

# D-Lab

## Equipment:

- 100 ml whirlpak collection bags (~2 x number of tests)
  - Use bags with sodium thio-sulphate tablets if water may have been chlorinated
- sharpie pens (3)
  
- filters (~1.2 x number of tests)
- petri dishes (~1.5 x number of tests)
- large syringe (2)
- luer fittings (2)
- tubing
- mesh screen (2)
- aluminum washer (2)
- rubber washer (2)
- sterile inserts (1.2 x number of tests)
- bottle
- spout (2)
- m-coli blue (should be kept refrigerated, if possible) (~1.1 x number of tests)
  
- candles (3)
- matches
- tweezers
- alcohol
- hand cleanser
- wipes
- tape
  
- PCM balls (1000)
- mesh bag
- insulated container
- thermometer

## Collecting water samples.

- If you are testing water that might contain traces of residual chlorine, be sure to use collection bags with sodium thio-sulphate tablets, this will neutralize the chlorine and allow your samples to grow properly.
- If testing water from a tap, let the tap run for several seconds before collecting the sample. Fill sample to the line, then whirl it and seal the tabs.
- If collecting from a river or stream, have the opening of the bag face upstream. Fill sample to the line, then whirl it and seal the tabs.

- If collecting from a well, try to lower the sample bag into the well at an angle so that it will fill easily. Pour out any excess then whirl the bag and seal the tabs.
- If you use an auxiliary collection vessel, make sure you rinse it at least three times with water from the source that you are testing before taking the sample.
- Mark the date, time and location of each sample
- Take at least three samples from each site.
- Keep samples cool until testing.
- Do not store samples for more than 6 hours before testing

### Preparing the Incubator

- Put about 1000 PCM balls in a mesh bag.
- Place the bag in a large pot of water and heat it until the phase change material in the balls has melted.
- Take the bag out of the pot and put the balls in the insulated container.
- Put the thermometer in the incubator.
- When the temperature has reached 38° C, put in the samples and seal the top.

### Testing

- Mark the sample numbers on the petri dishes before you get started.
- Make sure your work area is set up so that everything you need is at hand.
- It's important to keep sterile technique, light a candle, fill a petri dish with alcohol and make sure the hand cleanser and wipes are easily available. You should also have a bucket or other container for the waste water and another one for trash.
- Always start with a blank to verify that the equipment is not contaminated.
- Place the sterile insert in the bottle.
- Pour the water into the insert.
- Sterilize the tweezers by passing them through the flame.
- Sterilize the metal washer
- Place the metal washer on the rim of the insert.
- Remove a filter from the packet with the tweezers.
- Place the filter paper on the top of the bottle, with the squares facing down.
- Dip the mesh screen in alcohol then run it through the flame till all the alcohol burns away.
- Place the mesh on top of the filter.
- Screw on the lid, being careful to keep the filter and mesh properly aligned. It is very important that a good seal is made, so screw the lid on tightly.
- Attach the syringe to the end of the tubing and pull the water through the filter. If excessive air comes in through the edges of the lid, then the seal is not adequate and you should try to tighten the lid.
- Remove the syringe from the end of the tubing and empty it into the bucket.
- Unscrew the lid.
- Sterilize the tweezers by running them through the flame.
- Remove the mesh.

- Remove the filter and place it in a petri dish, with the squares facing up.
- Remove the lid from an ampule of m-coli blue and squeeze it onto the filter paper.
- Close the petri dish. When you have a stack of about 6 - 8 petri dishes, tape them together and put them into the incubator.
- Always finish with a blank to be sure that the equipment is not contaminated. If you are doing more than twenty samples at a time, run a blank midway through to verify the sterility of the test equipment.

### Getting results

- After 24 hours, take the petri dishes out of the incubator.
- Verify that the temperature was within an acceptable range (34 - 38° C)
- Count the number of colonies that were formed and record it on your sheet. *E. coli* are the blue, total coliforms is the sum of the red and blue,
- Truly safe water has no bacteria; less than 10 is considered okay; less than 50 is acceptable, but not good.

### Disposal

- Pour bleach on the samples to kill the bacteria
- Dispose of the petri dishes

