

OXFAM – DELAGUA

Portable Water Testing Kit

Users Manual (abridged web download version)

**Revised and updated 4th edition
2000**

This equipment was designed to conform to the parameters specified in *World Health Organisation Guidelines for Drinking Water Quality, Volume III*.

The equipment should only be used by trained personnel familiar with those guidelines.

For more details, please contact the Robens Centre for Public and Environmental Health

Full copies of this manual (with diagrams and appendices) can be obtained from the Robens Centre in the following languages:

English, French, Spanish,
Mandarin Chinese, Persian, Croatian and Bosnian

If you regularly use the OXFAM - DELAGUA water testing kit and have translated the manual into another language, please send us the translation. Under these circumstances, we normally organise printing and give free copies to the programme that provided the translation.

For advice and information on the OXFAM - DELAGUA water testing kit, spare parts or service, please contact us at:

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We are continually trying to improve the OXFAM – DELAGUA water testing kit and because of this, some components may be different from those which appear in the manual.

The Robens Centre offers one and two week training courses overseas which include water quality testing, sanitary inspection, water supply disinfection, and use and maintenance of the OXFAM – DELAGUA kit. Purchasers of the kit are also entitled to participate in a one-day course at the Robens Centre in the use of the kit, free of charge.

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1. Sampling Programmes

Selection of Sites and Frequency of Sampling

Samples should be taken from locations which are representative of the water distribution network and household connections. Where there are several sources and a mixed distribution system, it is necessary to take account of this. Where there is a branched distribution system, samples should be taken at random points evenly spread throughout the system. Where there are main branches and a remote periphery (as shown), greater attention should be devoted to the main branches and remote points in the network.

The recommended minimum frequencies for sampling for both piped supplies and point sources are shown in the tables below:

Minimum Frequency of Sampling and Analysis of Piped Water Supplies

Population served	Minimum frequency of sampling
Less than 5,000	1 sample monthly
5,000 to 100,000	1 sample per 5,000 population monthly
more than 100,000	20 samples monthly plus 1 sample per 10,000 population monthly

Minimum Frequency of Sampling and Analysis of Unpipd Water Supplies

Source and Mode of supply	Bacteriological	Physical/Chemical	Remarks
Open well	Sanitary protection measures and testing only if situation demands	Once initially for community wells	Pollution usually expected to occur
Covered well Shallow tubewell with handpump	Sanitary protection measures and testing only if situation demands	Once initially, thereafter as situation demands	Testing needed when environmental conditions change or when outbreak or increase in waterborne disease occurs
Deep tubewell with handpump	Once initially, thereafter as situation demands	Once initially, thereafter as situation demands	Testing needed when environmental conditions change or when outbreak or increase in waterborne disease occurs
Springs and piped supplies	Once initially thereafter as situation demands	Test periodically for residual chlorine if water is chlorinated	Testing needed when environmental conditions change or when outbreak or increase in waterborne disease occurs
Community rainwater collection systems	Sanitary protection measures and testing only if situation demands	Not needed	

Source: Adapted from *WHO Guidelines for Drinking Water Quality Volume III* Geneva, 1985.

2. Preparation of the Kit

2.1 Preparation of Culture Medium in the Laboratory

You will need the following items:

1. Membrane Lauryl Sulphate Broth (culture medium)
2. Distilled Water
3. Polypropylene Bottles
4. Measuring Cylinder
5. Pressure cooker, steriliser or autoclave
6. Heating element, stove or burner

*

Carefully wash the plastic culture medium bottles in clean, warm water before use. If necessary, use a little detergent and then rinse well with clean water to remove all traces of detergent.

1. Always read the instructions on the side of the container before you proceed.
2. Place 38.1g of Membrane Lauryl Sulphate Broth powder into a beaker and add 500ml of distilled water or clean, filtered rainwater¹. The broth powder is usually supplied in pre-weighed tubs of 38.1g. Tubs of 500g are available for those needing large quantities of culture medium. It is important to keep the powdered medium well sealed in its container until used, as the powder absorbs water and will deteriorate if left open to the atmosphere.
3. Mix and apply gentle heat if necessary to dissolve the powder completely. *Do not boil*. The culture medium will be a bright red colour when dissolved.
4. Decant approximately 40ml of culture medium into the polypropylene bottles. . Each bottle should contain enough medium for one days use. The kit contains enough petri dishes to process 16 samples per day. (40ml = 16 [samples] x 2.5ml)
5. Replace the screw caps loosely on the polypropylene bottles and place a small strip of autoclave tape over the loose lid. Over-tightening can result in leakage and the bottles to collapse.
6. If an autoclave is available, sterilise the bottles with the tops loose at 121°C for 15 minutes. Tighten caps carefully once cooled.

If there is no autoclave available, then a household pressure cooker or portable steriliser may be used. Place the bottles in a rack inside the cooker (they may melt if placed directly on the floor of the cooker), replace the lid and heat to full pressure (about 15psi). Once the cooker has reached full pressure, time 15 minutes using a stopwatch or clock. Switch off the heat and allow the cooker to cool. If the sterilisation process has been carried out properly then the autoclave tape will have dark (black) stripes on it. If the stripes are not dark then sterilisation has not been fully achieved and Step 6 will need to be repeated.

