

Water & a Healthy Environment

6. COLIFORM BACTERIA TEST



An indicator of health risk



What do we need?

- Nearby water supply
- Gloves (in pack)
- Clean cup/bottle
- Long plastic droppers (in pack)
- 6 bijou bottles (in pack)
- *Colilert* ampoule (in pack) (± 60 days shelf-life- it must be stored in a *cool place!*)
- Spirit burner (in pack)
- Methylated spirits
- Matches
- Zip-lock bags (in pack)
- Jik (in pack)
- Instructions for low-cost incubation chamber (in pack)
- Gas/electric stove
- Small cooking pot
- Aluminum foil

How do we use the kit?

Here, we are reflecting critically on what we have found out so far about the environmental risks. We are now probing more deeply to get a better sense of what is happening in our water supply. This *semi-quantitative test* can help us to get a sense of *how serious* the water pollution is.

Questions we are asking, and how we plan to take our investigation further:

In the *Colilert* test, coliform bacteria serve as an "indicator species". One kind in particular, *E. coli*, lives in faeces (it plays an important role in food digestion - in humans and other warm-blooded animals). If *E. coli* is found in the water, it warns us that the water has been contaminated by faeces, which means there may also be a risk of disease-causing bacteria, e.g. typhoid, cholera, and hepatitis.

Coliform bacteria grow and produce enzymes when *Colilert* is added to the sample and kept warm (close to body temperature). The enzymes react with the reagent in the *Colilert* - this leads to a colour reaction (Yellow = coliforms present. Bright yellow = *E. coli* present). If the 'control' bottle (sterile water only) also changed colour, what would this tell you about the hygiene of your methods?

What have we found out by doing this *semi-quantitative* test? We know that by diluting the water sample increasingly (dilution series), any bacteria in the water would become fewer and fewer. So if we get a positive result in even the most diluted sample (1/1000), then there must be *many* coliform bacteria in the undiluted sample!

If there was *no* colour change in any of the sample bottles, we could assume that there were no coliforms in the water, and that it is safe to drink. We should not forget that other kinds of micro-organisms may still be present. Also, not all micro-organisms are harmful.

What can our *Colilert* test tell us? What else do we need to find out? Is raw sewage contaminating the water? Are there large numbers of livestock in the area, or storm water runoff? Are there over-used pit toilets nearby? How could we work out if the water is safe to swim in? For farm animals to drink? To irrigate plants?

If we wish to compare our test with a seriously polluted sample, what could we test? Try testing a bowl of water in which everyone washed their hands. What else could we test in public toilets?

Where do we take our investigation from here? How can we bring our ideas into action?

Colilert test: is the water contaminated with Coliform bacteria?

Preparation:

- Wash hands well. Prepare a clean working surface: wipe with Jik and dry with clean paper towel.
- Sterilise all plastic droppers (wrapped in tin foil) and bijou bottles (with lids loosely on) by boiling *very* gently in a cooking pot for 5 minutes. Drain and leave to cool in a clean, dry place.
- Label the bijou bottles: '1', '1/10', '1/100', '1/1000', & 'C' (Control),
- Using a sterile plastic dropper, add **4½ml sterile boiled water** (which has cooled) to four of the bijou bottles: '1/10', '1/100', '1/1000', & 'C' (Control).

The water sample:

- Using a clean plastic container, collect a water sample from the water supply/river/stream/ pond.
- Using a clean plastic dropper, transfer a total of **5ml** of the water sample to bijou bottle '1' (**undiluted water sample**)

Dilution series (of the water sample):

- **Transfer ½ml** from bijou bottle '1', and add to bijou bottle '1/10' - this is now diluted ($\frac{1}{2}$ ml in 5 ml) 1 in 10 parts sterile water.
- **Transfer ½ml** from bottle '1/10' into '1/100'
- **Transfer ½ ml** from bottle '1/100, into '1/1000'
- **Discard ½ ml** from bottle '1/1000' so that it has the same amount as all the other bottles ($4\frac{1}{2}$ ml).

