

How does a remote testing kit designed for the Indian subcontinent compare with standard water testing kits in detecting pathogenic microbes?

A way to remotely test pathogenic microbes in water in the Indian subcontinent.

Objective: Find an easy and accessible way to detect harmful microbes in water and assess its safety and purity.

Background research:

Clean water is not a privilege; it is a necessity. However, the availability of safe water has continuously declined, and individuals who lack access are increasingly vulnerable to numerous diseases. India faces major water-related health challenges due to poor sanitation and hygiene in rural areas and rapidly developing urban areas. A large percentage of the Indian population (around 51%) relies primarily on polluted or contaminated water sources like rivers, lakes, and even ponds. (UNICEF)

Various pathogens are present in water bodies such as *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, and *Mycobacterium tuberculosis*.

- *Escherichia coli* is a bacterium that attaches to host tissues and produces exotoxins. Accidental consumption may cause diarrhoea, and abdominal cramps, and can lead to renal damage. (2024, May 1). Cleveland Clinic)
- *Salmonella typhi* leads to typhoid characterized by protracted fever, exhaustion, and gastrointestinal problems. (World Health Organization: WHO. (2023, March 30)
- *Vibrio cholerae* is a bacterium that produces a toxin that leads to diarrheal disease and essential fluids are lost.
- *Mycobacterium tuberculosis* results in tuberculosis which primarily affects an individual's lungs.

According to the World Health Organisation, in 2022, 1.7 billion people's drinking sources were contaminated with faeces. Over 500,000 deaths were attributable to contaminated drinking water in 2022. (World Health Organization: WHO. (2023, September 13)

What spiked our interest?

During a recent day camp in the outskirts of Mumbai, by the end of the day, we had consumed all the water that we had carried along. A friend urgently needed to quench her thirst and was questioning the safety of drinking water from a nearby stream. Due to her parched state, she consumed the water which looked clean. After a few days, she started feeling weak and feverish. Following a medical consultation, she was diagnosed with typhoid fever. The doctors suggested that she got the infection from the untreated stream water, leading us to question the current water safety protocols in developing nations. This incident serves as a reminder of the importance of the availability of clean and safe drinking water. In response to this experience, we decided to base this science experiment on developing a portable device, which would help detect the harmful microbes in water. Our goal is to eliminate diseases or illnesses caused due to the consumption of contaminated water.

As the current water testing kits in the market are universal and not tailored to the microbes that exist in specific areas, we sought to create an accurate water testing product that could be easily used and specifically for use in the context of the Indian subcontinent.

Test 1: Growing Cultures of Bacteria

Hypothesis:

Distilled water will have no bacterial growth, tap water will have some bacterial growth, and pond water will have a high amount of bacterial growth.

Variables:

Independent Variable Type of water used in each sample	Three setups were used: 1. Pond water 2. Distilled water 3. Tap water
Dependent Variable The type of bacteria grown, and number of bacteria grown	Bacteria colonies and the amount of bacterial growth on the agar samples
Controlled Variable 1. Mass of agar powder 2. Amount of water 3. Heat from Bunsen burner 4. Incubation temperature 5. Incubation time 6. Amount of curd	1. Using a weighing scale, 0.7 grams of agar powder was added to an empty beaker. 2. Using a measuring cylinder 25 ml of distilled water was poured into a beaker. 3. The beaker was heated over a Bunsen burner flame until the agar powder was fully dissolved. 4. The agar gel was added to Petri dishes and samples of 0.5 ml water was added to the dish. 5. The incubator was set to 37 degrees Celsius to ensure the optimal growth conditions for bacteria. 6. The samples were incubated for 48 hours ensuring enough time for development of visible colonies, while avoiding overgrowth or death of bacteria.
Uncontrolled Variable 1. The surrounding air (which had the presence of bacteria) in the biology and chemistry lab. 2. Ambient temperature of the biology and chemistry labs	The temperature of the surroundings was not monitored, and neither was the humidity or the temperature change across the biology and chemistry labs.

Materials:

Agar powder

Sterile Petri dishes

Water samples: Tap water, Pond water, Distilled water

Sterile pipettes (one per water source)

Incubator: Set to 37°C for 48 hours

Universal pH Indicator

Sterile gloves

Stirrer

Distilled water

Aluminium foil

Alcohol wipes for sterilizing equipment

Marker for labelling plates

Masks

Procedure:

Step 1: Preparation of Nutrient Agar Plates

We dissolved 2.8 grams of agar powder in 100 mL of distilled water and heated the solution in a beaker using a Bunsen burner. Once the agar powder had completely dissolved, we removed the beaker from the heat, poured 25 mL of the agar solution into 3 sterile Petri dishes, and allowed it to cool to room temperature into a gel-like substance.