Remove the bottles. Once the broth has fully cooled loosen one end of the autoclave tape, tighten the lid then re-attach the autoclave tape and store in a cool, dark place.

¹ If rainwater is not available then use the De-ionisation pack to “clean” the water. Please check the pH and residual chlorine levels of the water first as both of these may affect the broth quality.

2.3 Storage of Culture Medium

Culture medium which has been sterilised in an autoclave or pressure cooker and then stored in a cool, dark place, normally remains stable for several months, approximately 3 months. If signs of deterioration appear, e.g. cloudiness or yellow coloration, the contents of the bottle must be discarded. In cases where the medium is kept in a cold environment, e.g. refrigerator, a deposit may form which dissolves when the bottle is warmed and gently shaken. This deposit does not mean that the medium has deteriorated, it is a principle of lauryl sulphate.

2.4 Disposal of Contaminated Material

To avoid any risk of infection from contaminated materials, take care not to touch contaminated membranes directly with the hands. Do not eat, drink or smoke while handling contaminated materials. Wash hands immediately. Contaminated material, such as used membranes and pads, may be made safe by autoclaving or incineration. Do not discard non-sterile membranes and pads into the environment. The petri dishes must be carefully washed with detergent after use, rinsed with clean water and dried.

Sterilisation of the dishes may be carried out in several ways:

1. Autoclave at 121°C for 15minutes. Assemble the dishes and store away from sources of contamination.
2. Place the dishes in a conventional oven at 180°C for 30 minutes.
3. Plunge the bases and lids of the dishes into boiling water for 15 minutes. Pour away the water and assemble the dishes as they dry, while they are still hot.

Whenever possible, always use one of the above methods. If this is not possible, then the following method can be applied:

Flame the bases and lids of the dishes with a lighter or gas burner using the tweezers to hold the bases and lids. Assemble while still hot.

2.5 Absorbent Pads and Dispenser

The pads are supplied sterile in packs of 100 units. A pad dispenser is also supplied with the kit. Never leave the dispenser without a pack of pads attached as it will increase the possibility of contamination.

It is preferable to dispense pads into the petri dishes at base and not to use the dispenser and pads in the field.

If it is necessary to dispense pads in the field, take care not to contaminate the dispenser assembly. If the dispenser is lost or damaged, pads may be dispensed in the field using the sterile tweezers. Some kit operators prefer this method to using the dispenser.

In order to cut down in possible contamination either in the office or field it is easier to sterilise the absorbent pads in the petri dish as a single unit.

Methodology

1. Wash and dry the petri dish in warm water.
2. When the dishes are fully dried then place an absorbent pad on the bottom of each dish and replace the lids

3. Seal the plate with a strip of autoclave tape – making sure the lid is firmly attached to the base of the dish. Keep a small section of the autoclave tape folded over (but not attached to the petri dish) so that it can be opened easily while preparing samples in the field.
4. Place the sealed petri dishes on a small stand within an autoclave or pressure cooker and sterilise at 15lbsi (121°C) for 15 mins.
5. Once the autoclave or pressure cooker has cooled down, check that fully sterilisation has occurred (as before)
6. Store the petri dishes in a dry cool area until ready to use.
7. The petri dishes will remain sterile for upto 4 weeks as long as the tape seal is not broken.

This method has many advantages as it cuts down on possible areas where contamination can occur such as trying to keep the pad dispenser sterile, trying to keep the petri dish sterile until the sample is ready to be processed etc.

2.6 Methanol Dispenser

Methanol is highly flammable. *Keep methanol away from naked flame.*

The methanol dispenser is supplied with a plastic cap and dispensing nozzle. The dispenser should be half-filled with methanol using a small funnel, pipette or syringe to avoid spillage of methanol. *Do not overfill the methanol dispenser as it may leak in hot weather.*

To dispense methanol, lever the dispensing nozzle into the upright position with the tip of the tweezers. To seal off the flow of methanol, push the nozzle down into the recess in the cap. Be sure not to leave the dispensing nozzle in the upright position after using the kit as the methanol will leak.

3. Sampling Methods

3.1 Sampling from a Tap

1. Remove any attachments e.g. nozzles, pipes, etc., from the tap. Check that the tap does not leak and that all seals are in good condition.
2. Carefully clean the mouth of the tap with a clean cloth or tissue to remove any dirt or grease. It may also be advisable to wipe the tap on the outside, and internally with methanol then flame the methanol to ensure that the tap is fully “sterilised”² *Open the tap and leave running for at least one minute before taking a sample.* This ensures that any deposits in the pipes are washed out.
3. . Analyse the sample for chlorine residual and turbidity levels from water taken directly from the running water
4. If the chlorine residual and turbidity results suggest, then take a second sample for bacteriological analysis using the sterile sample cup (the cup with the hole).

3.2 Sampling from a Lake, Reservoir or other Surface Water Source

Where there is adequate access it may be possible to take samples by hand. In many cases it may be inconvenient or dangerous to enter the water. Grasp the sample cup firmly and dip the open mouth of the cup into the water. Submerge the cup about 30 cm below the surface of the water and scoop up the water sample.

This scooping action ensures that no external contamination enters the sample cup.

