OXFAM - DELAGUA

Portable Water Testing Kit

VERSION 4.3
REVISED 2012

CONTENTS

OXFAM - DELAGUA

Portable Water Testing Kit
Users Manual

Revised 2012

This equipment was designed to test for the critical parameters specified in World Health Organization (WHO) Guidelines for Drinking Water Quality, Second Edition, Volume III.

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

For more information about the kit and for technical help and guidance, please contact DelAgua Water Testing Ltd.

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Copies of this manual are available in several languages. Please consult our website for current availability. Abridged versions of the manual are available to download.

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 which provided the translation.

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. We also welcome suggestions from users about ways for improving the kit to meet their own particular needs.

Training

Purchasers of the kit are entitled to participate in a one-day course at the DelAgua office in the use of the kit. The course is free of charge.
DelAgua also offers one and 2-week training courses overseas that include water quality testing, sanitary inspection, water supply disinfection, and use and maintenance of the OXFAM - DELAGUA kit.

Please consult our website for further details or contact us at info@delagua.org

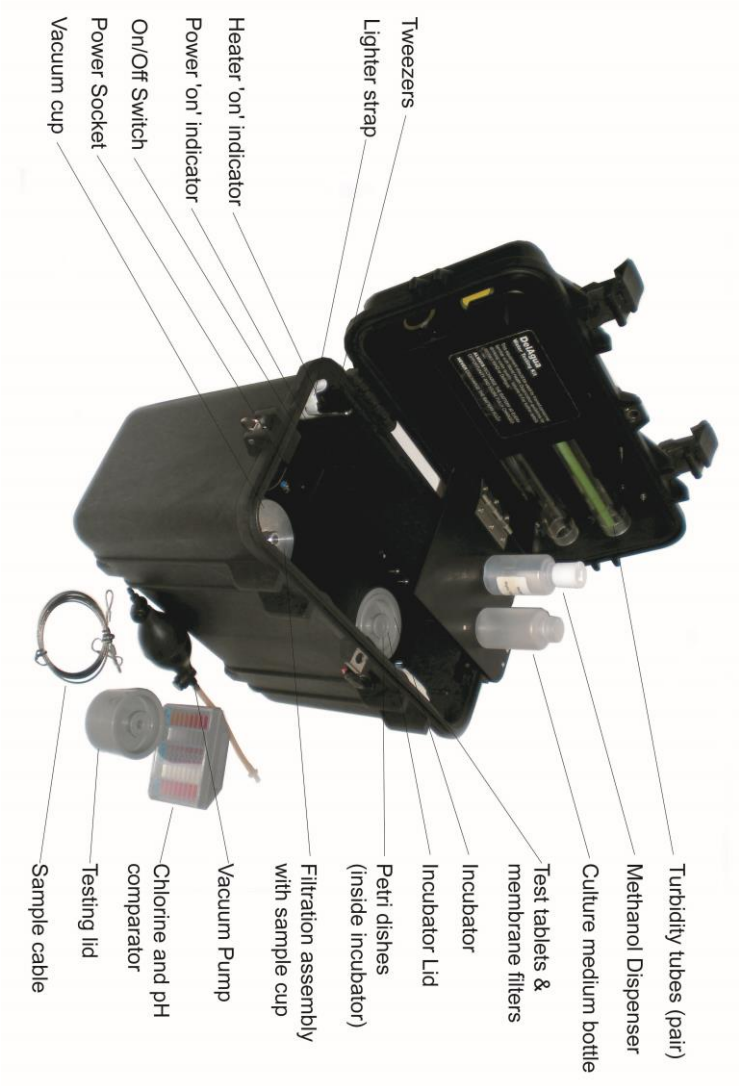
Instructional CD-ROM

This version of the manual includes a CD-ROM that provides guidance on the use and maintenance of the kit and includes video sequences that demonstrate the procedures described in the manual. The CD-ROM will start automatically after it is inserted into your CD-ROM drive. This is the first time we have produced a training CD-ROM for the kit and so we would welcome your general comments and any suggestions that would help us to improve the product in the future.

Note: The main sections of this manual describe the use and maintenance of the single incubator water test kit. The additional procedures for the operation of the dual incubator kit are described in Appendix F.