Step 2: Collection of Water Samples

Tap Water: We collected 10 mL from the laboratory faucet in a sterile container, ensuring no external contaminants entered the sample.

Distilled Water (Control): We collected 10 mL from a commercial supplier. As this water had undergone purification, it was expected to contain no microbial life. To prevent contamination from the air it was covered with aluminium foil.

Pond Water: We used a sterile container to collect 10 mL from a local pond. This sample was anticipated to have the highest microbial load due to environmental exposure to other organisms.

Step 3: Inoculation of Water Samples

We hatched the agar gel on the Petri dishes in four sections each to isolate the colonies, ensuring not cut through the agar.

Using a sterile pipette, we transferred 0.5 mL of each water sample (tap, distilled, and pond) onto separate nutrient agar plates and labelled each plate accordingly. We covered them immediately to avoid contamination from bacteria present in the surroundings.

We incubated all plates at 37°C for 48 hours, allowing for the formation of bacterial colonies.

Step 4: Preliminary Results and Observation

After 48 hours, we inspected the plates for microbial growth using (a) visual references, (b) microscope examination and (c) testing the pH.

Precautions:

1. Laboratory Safety Measures

Personal Protective Equipment:

- a. Gloves: Sterile gloves were used at the time of working with the water samples and during inoculation to avoid contamination or exposure to pathogens.
- b. Masks: Masks were used to prevent the bacteria (which contaminated the air once exposed) from entering our nasal pathways

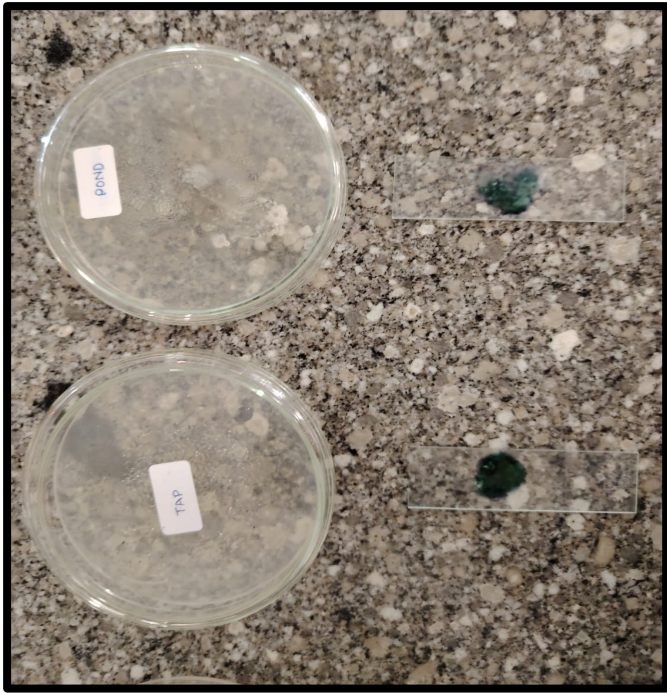
2. Bacterial Growth Conditions:

Bacterial growth is affected by different environmental factors:

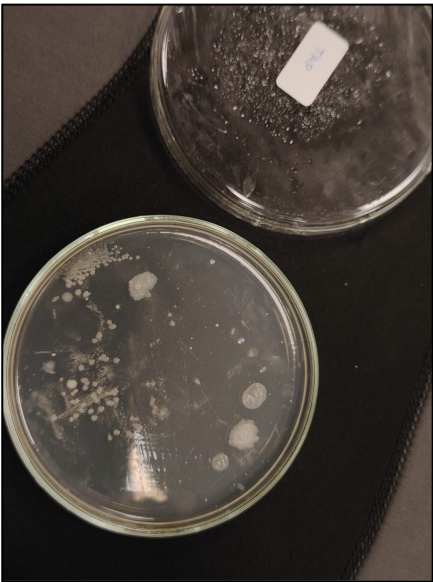
- a. Temperature - Each species of Bacteria has an optimal temperature for growth. Low and high temperatures reduce the metabolism of an organism, thus delaying its capacity to produce acidic by-products required for pH change. We used an incubator for the same, to ensure a constant/optimal temperature for bacterial growth.
- b. Availability of Nutrients: To grow, bacteria need nutrients. If the growth medium is deficient in some essential nutrients, the production of metabolic by-products that affect pH might be impaired. We provided the nutrients in the agar gel.