Lift the sample cup carefully and place on a clean surface where it cannot be knocked over.

In areas where there is a current flow, e.g. rivers and streams, the sample should be taken against the current flow.

In all cases, it is vital to obtain a sample that is representative of the main body of water. For example, when sampling from a river, do not sample the quiet or stagnant areas near the bank, as these do not represent the main body of water. Furthermore, it is vital not to introduce external contamination into the sample. For these reasons it is often better to sample with the help of the cable supplied with the kit.

3.3 Sampling from an Open Well or Storage Tank

1. Fasten the sampling cable to the hole in the lip of the sample cup by means of the clip on the end of the cable. It may be necessary to increase the length of the cable by attaching a rope or string to the sample cable. Take care not to lose the sample cup. The cable should be attached to the sample cup from the outside the cup, you should never try to attach the cable from inside the cup as this will lead to possible contamination of the sterile sample cup.
2. Lower the sterile sample cup into the well or tank, taking care not to allow the cup to touch the walls of the structure where it may pick up dirt. Submerge the cup to a depth of 30cm.
3. Lift the sample cup carefully and place on a clean surface where it cannot be knocked over.

² Follow this method if it is thought the contamination is from the water itself. However, if you want to identify the source of contamination then a sample should be taken before sterilising the tap then one after so that comparisons can be made.

4. Processing of Samples Using the Kit

4.1 Introduction

The first tests that must be carried out on a drinking water sample are the determination of chlorine residual and turbidity. The sample must be taken in a clean, but non-sterile container or in the pooltester and turbidity tubes where possible, never use the vacuum cup. Rinse the non sterile container several times with the water that is to be analysed before taking a sample for analysis.

If the results of the analysis are as follows:

Residual free chlorine greater than 0.2mg/litre (0.2ppm) *and*

Turbidity less than 5TU

it is highly *unlikely* that the sample will contain thermotolerant (faecal) coliform bacteria and therefore it may not be necessary to carry out thermotolerant (faecal) coliform analysis.

Where results are not within the above criteria, it is necessary to carry out thermotolerant (faecal) coliform analysis. Under these circumstances, samples for analysis must be taken with the sterile sample cup.

4.2 Analysis of Chlorine Residual and pH

1. Wash the comparator cells three times with the water that is to be analysed and finally fill all three cells with the sample.
2. Drop a DPD No. 1 tablet into the right hand cell (C₁₂) and a Phenol Red tablet into the left hand cell (pH). Never let your fingers come into direct contact with either of the tablets – sweat etc on your fingers may result in false results
3. Replace the lid of the comparator and push down firmly to seal. Invert the comparator repeatedly until the two tablets have dissolved completely. Do not shake, as this will introduce air.
4. Immediately read the free chlorine residual and pH concentrations by holding the comparator up to daylight and matching the colour developed in the cells with the standard colour scale in the central part of the comparator. If the colour falls between two standard colours, then it will be necessary to estimate the concentration. Record the result on the daily report sheet.
5. To test for total chlorine residual, do not discard the liquid in the comparator, but remove the lid and add a tablet of DPD No. 3 to the right hand cell (C₁₂).
6. Again, invert the comparator repeatedly to dissolve the tablet. The colour developed after 10 minutes represents the total chlorine residual in mg/litre.
7. Subtract the free chlorine result from the total chlorine result to obtain the combined chlorine concentration:

DPD No. 1	=	Free chlorine residual
DPD No. 1 + DPD No. 3	=	Total chlorine residual
Total – Free	=	Combined chlorine

4.3 Turbidity Analysis

Note: The turbidity tubes cover the range 5 to 2,000 TU

1. Remove the two turbidity tubes from their clips in the lid of the test kit case. Carefully push the upper tube (open at both ends) squarely into the lower tube. Look through the open end of the tube at the black circle printed on the base of the tube. Ensure that there is good illumination available. Normal daylight is adequate for this purpose.
2. Fill the turbidity tube with the water sample to the very top. Allow a few moments for the water to settle (as there may be formation of bubbles) before you read the tube. Hold the tube between the thumb and index finger at the joint of the two tubes. Have your arm fully extended. Do not strain to see the black circle as this can sometimes cause biased results. Continue to pour small amounts of the water slowly out of the tube checking each time to see if you can see the black circle. As soon as you can clearly see the black circle then read the level on the outer markings on the tube.
3. The turbidity tubes are graduated in a logarithmic scale with the most critical values. The result is the value of the line nearest the water level. This permits a reasonable estimation of the turbidity of the water sample. As the scale is Logarithmic it will be difficult to get an accurate reading when the water level is between scales. It is always better to take the reading which appears below the water level.

4.4 Bacteriological Analysis of Water

The analysis of water samples for thermotolerant (faecal) coliforms is carried out by passing a measured quantity of water through a sterile filter. Any bacteria present in the water are caught in the filter. The filter is then placed onto a paper pad soaked in a liquid growth medium that feeds coliform bacteria, but inhibits the growth of any other bacteria caught in the filter. To ensure that only thermotolerant (faecal) coliform bacteria are allowed to grow, the filter is kept at 44°C in the kit's incubator until the bacteria multiply many times to form colonies of bacteria which can be seen with the naked eye. Thermotolerant (faecal) coliforms are recognised by their ability to produce a colour change (from red to yellow³) in the culture medium at 44°C. Results are expressed as colony-forming units per 100ml of water (CFU/100ml).

