DIRTY WATER

Dirty water kills millions of people across the world every year. Can you make water safe to drink? Water can be cleaned by purification and filtration. Purification kills micro-organisms and pathogens. Filtration improves the colour and taste of bad water. It removes or significantly reduces debris, large pathogens and chemicals.

You will test the levels of bacteria in water treated by filtration and/or chemical purification.


WaterAid / Marco Betti

EQUIPMENT

- large plastic bottle
- cotton wool
- activated charcoal
- washed fine sand
- washed gravel
- clamp stands, clamps and bosses
- water sample
- labels
- chlorine tablets
- iodine tablets
- sterile container (for collecting filtered water)
- support for the filter
- sterile bottles
- measuring cylinders
- sterile dropper pipettes
- Petri dishes containing nutrient agar
- water samples for analysis
- discard pot containing disinfectant
- incubator at 25-30 °C
- Bunsen burner
- spirit marker pen
- sticky tape to tape the lid on Petri dishes
- ruler
- eye protection
- disposable gloves

SAFETY NOTES

Do not drink any of the water samples even after filtration/purification. Wear protective clothing and eye protection.

All micro-organisms present in a sample multiply during incubation, including any harmful ones. Therefore, after incubation samples MUST NOT be opened and must be disposed of safely.

When the Bunsen burner is not being used, close the air hole so you can see a yellow flame. Move it away from where you or others are working.

Take care when using sharp instruments such as scissors.

Make sure you're familiar with the aseptic technique that you will need to use:

- flaming the neck of tubes or bottles as they are opened or closed
- correct handling of pipettes and Petri dishes
- safe disposal of waste.

You must use aseptic technique throughout the procedure.

METHOD 1: MAKING A FILTER

1. Cut off the bottom of a large plastic bottle.
2. Plug the neck of the bottle with cotton wool or other material/fabric that allows water through.
3. Clamp the bottle upside down. Attach one clamp to the neck of the bottle and one higher up. You may need to construct additional support for the bottle.
4. Place 2 cm layers of the following materials into the bottle on top of the cotton wool: activated charcoal, washed sand and lastly gravel.
5. Put a sterile container under the opening in the bottle to catch the filtered water.
6. Slowly add the water sample to the top of the filter.
7. Measure 500 cm^3 of filtered water into each of four sterile bottles. Label them 'filtered water'.

Water samples must not be contaminated with micro organisms from you or the laboratory. They should be stored in sterile bottles, sealed, kept cool and tested as soon as possible.

METHOD 2: CHEMICAL PURIFICATION

You will use iodine tablets and chlorine tablets to purify the water.

1. Measure 500 cm^3 of the original unfiltered water sample into each of four bottles.
2. Label the bottles of filtered and unfiltered water (see table below) and add the relevant chemical tablets.

You need to make sure you measure out the correct volume of water and add the correct amount of tablet.

Check with your teacher before you begin.

label	description	chlorine tablet added	iodine tablet added
A	control	no	no
B	chlorine	yes	no
C	iodine	no	yes
D	chlorine + iodine	yes	yes
E	filtered control	no	no
F	filtered + chlorine	yes	no
G	filtered + iodine	no	yes
H	filtered + chlorine + iodine	yes	yes

METHOD 3: TESTING THE BACTERIAL CONTENT

Use aseptic technique

You'll need to set up a separate test plate for each water sample being tested (A-H).

