-type: none"> <li>• Demonstration of sediment sample processing and analysis</li> <li>• Each student performs enzyme substrate test One student uses Colilert-18, the other uses Enterolert</li> <li>• Students incubate trays</li> <li>• Clean up</li> <li>• Students count demonstration trays and calculate results</li> </ul>	Group B (2 people per team)
11:45 – 12:30	<b>Lunch</b> (provided) in lecture room	
12:30 – 1:30 pm	<b>Switch activities</b>	Membrane filtration: Group B Sediment analysis: Group A
1:30 – 3 :45	Return to lecture room for presentations	
3:45 – 4:30 pm	<b>Demonstration</b> —coliphage, qPCR, IMS/ATP rapid method	Split into three groups

**MEMBRANE FILTRATION HANDS-ON ACTIVITY**

1. **Obtain supplies.** Each team receives the following:
  - Modified mTEC—6 plates
  - mEI—6 plates
  - Sample—1 bottle
  - Other supplies can be found at your station
2. **Put on gloves and wipe down your work area with sanitizing spray.**
3. **Label your plates.** On the bottom of the plate, record the analyst’s initials, date, sample identifier, and the sample volume as shown in the following examples.



Each team will need plates as listed on table 2. One team member will plate on modified mTEC and the other member will plate on mEI.

**Table 2:** Membrane filtration method samples—volumes and order of filtration

Order	Volume (mL)	Source
1	50 FB (filter blank)	buffer water
2	0.3	30 mL of 1:100 dilution
3	1	sample
4	3	sample
5	10	sample
6	50 PB (procedure blank)	buffer water

4. **FILL OUT DATA SHEETS FOR SAMPLES!** The needed information includes sample identifier, collection date and time, analyst’s initials, media lot number (date plates poured), filter lot number, and buffer water lot number.
5. Each student will **prepare one dilution** of the sample. Label a 99 mL bottle of phosphate buffer. Transfer 1 mL of the sample to the labeled dilution bottle to prepare a 1:100 dilution.
6. **Assemble membrane filtration apparatus.** Use the manifolds with filter funnels attached to a peristaltic or vacuum pump.
7. Each person filters the sample as described in table 2. In each team, one student filters the sample on modified mTEC (6 plates) and the other student filters the sample on mEI (6 plates). Each team member uses a different filter funnel. The order of filtering is as follows:
  - a. First, a filter blank is filtered. For the filter blank, pour approximately 50-mL of buffer water into the funnel, being sure to rinse the sides of the funnel as you pour. This tests the sterility of the buffer, filter funnel, and filter.

- b. Then, filter the sample volumes starting with the smallest and progressing to the largest sample volume (see table 2). Use a graduated cylinder to measure volumes greater than 30 mL. Use a 10-mL pipet to measure volumes from 2 to 10 mL. Use a 1-mL pipet to measure volumes of 1 mL. **FOR VOLUMES LESS THAN 10 ML, PUT ABOUT 10 ML OF BUFFER INTO THE FUNNEL BEFORE ADDING THE SAMPLE.** This ensures even distribution of the sample across the membrane. For each volume, rinse the filter funnel with phosphate buffer after filtering the sample and before removing the filter.
- c. Filter a procedure blank. For the procedure blank, pour approximately 50 mL of phosphate buffer into the funnel, being sure to rinse the sides of the funnel as you pour. This tests the analyst's rinsing procedure to ensure it is adequately rinsing all of the bacteria onto the filter.
8. **Incubate the sample plates.** Incubate the plates (upside down) for the prescribed period of time at the indicated temperature of the test procedure. *E. coli* on modified mTEC is incubated at 35°C for 2 hours and then at 44.5 °C for 20-22 hours. The instructors will move the *E. coli* plates for you after two hours. enterococci on mEI agar are incubated at 41°C. Record times on appropriate forms
9. **Clean up.** Place all dirty dishes on the cart marked "Dirty Dishes", and all of the sample bottles need to be placed on the "Sample" cart. Use the sanitizing spray to clean your work area.
10. **Paperwork.** Ensure all data sheets are complete and accurate
11. **Count demonstration plates.** Each team will be given a set of demonstration plates (plated yesterday so that you have plates to count today). Count the colonies and calculate results.