Thermotolerant (faecal) coliforms are of sanitary significance when present in drinking water supplies. Users should refer to country-specific water quality standards or guidelines, or to World Health Organisation *Guidelines for Drinking Water Quality Volume III* to decide when action should be taken to improve contaminated water supplies.

Some users may need to analyse for total coliform bacteria which, although of less sanitary significance than thermotolerant (faecal) coliforms, can be used to indicate problems in large distribution networks. Total coliform analysis is carried out using the same process as for thermotolerant (faecal) coliforms, the only difference being that the filters are incubated at 37°C.

The OXFAM – DELAGUA incubator can be recalibrated to 37°C by following the recalibration procedure. However, this is not satisfactory when carrying out both thermotolerant (faecal) coliform and total coliform analysis on a regular basis. A dual temperature incubator is available from the Robens Centre that allows both tests to be carried out simultaneously.

³ Most thermotolerant faecal coliforms ferment lactose producing acid as a bi-product. This acid then changes the pH indicator (Phenol Red) present in the media broth, hence why the faecal coliforms are yellow.

4.5 Selection of Appropriate Sample Volumes for Thermotolerant (Faecal) Coliform Analysis

The most appropriate volume to process is that which allows the most accurate count of the bacteria. The most statistically valid range of colonies to count for contaminated water supplies is between 20 and 200 colonies per membrane filter. When there are less than 20 colonies there is a possibility of statistical error, and it may prove difficult to count more than 200.

Water for Human Consumption

For treated waters or water in a distribution network, it is likely that the number of thermotolerant (faecal) coliforms per 100ml will be around zero. For these waters the standard volume used is 100ml, and a count of zero thermotolerant (faecal) coliforms per 100ml is indicative of a safe water supply. If the count exceeds 50 colonies per 100ml, then the supply is heavily contaminated and requires immediate remedial action. The same action is required when waters treated with disinfectant, e.g. chlorine, give a count of greater than 1 thermotolerant (faecal) coliform per 100ml.

Other Water Sources

The selection of the most appropriate sample volume for a given source, treatment plant or distribution system is normally best made in the light of previous experience.

For piped supplies and partially treated waters (including those derived from ground water sources), it is possible to adjust the sample volume to obtain a final count in the range of 20 to 200 faecal coliforms per 100ml. Recommended sample volumes for each type of source are shown below.

Suggested sample volumes for thermotolerant (faecal) coliform analysis by the membrane filtration technique (alternative volumes are shown in brackets).

Lakes, ponds and other surface waters	10ml (5ml* or 1ml*)
Protected ground waters e.g. wells and springs	50ml (10ml or 5ml*)
Unprotected ground waters e.g. open dug wells and springs	50ml (10ml)
Waters in treatment plants after partial treatment	50ml (100ml or 10ml)
Waters in treatment plants after full treatment	100ml
Reservoirs, distribution networks and household tap	100ml

*Note: This volume will require the use of sterile syringes or pipettes..

Please note, these are guide values only. They do not represent absolute recommendations to be applied to sampling programmes. It may be useful to analyse different volumes of the same sample in order to decide the best range in which to count the bacteria. It is not necessary to sterilise the filtration apparatus and sample cup between two analyses of the same sample provided that the smaller volume is processed first.

4.6 General Hygiene in the Field

Although all components of the kit should be kept free from dirt and other contamination, there are some parts of the kit that must always be kept *clean and sterile*. These are as follows:

- a) All those areas in direct contact with the water sample, e.g. the internal surface of the sample cup, the internal surface of the filter funnel, the upper part of the filtration base and the surface of the bronze disc.
- b) Surfaces in contact with the culture medium, e.g. the internal surface of the petri dishes and the absorbent pads.
- c) Parts in contact with the membrane filters, e.g. the filtration apparatus, the absorbent pads and the tweezers.

Under no circumstances should any of these components be allowed to come into contact with dirt, dust or external objects that may contaminate them and interfere with the bacterial count.

Before handling a membrane filter and after processing a sample, the tips of the tweezers should be flamed with a lighter. Hold the tips in the flame for five seconds and allow to cool before handling a membrane filter. After sterilising the tweezers in this way, they should be placed so that the tips do not touch any other object. - the easier place to hold the sterile tweezers is in the locking mechanism on the lid. Press the base of the tweezers in to this catch as far as it will go – this will close the internal surface of the tweezers helping reduce the likelihood of contamination while the sample is being processed.

4.7 Sample Processing for Thermotolerant (Faecal) Coliform Analysis in the Field

1. Take a sterilise petri dish and gently peel off the tape from the bottom of the plate. Place the plate on the side of the incubator attaching the plate to the surface with the peeled section of the autoclave tape.
2. With a sterile pipette or syringe – remove approximately 2.5ml of the broth and place on to the absorbent pad in the petri dish to soak the pad and leave a slight excess Replace the needle sheath and place the needle and syringe to one side within the kit. If the needle has not come in contact with any other surface then the needle can be reused for the next sample. The needle and syringe must never be used for more than 1 days sampling.
3. Replace the bottle cap immediately. Do not allow the bottle neck to come into contact with any external objects. Immediately before processing a sample, drain off most of the excess medium. Always ensure that a slight excess remains to prevent the pad drying out during incubation.