1. Arrange a clean workspace so you have, within reach, a Bunsen burner with a blue flame and the Petri dishes of nutrient agar (don't take off the lids yet).
2. Label the bottom of the plates. Write your name, date and the water sample (A-H), but keep the labelling small and near the edge or it will be difficult to see what grows on the plate.
3. Collect your first water sample bottle.
4. Loosen, but don't remove, the cap of the bottle.
5. Using aseptic technique, remove the cap and pass the neck of the bottle briefly through the hot part of the Bunsen flame. Using a sterile pipette, draw up a little of the water sample.
6. Lift the lid of the Petri dish and drip one drop of sample onto the centre of the agar plate. Immediately replace the lid of the Petri dish.
7. Replace the cap of the bottle, put it to one side and put the pipette into the waste beaker of disinfectant.
8. Now repeat steps 4-7 to inoculate the plates with other water samples.
9. Once the plates have dried, use two short pieces of tape to tape the lids onto the Petri dishes, making sure a complete seal is avoided. Check with your teacher if you are not sure how to do this. Turn the Petri dishes over (this prevents condensation dripping onto the agar surface during incubation).
10. Incubate the plates at 25 to 30 °C for 48 hours. Your teacher or technician will probably fix (stop) the growth of the cultures before giving the plates back to you.
11. Without opening your plates, examine them and note:
    - general appearance of any growth (colour, texture)
    - number of areas of growth
    - diameter of the main area of growth. Measure this with a ruler.
14. After examination, dispose of your agar plates safely. Your teacher will tell you how.

RESULTS

Write your results into a table similar to this one.

	sample	appearance of water sample after stage 2	general appearance of growth (colour, texture)	number of areas of growth	diameter of the main area of growth
A	control				
B	chlorine				
C	iodine				
D	chlorine + iodine				
E	filtered control				
F	filtered + chlorine				
G	filtered + iodine				
H	filtered + chlorine + iodine				

Describe any differences between the clarity (clearness) of (a) the filtered water, (b) the original sample, (c) tap water.

SOME MORE QUESTIONS

1. Find out why there are three different layers in the filter.
2. Compare the advantages and disadvantages of using a filter and/or chemical methods to treat water.
3. List factors in addition to bacterial content that should be considered, when testing water quality.

SOME MORE THINGS TO TRY

Scaling up

Compare your water treatment system with a large scale waterworks such as the one in the video (think about the materials used in your filter).

What additional features are required in the design of the waterworks cleaning system?

Scaling down

Some people travel to places where there may not be sufficient clean water to drink. For example, on a camping trip in a remote area.

Design a light weight bottle or container system for cleaning water. Draw a diagram of your design. Below are some websites of companies that produce this kind of water purification system. They may help with your design.

Katadyn:

http://www.katadyn.com/brands-products/katadyn/tab/product-categories.html

Aquamira:

http://www.aquamira.com/

MSR:

http://www.msrgear.com/watertreatment/

Hydropal:

http://www.hydropal.co.nz/index.pasp

INFORMATION SHEET: ASEPTIC TECHNIQUE

Aseptic technique is the most important skill a microbiologist needs to learn. Using aseptic technique makes it unlikely that samples are contaminated with micro-organisms from the environment (in the air or on surfaces) and the micro-organisms being studied do not escape to cause infection.

Basic guidelines

- Wear gloves and a clean laboratory coat (one which stays in the laboratory).
- Before beginning work, make sure your work place is clean (use an appropriate disinfectant spray or wipe over surfaces using a suitable disinfectant cleaner).
- Work quickly but carefully to reduce the time specimens are open/out of incubators.
- Avoid working in draughts or next to open windows, where micro-organisms may blow across your work place.

Aseptic technique

- Sterilise equipment in an autoclave before use or use disposable sterile equipment.
- Once removed from its packet, avoid contaminating sterile equipment. For example, do not put down on the laboratory bench or walk around the room with it.
- If sterile equipment accidentally touches a non-sterile surface (such as the outside of a bottle) discard it and start again with a sterile item.
- When you remove caps and lids from containers, do not place them on the workbench; keep them in your hand while the sample is being processed, taking care not to contaminate hand or cap. Hold the lid the right way up. Replace caps and lids as soon as possible.
- If you are carrying out the work on the open bench, a Bunsen burner should be close by, to flame bottle necks.
- When opening culture containers, keep samples away from your face.