3. All sampling and inoculation instruments, and apparatus were always kept sterile to reduce contamination risks, and once used we thoroughly cleaned all the apparatus and the tables using alcohol and cotton.

4. We clearly labelled all the samples and cultures to avoid mix-ups and ensure accurate tracking of results.



PH TEST RESULTS WITH LABELLED PETRI DISHES



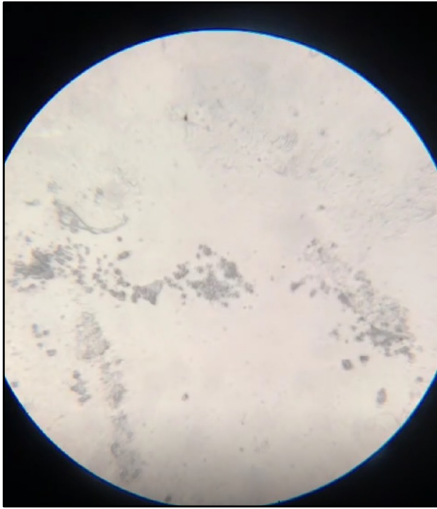
TAP WATER BACTERIA



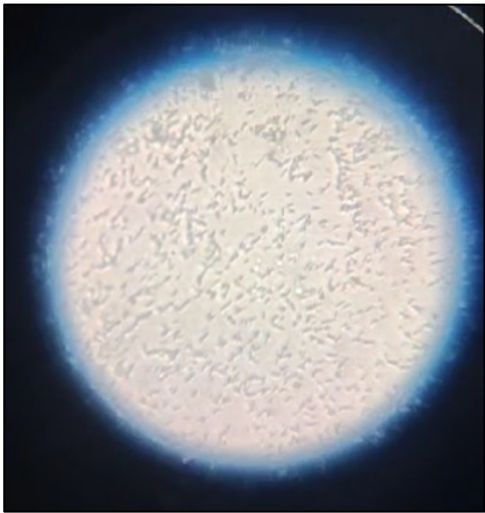
DISTILLED WATER BACTERIA



POND WATER BACTERIA



**POND WATER BACTERIA
UNDER MICROSCOPE**



**TAP WATER BACTERIA
UNDER MICROSCOPE**

Conclusion and Results

This experiment aimed to determine and compare the contamination in different types of water. This was achieved by inoculating nutrient agar plates with the samples of tap, distilled, and pond water and incubating them to observe bacterial growth.

Result analysis:

1. Pond water

Matching our hypothesis the pond water had the highest amount of bacterial growth, with numerous visible colonies around the plate. This is likely due to high exposure to environmental factors such as soil, and interaction with animals and insects, which introduced various bacteria and microorganisms to the water. We also conducted a pH test on the bacteria using Universal Indicator and it resulted in a green colour demonstrating the bacteria's alkaline nature.

2. Tap water

Matching our hypothesis the tap water had bacterial growth, but lower than that in pond water, again with numerous visible colonies around the plate but with a larger variation of type of bacteria. We conducted a pH test on the bacteria using Universal Indicator and it resulted in a dark green colour, slightly darker than that of pond water's bacteria, demonstrating the bacteria's more alkaline nature when compared to pond water.

3. Distilled water

Unlike our hypotheses, distilled water grew bacteria. We believe this is because of its exposure to air and other factors that we did not foresee. Since we were not expecting the bacterial growth and considered it an anomaly, we did not test for the pH.