Note: Once the bottle of culture medium has been opened, it is recommended that the contents are used within one day. It is not advisable to use the medium in one bottle over several days since this can lead to contamination.

3. Flame the tips of the tweezers with the lighter and leave to cool.
4. Place the heel of the tweezers into the test kit case as indicated. This ensures that the tips are kept away from all sources of contamination whilst analyses are in progress.
5. Remove the sterile sample cup from the filtration apparatus. Push the filtration apparatus firmly onto the vacuum cup (if this is difficult, then it is likely that the sealing ring needs lubrication with silicone grease) Place the assembly in an upright position in a convenient place in the kit. Do not place the apparatus on the ground where it may become soiled.
6. Unscrew the plastic collar and filtration funnel in order that these may be easily removed. Do not place these on any surface other than the filtration base.
7. Using the sterile tweezers, carefully remove a sterile membrane filter from its packet. Hold the membrane only by the edge.

8. With one hand, lift the filtration funnel and plastic collar above the filtration base. With the tweezers in your other hand, place the membrane filter (grid side uppermost) onto the bronze disc filter support. Replace the filter funnel and collar immediately, without allowing them to come into contact with any external objects. It is normally convenient to hold the funnel between the thumb and forefinger which ensures that the collar will not slip off and that the fingers do not come into contact with the interior surface of the funnel.

9. Screw the plastic collar down tightly to hold the membrane and to provide a water tight seal.

Note: The plastic collar has three adjustment positions:

1. Completely free – the apparatus can be dismantled when in this position.
2. Loose but not free – all interior surfaces are exposed to the atmosphere. This is the position used when sterilising the apparatus.
3. Fully tightened – the funnel forms a tight hermetic seal between the membrane support and the membrane filter. This is the position for filtration.

10. Rinse the sterile sample cup once with the water to be sampled and then fill the cup with the water. Take care not to allow external contamination to enter the sample cup, e.g. dirt and debris.

11. Pour the sample into the filtration funnel up to the appropriate mark (10, 50 or 100ml) engraved on the internal surface of the funnel. Take care not to allow external debris to enter the funnel. It may be difficult to see the 10ml mark on the funnel therefore a sterile syringe can be used to get the accurate volume of water.

Each sample must use a new syringe.

12. Insert the plastic connector of the vacuum pump into the vacuum connection on the filtration base. Squeeze the pump bulb several times to draw a vacuum, then squeeze as required to draw all the water through the membrane filter. When all the water has passed through the filter, disconnect the pump from the filtration apparatus.

13. Unscrew the collar and remove the funnel and collar with one hand. Using the sterilised tweezers in the other hand, lift the membrane carefully from the filtration base. Hold the membrane by the edge only.

14. Remove the lid of a prepared petri dish and place the membrane, grid side uppermost, onto the absorbent pad soaked in culture medium. Start at one edge and lower the membrane by 'rolling' so as to avoid trapping air bubbles under the membrane. Never touch the area of the membrane where the water has been filtered through. This will disturb any bacteria on the surface of the membrane and give false counts.

15. Replace the lid of the petri dish and mark the sample number on the lid. A wax pencil or indelible marker pen is suitable for this purpose. Fill all the details on the Daily Record Form e.g. volume of water samples, source, colour, odour etc

16. Place the petri dish with the lid uppermost into the carrier and return the carrier to the incubator pot. Replace the incubator lid.

4.8 Re-Sterilisation of the Filtration Apparatus

The sample cup and the filtration apparatus must be re-sterilised between samples when analysing water from two different sources.

Sterilising the equipment in the field presents some practical problems and must be carried out using simple methods. The most appropriate is the use of *methanol*, which is described below. Where methanol is not

available, the filtration apparatus and sample cup can be sterilised by immersion in boiling water for five minutes.

Note: **Methanol is the *only* alcohol suitable for sterilising the filtration apparatus; there is no substitute.** When burnt in a shortage of oxygen – in the closed sampling cup for example – formaldehyde gas is produced which is a very effective disinfectant. Methanol is expensive to freight and requires special transport conditions. It is therefore usually best obtained in-country from a pharmaceutical supplier, a local hospital, medical NGO or university laboratory, and is not normally supplied with the kit. However, if necessary, methanol can be supplied by the Robens Centre on request.

1. Carefully dry the sample cup and filtration assembly with a clean dry towel or tissue.
2. Using the plastic collar, secure the filtration funnel in the second position, which allows the sterilising agent to penetrate.

5 Pour about 1ml (approximately 20 drops) of methanol into the sample cup.

12 Carefully ignite the methanol in the sample cup using the lighter. *Caution: keep the mouth of the cup away from your face.* Place the cup on a flat surface that will not be harmed by heat. Allow the methanol to burn for several seconds and, when almost completely burned up, place the filtration apparatus over the sample cup and push firmly into place to form a good seal. After about 30seconds place the filtration cup loosely on top of the unit – this will also be partially sterilised by the formaldehyde escaping from the filtration unit.