Minimise airborne contamination:

- Open caps slowly as the contents are sometimes under pressure.
- Avoid vigorous swirling or shaking before opening bottles.
- Avoid expelling the last drop from a pipette.
- Petri dish lids should only be removed when necessary and for the minimum time only.
- Hold lids over the dish during transfer operations.
- Seal agar plates with two pieces of sticky tape placed vertically over the rim. Do not totally seal around the rim as this can lead to the growth of more dangerous anaerobic bacteria. Store the plates upside down to prevent moisture build up.

Disposal

Treat all cultures as potentially pathogenic. All waste that has come into contact with micro-organisms should be classed as a biological hazard. It will need to be sterilised before disposal. Follow your laboratory guidelines for disposal.

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HEALTH AND SAFETY

A risk assessment must be made before starting any practical work. Students should wear protective clothing and eye protection. Disposable gloves, preferably nitrile ones should be worn when using aseptic technique. Students must not taste water samples, even after purification.

It is assumed the teacher is familiar with all aspects of the safety guidelines for working with micro-organisms at this level.

For guidelines on working with and disposing of micro-organisms, refer to section 15.2 of the CLEAPSS Laboratory Handbook (revised 2006) and the handbook section of the CLEAPSS Science Publications CD Rom 2008, both of which should be available in schools.

The laboratory should be set up to comply with good microbiological practice including procedures for waste disposal and spills management. Detailed advice can be found in the Society for General Microbiology (SGM) booklet *Basic Practical Microbiology: a manual*, which can be downloaded from the Internet: www.microbiologyonline.org.uk/forms/BPM.pdf.

In these tasks, as in all laboratory activities, the students should have been carefully briefed in good laboratory practice, with particular respect to the personal safety precautions necessary when working with micro-organisms.

To reduce the number of agar plates required, ask each group to test two purification methods (e.g. iodine and filtered + iodine) plus the control. Results can then be pooled.

THE INVESTIGATION

Students should work in twos or threes.

Students could test water collected from a local source, such as a pond, river or stream. Samples should not be collected in areas that might be contaminated with sewage or farm waste. Water samples should be kept in sterile bottles, kept cool and tested as soon as possible after collection. If the students collect the samples, it should be under the guidance of a teacher.

Alternatively you could dig up some soil from a garden and mix into tap water. You shouldn't purposely add micro-organisms from dangerous sources (such as human or animal body fluids/excrement).

Iodine and chlorine tablets can be bought from camping shops (or ordered online). You will need to check the manufacturer's guidelines on how much water to add to one tablet. Students' worksheets may need to be adjusted to fit in with this value.

You will probably need to demonstrate inoculating an agar plate to students before they begin, highlighting how to perform aseptic technique. The information sheet can be given to students, to help them remember the key points.

All cultures should be considered potentially pathogenic. It is advised that, after incubation, micro-organisms on plates are killed before they are given back to students. This is done in case students disobey instructions and open plates. The micro-organisms can be killed by exposing them to an atmosphere of methanal (formaldehyde), see section 15.2.11 of the CLEAPSS Laboratory handbook for details.

Time required

As the agar plates will need to incubate for 48 hours, this activity will take two sessions to complete.

SOME MORE THINGS TO TRY

You might ask students to complete additional tasks on large or small scale water purification systems. They may need to carry out some web-based research for these tasks.

TECHNICIAN EQUIPMENT LIST

per group

- water sample
- activated charcoal
- washed fine sand
- washed gravel
- chlorine tablets
- iodine tablets
- large plastic bottles
- scissors
- cotton wool
- clamp stands, clamps and bosses
- labels
- large sterile containers for collecting water from the filter
- support for the filter
- sterile 500 ml bottles
- measuring cylinders
- sterile dropper pipettes
- Petri dishes containing nutrient agar (could be bought pre-prepared or prepared yourself)
- discard pot containing disinfectant such as VirKon [HARMFUL]
- incubator at 25-30 ^{o}C
- Bunsen burner
- spirit marker pen
- sticky tape to tape the lid on Petri dishes
- ruler
- eye protection
- disposable gloves (preferably nitrile)

additional materials not to be given to the students

- methanal (formaldehyde)
- filter paper

Chlorine and iodine tablets are available from camping shops, including online.