Adding the Colilert (reagent):

- Snap open the *Colilert* ampoule, and pour contents into a dry, sterile bijou bottle. The powder must remain dry - keep the lid closed!
- Light the spirit burner and sterilise the "measuring doppie"
- Add a flat measure of *Colilert*, using the sterile "measuring doppie", to bijou bottle 'C'. Shut lid.
- Repeat for each bijou bottle: '1/1000', then '1/100', '1/10', & '1'.

Incubation:

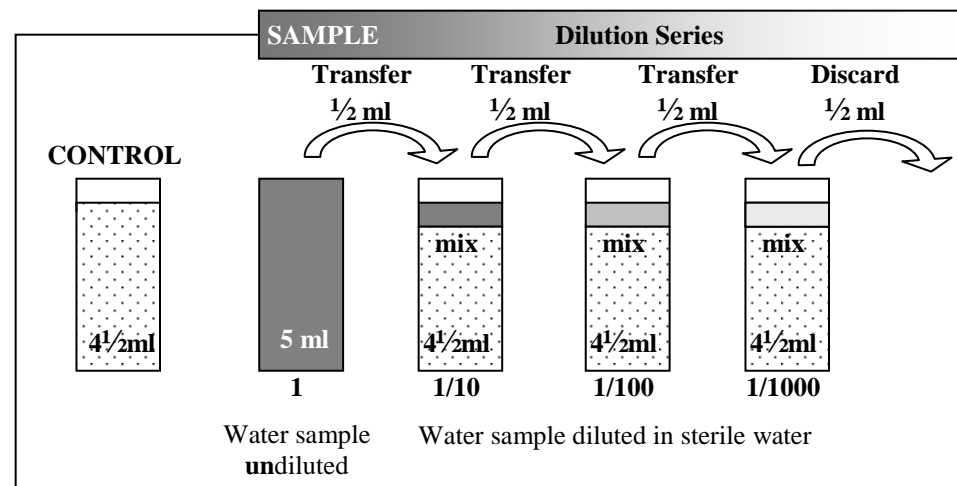
- Place the five bijou bottles inside zip-lock bags with a drop of Jik, and place inside a second zip-lock bag. This is a health safety precaution!
- Incubate the bottles in a dark, warm place ($\pm 37^{\circ}\text{C}$ - e.g. your pocket, or in a home-made incubator).

Reaction:

- Check if the colour changes after 18, 24, then 48 hours.
- How many, if any, of the bottles had a 'positive' result/colour change? (**Yellow** = *coliform* bacteria present. **Shiny yellow** = *E. coli* bacteria present)
- How soon did the colour change take place?
- Was there a difference in colour intensity between dilutions?

Safe disposal:

- Wearing protective gloves, empty the contents of the bottles into the toilet, and rinse with water.
- Soak bottles overnight in a Jik solution (1 part Jik in 10 parts water) **[is this strong enough to kill bacteria?]**



Interpreting the results....

6. COLIFORM BACTERIA TEST

Bijou bottles	1	1/10	1/100	1/1000	Interpretation	
Colour after 18 (or 48) hours	Clear	Clear	Clear	Clear	0 coliforms (cfu). No contamination. Safe to drink.	
	Yellow	Clear	Clear	Clear	0 - 10 000 cfu/100 ml	Some contamination. Not safe for humans to drink.
	Yellow	Yellow	Clear	Clear	10 000 - 100 000 cfu/100 ml	More contamination. Not safe to swim, fish or boat.
	Yellow	Yellow	Yellow	Clear	100 000 - 1 000 000 cfu/100 ml	Critical contamination. Not safe to irrigate crops.
	Yellow	Yellow	Yellow	Yellow	>1 000 000 cfu/100ml	Serious health hazard!
	<p>Yellow = coliform bacteria present. Shiny yellow = <i>E.coli</i> bacteria present</p>					

Your results:

Bijou bottles	1	1/10	1/100	1/1000	Control
Colour after 18 (or 48) hours					
Comments					