6. Keep the filtration apparatus in the sample cup for at least 15 minutes before using to process a further sample.

Once the 15 mins sterilisation time is up, push the cups tight into the filtration unit and turn the whole unit so that the sample cup is on top – this means that the unit is in the correct position and immediately ready to process for the next sample.

Note: The use of too much methanol will result in a residue being left in the sample cup and filtration apparatus after sterilisation. The ideal volume of methanol to use will be determined in the light of experience.

Note: It is best to sterilise the filtration apparatus immediately after each analysis and to keep the filtration apparatus in a sterile condition during transport and storage. In this way, the filtration apparatus is always ready for use.

4.9 Resuscitation of Bacteria

Once the last sample of the day has been taken, wait for a minimum of 60 minutes before switching on the incubator (resuscitation time). Try to plan the day so that the time between the processing of the first and last samples is not more than 3 hours. This restricts the resuscitation time to a maximum of 4 hours.

Note: Resuscitation time is particularly important for chlorinated waters or marine water where the thermotolerant (faecal) coliform bacteria are 'stressed' due to environmental exposure. For these types of waters it is beneficial to leave processed membranes for 4 hours after the last sample has been processed before switching on the incubator.

4.10 Sample Incubation

Incubate the samples for 16 to 18 hours. The incubator is designed to maintain a temperature of 44°C +/- 0.5°C. In order to maximise battery life, do not leave the incubator on for more than the specified period, e.g. 4.00 p.m. to 8.00 a.m.

There are three alternative power sources for the incubator:

1. Mains electricity supply via the charger unit
2. Internal battery

3. External 12v battery

Note: It is recommended that the mains supply option be used wherever possible. When used in this way, the charger unit will operate the incubator and charge the battery. If the mains electricity fails, the internal battery will operate the incubator automatically.

Using Mains Electricity or Generator via the Charger Unit

When using mains electricity to power the incubator, the incubator can be operated and the internal battery charged simultaneously. If the power fails for any reason, the internal battery continues the incubation cycle. When operating from mains electricity, connect the three pin plug to the socket in the left hand side of the incubator console. Plug the incubator into the mains electricity socket using an appropriate plug and switch on the mains. Switch on the incubator and leave until the incubation cycle is complete.

Internal Battery

When planning to work for several days in the field, it is possible to obtain up to five incubation cycles from the internal battery. When using the internal battery in this way, *never* try to use the incubator for *more than five cycles* without recharging the battery or run the incubator for more than 18 hours during any cycle. Always recharge the battery fully at every opportunity using mains electricity.

External 12v Battery

When planning to work in the field for more than five days, or when working in remote areas, it is possible to operate the incubator using an external 12v battery, e.g. vehicle battery, using the connection lead provided in the spares case or the auxiliary battery pack (available as an optional extra).

An external battery cannot be used to recharge the internal battery, only to operate the incubator. When incubating, very little current is drawn and it is usually safe to operate from a vehicle battery for one incubation cycle without risk of discharging the vehicle battery excessively. Never run the incubator from a vehicle battery for more than one cycle if the vehicle is not being driven regularly. Repeated use of the incubator will drain the vehicle battery.

To operate the incubator from an external battery, connect the crocodile clips on the external battery lead to the correct terminals on the external battery (Red to Positive or '+', and Black to Negative or '-'). Connect the three pin plug to the left hand side of the incubator console. Switch on the incubator and check that the 'Power on' indicator is lit.

Note: A poorly maintained external battery may cause the internal battery to discharge.

Always incubate the petri dishes with the incubator and case lids firmly closed. This reduces heat loss and saves battery power.

Always keep the kit in a normal environment, e.g. place on a chair or table to prevent heat loss through the floor and avoid incubating samples outdoors during cold weather.

4.11 Counting Colonies and Recording Results

1. Once the incubation period is complete, remove the petri dishes and their holder from the incubator pot. Remove the lid of a petri dish and observe the surface of the membrane in good incident light.

2. Count all the yellow colonies that have a diameter of between 1 and 3mm. Do not count colonies that are transparent or red/pink on cooling. These are bacteria that do not ferment and cannot be identified without further study. They are not thermotolerant (faecal) coliforms.

Colonies may vary considerably in size. Generally, where the membrane contains a large number of colonies, the colonies are smaller in diameter. Where colonies are fewer, they tend to be larger. This is because the colonies compete for nutrients and will grow larger where there is no competition.

If there are large numbers of yellow colonies, count methodically using the horizontal grid lines. In this way it is possible to count between 1 and 200 colonies per membrane.

3. Convert the count into number of thermotolerant (faecal) coliforms per 100ml and record the result on the daily report sheet. The calculation is made as follows:

Volume filtered	Thermotolerant (faecal) coliforms per 100ml
100ml	Number of colonies x 1
50ml	Number of colonies x 2

5. Care and Maintenance of the Kit

5.1 The Battery

Never

Allow the internal battery to discharge completely.

The useful life of the battery will be maximised if the battery is always kept in a well-charged state. In order to ensure this, it is advisable to recharge the battery fully at weekends whenever possible.

Never

Leave the incubator switched on for more than 18 consecutive hours.