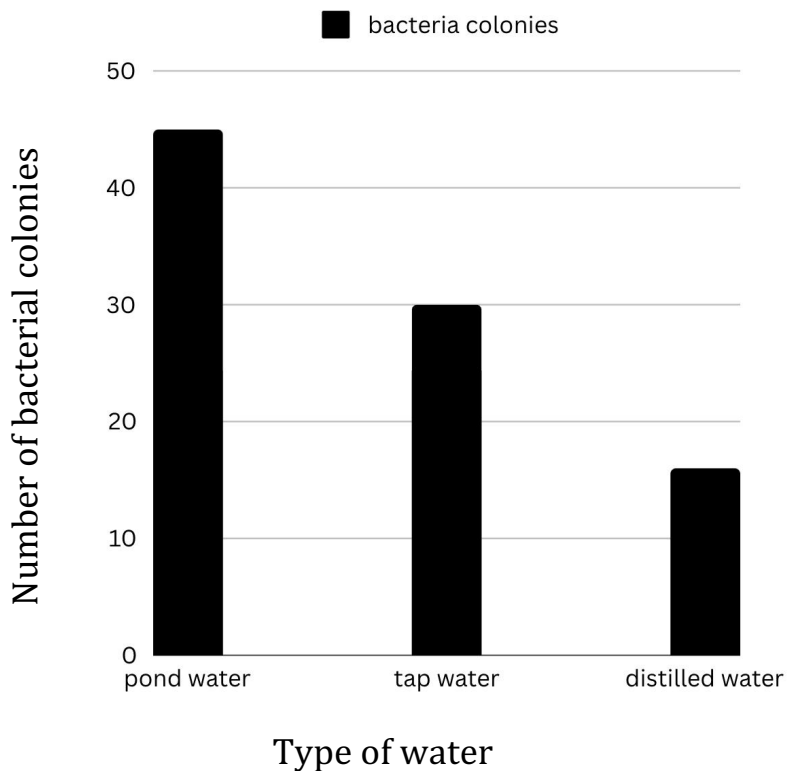
The experiment successfully demonstrates that different sources of water carry different levels of mycobacterial life.

- Pond water being the most exposed to the environment, contained the highest microbial load.
- Tap water that was treated still contained some bacteria.
- Distilled water that has undergone purification showed lower bacterial growth.

This confirms the effectiveness of water treatment but starkly brings out the contaminations of tap water and other non-purified water sources in general.

Errors:

1. Distilled water contamination: Although we hypothesized that distilled water would have no bacterial growth, there was some due to contamination due to air exposure.
2. Lack of sterilisation prior to the experiment: We washed our equipment with distilled water prior to the experiment but did not clean them with alcohol. This could have resulted in contamination.
3. Humidity: We were unable to control the humidity of the air in our incubator, which changed and could result in higher or lower growth rates of bacteria
4. Time that each sample was exposed to air: We did not control the time each sample was exposed to air. This could lead to unequal levels of contamination across samples and change our results.



Bar graph comparing the colony counts in each petri dish.

Test 2: Testing for the presence of bacteria

These tests were conducted to prove that pathogenic bacteria can be tested by simple chemical tests too, since we were unable to conduct tests for pathogenic microbes in our school labs.

We wanted to identify the presence of *Lactobacillus* bacteria in curd using two tests: a pH test with a universal indicator solution and a catalase test with hydrogen peroxide.

Materials:

1. Curd sample
2. Universal pH indicator
3. Hydrogen peroxide
4. Test tubes
5. Distilled water
6. Pipette
7. Stirring rods
8. Glass beaker
9. Safety equipment (gloves, goggles)

Test A: pH Test with Universal Indicator

1. We took 5g of curd and placed it in a clean glass dish.
2. We added 5 ml of distilled water to dilute the sample, mixing with a stirring rod.
3. We used Universal Indicator to check the pH of the curd
4. We were able to detect a pH of 4.5, which indicated that the bacteria was acidic and that *Lactobacillus* was present.

Test B: Catalase Test with Hydrogen Peroxide

1. We took 1g of curd and placed it in a clean test tube.
2. Using a pipette, we added 1 mL of hydrogen peroxide to the test tube containing the curd sample.

3. We then carefully observed the reaction for any bubbles or foam formation.
4. A negative result (no bubbling) indicated that the bacteria present did not produce the enzyme catalase, which is typical for Lactobacillus, showing that it was present.