#### Calculating concentraions rules

1. **One filter within the ideal range**

$$CFU / 100mL = \frac{\# \text{ colonies} \times 100}{\text{volume plated}}$$

2. **Two or more filters within range**

$$CFU / 100mL = \frac{\text{SUM of counts} \times 100}{\text{SUM of sample volumes filtered}}$$

3. **All filters have counts < and (or) > the ideal range**

Use the same formula as in example 2

4. **No filters with colonies**

Assume 1 colony at largest volume plated

- Data are qualified with "<"

$$CFU / 100mL = \frac{1 \text{ colony} \times 100}{\text{Largest volume plated}}$$

5. **All filters have colonies too numerous to count (TNTC)**

Assume a maximum count (60, 80, or 100) at the smallest volume plated. Refer to the Ideal ranges of colony counts chart to determine maximum count.

- Data are qualified with ">"

$$CFU / 100mL = \frac{60, 80, \text{ or } 100 \text{ colonies} \times 100}{\text{Smallest volume plated}}$$

**ENZYME SUBSTRATE ANALYSIS HANDS-ON ACTIVITY**

1. **Obtain supplies.** Each team receives the following (some items may be located at your station):
  - Quantitray 2000—2
  - Colilert media packet—1
  - Enterolert media packet—1
  - 125 mL sterile bottle—2
  - Graduated cylinder—2
  - Sample—1 bottle
2. **Put on gloves and wipe down your work area with sanitizing spray.**
3. **Label your Quantitrays.** On the back of each tray, record the analyst’s initials, date, sample identifier, and media type as shown in the following examples. One analyst will process using Colilert, and the other will process using Enterolert

CMK	AMGB	RNB
4/11/08	4/11/08	4/11/08
Sample E	Sample F	Sample G
Colilert	Enterolert	Colilert

3. **FILL OUT DATA SHEETS FOR SAMPLES!** The needed information includes sample identifier, collection date and time, analyst’s initials, media lot number, and Quantitray lot number.
4. Each person will process a water sample as follows:
  - a. Add exactly 100mL of your sample (shake well before pouring) to the 125mL sterile bottle
  - b. Pour the contents of the media packet into the 125mL sterile bottle and shake well until dissolved. Try to avoid getting your sample foamy.
  - c. Without touching the inside of the tray, pour the contents of the 125mL bottle into the tray.
  - d. Run the tray through the sealer (use the orange tray holder).
  - e. Incubate the tray at 41°C for 24-28 hours for Enterolert or 18-22 hours for Colilert-18.
  - f. Record the time the tray went into the incubator on your data sheet.
5. **Clean up.** Place all dirty dishes on the cart marked “Dirty Dishes”, and all of the sample bottles need to be placed on the “Sample” cart. Use the sanitizing spray to clean your work area.
6. **Paperwork.** Ensure all data sheets are complete and accurate.
7. **Count demonstration trays.** Each team will be given a set of demonstration trays (analyzed yesterday so that you have trays to count today). Two demonstration trays will be water samples, and two trays will be sediment samples. Count the number of large wells (the extra-large well along the top of the tray is counted as a large well) and the number of small wells that are positive. Use the provided MPN tables to obtain concentrations. Be sure to finish the sediment sample calculations using the proportional dry weight of the sediment and the sediment dilution factor (calculation shown on the the data sheet).
  - a. **For Colilert:** Yellow wells are positive for total coliforms. Fluorescent under UV light are positive for *E. coli*.
  - b. **For Enterolert:** Fluorescent blue wells under UV light are positive for enterococci.