Always

Incubate samples with the incubator lid firmly in place and the kit closed.

Always

Operate the incubator in a vehicle or indoors, on a chair or table to prevent heat loss through a cold floor if possible. Do not operate outside in cold weather.

Always

Recharge the internal battery at the end of a period of work in the field.

Always

Leave the battery in a charged state when the kit is out of use or in storage. ***During storage, recharge monthly.***

To recharge the battery, connect the small three pin plug from the charger to the left hand side of the incubator console. Plug the charger into the mains electricity supply and switch on. Check that the incubator is switched off unless it is in use. Leave the kit and charger until the green light on the charger goes off, indicating that the battery is completely charged. This process may take 12 to 36 hours, depending on the state of charge of the battery. When the battery is completely charged, switch off the charger, disconnect from the mains electricity supply and the incubator, and store in a safe place.

When using the kit in low temperature environments, e.g. less than 10°C, the maximum number of 18 hour incubation cycles on one battery charge should not exceed three.

If any faults or malfunctions are apparent in the kit, refer to the section on ‘Fault Finding in the Incubator, Battery and Charger’.

5.2 Electronic Components and the Incubator

Do not allow water to enter the base of the kit

The electronic components are sealed during construction. This allows a certain tolerance of moisture. However, always immediately dry any spillage of water or other liquids inside the kit.

The temperature of the incubator should be checked periodically, e.g. every three months, as indicated in the section ‘Checking and Recalibrating the Incubator’.

5.3 Filtration Apparatus

At the end of each day, it is good practice to carefully dry all components of the filtration apparatus, including the vacuum and sample cups, and to sterilise the apparatus. This practice prevents corrosion of the metal components of the filtration apparatus.

5.4 Chlorine and pH Comparator and Turbidity Tubes

Avoid scratching the comparator and turbidity tubes. They rely on an adequate transmission of light for accurate results.

Keep the surfaces clean and dry and free of residues that may prove difficult to remove once dry. After use, always wash in clean water. *Never* use detergents, acids or organic solvents.

5.5 Kit Case

The outer case is robust and resilient and will withstand a certain amount of harsh treatment. However, try to avoid abrasion and hard impacts.

5.6 Maintenance

Weekly

1. Wash, rinse and dry the filtration apparatus
2. Apply a smear of silicone grease to the black rubber O-ring
3. Charge the internal battery fully at the end of each week

Quarterly

Check the incubator temperature and recalibrate if necessary.

6. Evaluation and Repair of the Kit

6.1 Fault Finding in the Incubator, Battery and Charger

1. Connect the battery charger unit to the incubator. Do not switch on the incubator. Connect the charger to the mains electricity supply.

Do the red and green lights on the charger unit light up when the mains supply is switched on?

Yes Go to Step 2

No Go to Step 6

2. Charge the internal battery according to the instructions.

How many hours does it take from switching on the charger to the point where the green light is permanently extinguished, i.e. the battery is fully charged?

Note: If after 72 hours the green light is still on, the battery is completely damaged or worn out and will require replacement. Battery replacement should be carried out only by a qualified electronics technician. A battery replacement kit is available from the Robens Centre. Generally, the total discharge of the battery is a sign of misuse.

Go to Step 3.

3. Prepare the kit for temperature checking and calibration as described in the section ‘Checking and Recalibrating the Incubator’.

Disconnect the charger from the mains electricity supply.

Disconnect the charger from the incubator unit.

Switch on the incubator unit.

Do the two red lights on the incubator console light up brightly?

Yes Go to Step 4

No Go to Step 7

4. Leave the incubator switched on until the temperature reading is stable over a period of at least 30 minutes. The time taken for the incubator to reach this point will depend on the ambient temperature, but is usually no more than 3 hours.

Does the incubator hold a temperature of between 43.5 and 44.5°C?

Yes Go to Step 5

No Go to Step 10

5. Does the incubator hold a temperature of between 43.5 and 44.5°C for 4 incubation cycles of 18 hours each, without needing to recharge the battery?

Note: Between each cycle, leave the incubator to cool for at least 8 hours.

Yes Your incubator and battery charger are in good condition

No Go to Step 9

6. The charger fuse may have blown. Ensure that the charger unit is disconnected from the mains electricity supply. Replace the internal fuse in the charger. Reconnect the charger to the mains electricity supply.

Do the red and green lights on the charger light up?

Yes Go to Step 2

No The charger is damaged. Replace with a new unit or organise repair through the Robens Centre or a qualified electronics technician. Then go to Step 1

Note: The battery charger unit is not a normal battery charger. Always use the correct spare parts, which may be obtained from the Robens Centre. The use of a car battery charger on the equipment will cause permanent damage to the battery.

7. Reconnect the battery charger to the incubator unit. Connect the charger to the mains electricity supply. Switch on the incubator.

Do the two lights on the incubator console light up brightly?

Yes Go to Step 8

No Go to Step 11

8. Check the reading on the thermometer.

Does the incubator hold a temperature of between 43.5 and 44.5°C?

Yes Go to Step 9

No Go to Step 10

9. The battery is damaged or worn out. Battery replacement should be carried out by a qualified electronics technician. A Battery Replacement Kit is available from the Robens Centre.