CATALASE TEST

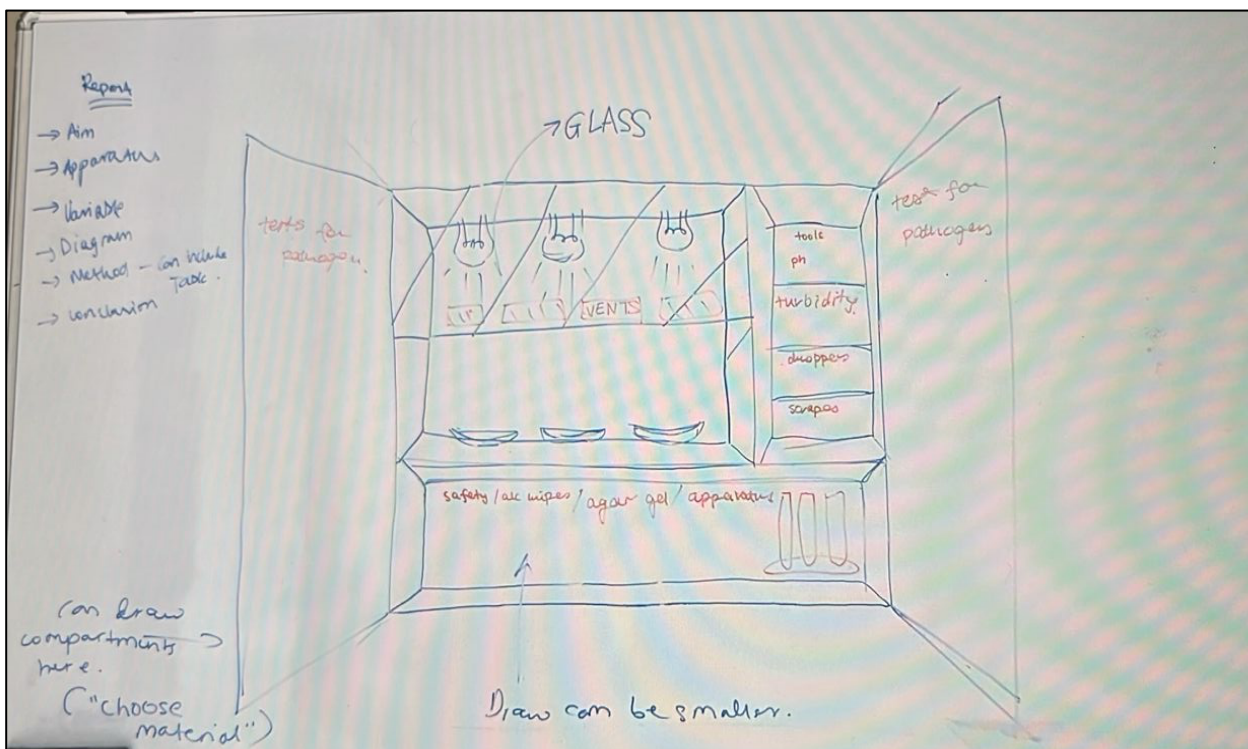


pH TEST

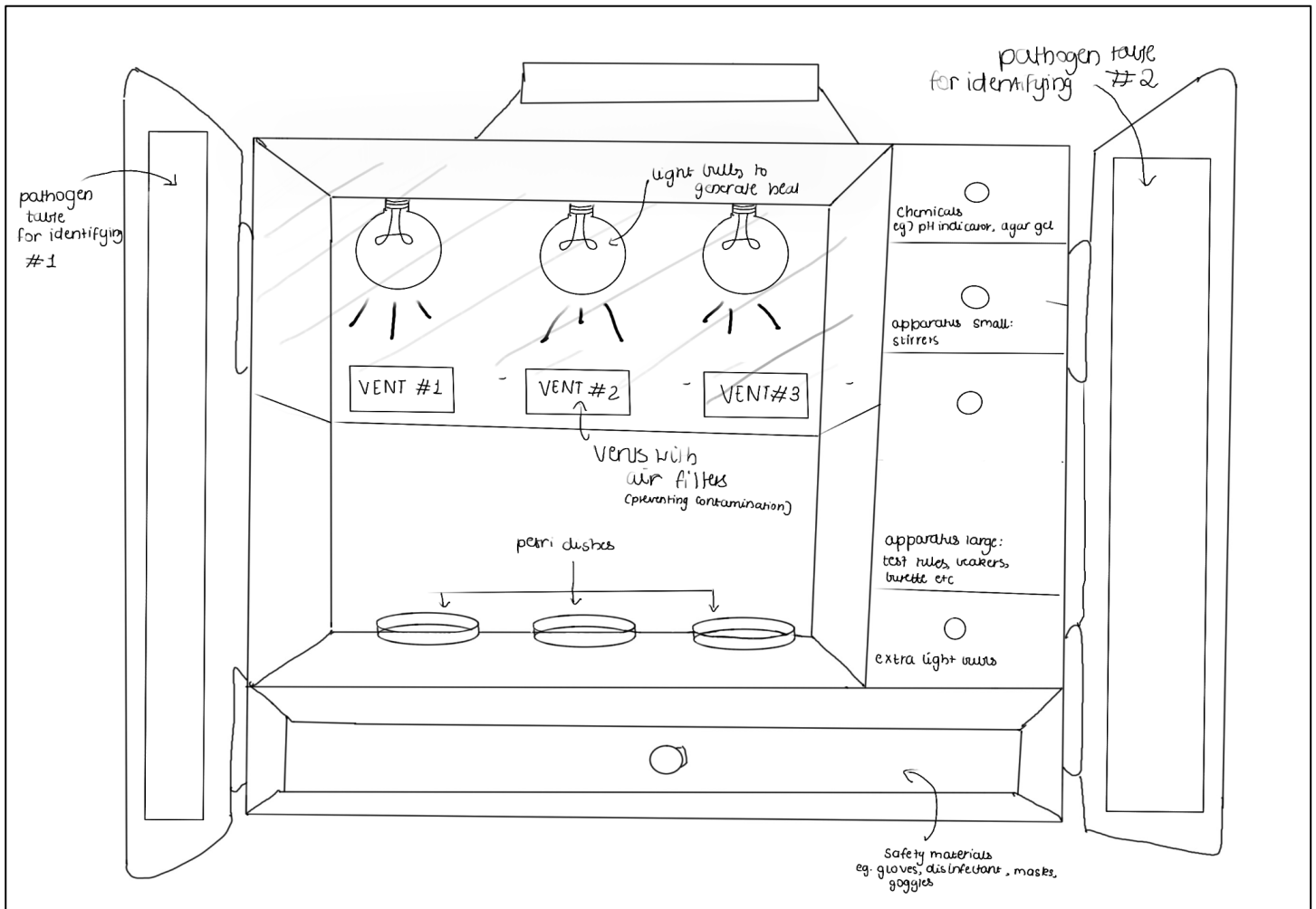
Conclusion

Both tests A & B proved the presence of Lactobacillus bacteria in the curd sample. The pH test showed a mildly acidic pH, and the catalase test produced no bubbling upon the addition of hydrogen peroxide.

Proposed Solution: Remote Testing Kit



Brainstorming



Final Sketch

Components:

Petri Dishes
 Agar Powder
 Tools
 Droppers
 Safety equipment - Gloves, Mask
 Apparatus
 Alcohol
 Tests for pathogen
 pH Indicator
 Light Bulbs

Procedure:

We propose the following procedure for our remote testing kit.

- 1) Collecting the contaminated water using a dropper. Placing 3 drops on a clean petri dish and adding 3 drops of universal indicator. Using a pH colour chart which is attached to the bottle itself. An acidic or alkaline test proves the contamination of the water and does not require further testing.

If the pH is neutral (or between 6.5 and 7.5), then move to Step 2 below.

- 2) Prepare the agar powder and the triple sugar iron agar in two different petri dishes using an available heat source. Using the dropper, place 4 drops of the water on both remaining petri dishes. Make sure

the light bulbs are turned on. Wait for 48 hours (be sure to close the kit during this period and place a lid on the Petri dishes)

- 3) Once 48 hours have elapsed, carefully remove the petri dishes. Using a scalpel, add samples of bacteria to 5 test tubes.
- 4) Do a visual inspection and colony count.
- 5) The tests are pathogen-specific and are listed below. They must all be carried out to successfully check for the presence of these 5 microbes.