10. Follow the incubator recalibration procedure in the section ‘Checking and Recalibrating the Incubator’.

Does the incubator hold a temperature of between 43.5 and 44.5°C after adjustment?

Yes Go to Step 5

No The incubator is damaged. A Repair Kit is available from the Robens Centre. Contact an electronics technician to carry out repairs, or return the kit to the Robens Centre for repair.

11. Disconnect the battery charger from the incubator unit. Connect the incubator unit to a well charged 12v battery using the lead with crocodile clips supplied with the kit. Switch on the incubator unit.

Do the two lights on the incubator console light up brightly?

Yes Go to Step 12

No The incubator is damaged. A Repair Kit is available from the Robens Centre. Contact an electronics technician to carry out repairs, or return the kit to the Robens Centre for repair.

12 Check the reading on the thermometer.

Does the incubator hold a temperature of between 43.5 and 44.5°C?

Yes The charger unit is damaged. Replace with a new unit or repair through the Robens Centre or a qualified electronics technician, then go to Step 1.

No The incubator is damaged. A Repair Kit is available from the Robens Centre. Contact an electronics technician to carry out repairs, or return the kit to the Robens Centre for repair.

6.2 Replacing the Charger Fuse

To replace the charger fuse you will need the following items:

1. Spare fuse. Spare fuses are supplied with new kits in the spares box.
2. Flat blade screwdriver. The tweezers supplied with the kit can be used if a screwdriver is not available.

Procedure for Replacing Charger Fuse

- a) Ensure that the charger unit is not connected to the mains supply or to the kit.
- b) Place the charger on a firm surface with the indicator lights facing you.
- c) Unscrew the four recessed plastic bolts at the corners of the charger using a screwdriver or the points of the tweezers.
- d) Carefully pull off the lid of the charger. As the charger unit is designed to be shower proof, the lid may be quite tight. It may help to break the seal by carefully inserting one point of the tweezers between the lid and the charger box. Take care not to lose the four plastic bolts which may fall out when the lid is removed.
- e) The fuse is situated on the rear left hand side of the charger. Prise up one end of the fuse with a screwdriver or tweezers and lift the fuse out of its spring clips.
- f) Place the new fuse onto the two spring clips and press into place with a finger.

Replace the charger unit lid and tighten the four plastic bolts with the screwdriver or tweezers. Return to Step 6 of the Fault Finding guide.

6.3 Checking and Recalibrating the Incubator

The equipment supplied for checking and recalibrating the incubator includes the following items:

1. Testing incubator lid with centre hole
2. Thermometer
3. Trimmer tool (similar to a small screwdriver)

Note: It is recommended that the temperature of the incubator is checked once every 3 months.

Procedure for Checking the Incubator Temperature

- a) Remove all contents from the kit and wipe clean the internal surfaces with a clean, damp cloth or paper towel. Pour approximately 20ml of clean water into the incubator pot.
- b) Push the thermometer through the hole in the testing lid.
- c) Replace the incubator lid with the testing lid and thermometer assembly, allowing the bulb of the thermometer to be completely immersed in the water.

Carry out the following procedure at an ambient temperature of between 15 and 25°C.

- d) After ensuring that the internal battery is completely charged, or that the kit is operating from a mains electricity supply or well-charged external 12v battery, switch on the incubator.
- e) Check the temperature of the incubator and observe over a period of 30 minutes to make sure that it has stabilised. The incubator normally takes no more than 3 hours to reach a stable temperature, depending on the ambient temperature.
- f) If, once the incubator has stabilised, the temperature is between 43.5 and 44.5°C, then recalibration is not necessary. If the temperature is not within these limits, follow the recalibration procedure below.

Procedure for Recalibrating the Incubator

- g) Leave the testing lid and thermometer assembly in place and keep the incubator switched on.
- h) Peel off the small black triangle on the right hand side of the incubator console and keep it in a safe place. Insert the trimmer tool into the hole beneath the black triangle and locate the tool in the calibration screw.

Note: Small adjustments of the screw make large adjustments to the temperature. A quarter turn (90°) results in a temperature change of approximately 1°C.

To increase the temperature, turn the adjustment screw anti-clockwise (+). To decrease the temperature, turn the adjustment screw clockwise (-). Make any adjustments in stages, a little at a time. After each adjustment, leave the incubator to stabilise for at least 30 minutes. *The complete recalibration procedure may take several hours. Be patient.*

- i) Once the incubator has been recalibrated to read between 43.5 and 44.5°C, leave it switched on for at least 3 hours. Take note of the temperature at 30 minute intervals to ensure that the temperature is stable.
- j) Switch off the incubator and leave to cool. Do not disconnect the incubator from the mains electricity supply.

- k) The following day, switch on the incubator and allow to reach a stable temperature. If the temperature is not within the correct limit, repeat the checking process detailed in steps (g) to (j).
- l) Dismantle the temperature checking equipment and store in a safe place.
- m) Replace the black triangle over the calibration screw hole.

Note: The above procedure guarantees an average incubator temperature $\pm 0.5^{\circ}\text{C}$. After reaching the set temperature, the temperature in the incubator may vary within $\pm 0.5^{\circ}\text{C}$ during incubation.