Bacteria	Visual Indication	Positive Chemical Test
E. Coli	Pink Colonies	Adding Kovac's reagent and a red ring appearing
Salmonella	Clear colonies with black centres	Inoculating Triple Sugar Iron agar and a black precipitate forming
Vibrio Cholerae	Yellow colonies	Adding a reagent for Oxidase testing and a colour change to purple
Mycobacterium tuberculosis	Non-pigmented or buff-coloured colonies	Staining using Ziehl-Neelsen stain and the bacteria turning red
Shigella	Slightly pink colonies	Urease Test - if urease is absent then it suggests the presence of Shigella. If there is no colour change it indicates a negative test.

Safety for home testing -

- 1) Wear gloves at all times
- 2) Wear a mask while opening the Petri dishes with the grown bacteria.
- 3) Make sure all equipment is sterile.
- 4) Ensure the room is well-ventilated.

Practical application:

As stated before, we hoped to find an easy and accurate way to analyse the contamination of water in rural areas in India. We believe that, although our kit can take time to cultivate bacteria, it is much more effective than current water testers since the proposed tester, tests for bacteria that grow specifically in the Indian subcontinent. This is important since the current kits on the market are either:

- for filtration: which works on a small scale and cannot assess the quality of water in large water bodies,
- pH tests (which are risky since bacteria can grow in neutral conditions as well)
- test for only one species of bacteria.

Our kit tests the pH and tests for the 5 most common microbes in the Indian subcontinent that cause damage and can therefore mitigate their effects.

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