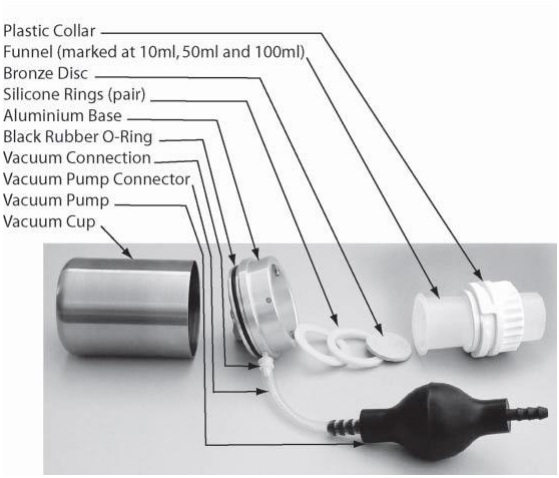
1 The OXFAM - DELAGUA Kit Components

1.1 General

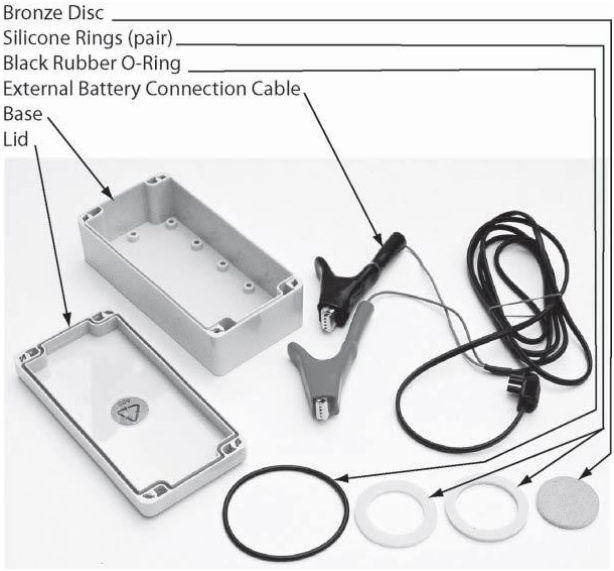


The kit also includes an electronic timer with its own instructions for use.

1.2 Filtration Apparatus and Components



1.3 Contents of the Spares Case



1.4 Materials Needed for Testing

Before you start to use your OXFAM - DELAGUA water testing kit, you will need the following materials:

For preparation of culture medium:

1. Pressure cooker, portable steriliser or autoclave.
2. Electric heating element, gas burner, stove or similar to heat the steriliser.
3. Distilled water (for alternatives see Appendix E).
4. Means of measuring distilled water eg. Measuring cylinder, or graduated beaker.

For using the kit in the field:

1. Methanol (for alternatives see Section 5.8).
2. Paper towels or clean cloths.
3. Wax pencil or marker pen.
4. Report sheets (see Appendix D).
5. Cigarette Lighter (any fluid filled lighter is suitable).

On receipt of a new Oxfam - Delagua kit we would recommend that you recharge the battery fully (Section 6.1) and check the operating temperature of the incubator (Section 7.3).

2 Sampling Programmes

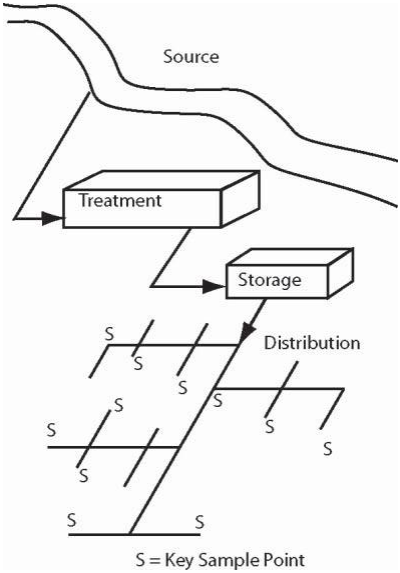
2.1 Selection of sites and frequency of sampling on a network supply

Samples should be taken from locations that 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 the variation that may exist in the system and incorporate this into the sampling programme.

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.



Location of key sample points on a network distribution system.

The recommended minimum frequencies for sampling of 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	One sample per month
5,000 to 100,000	One sample per 5,000 population per month
More than 100,000	20 samples monthly plus one extra sample per 10,000 population

Minimum Frequency of Sampling and Analysis of Unpiped 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 an 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 an 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 an 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 Guide lines for Drinking-Water Quality Volume III. Second Edition, Geneva, 1985.*

We would recommend that you refer to the WHO website (www.who.int) for the latest advice regarding sampling and analysis of water supplies.

3 Preparation of the Kit

3.1 Sterilising the Filtration Apparatus

The sample cup and the filtration apparatus must be sterilised before use and re-sterilised between samples when analysing water from 2 different sources.

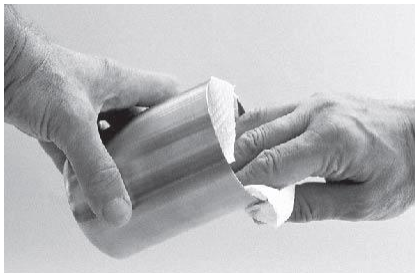
Sterilising the equipment in the field presents some practical difficulties and must be carried out using simple methods. The most appropriate is the use of methanol, which is described below. When methanol is burnt in a low oxygen atmosphere - for example, in the closed sample cup - formaldehyde gas is produced as a by-product of combustion.
Formaldehyde gas is a very effective disinfectant. Methanol is expensive to freight and requires special transport conditions. We would recommend that you first try to obtain methanol in-country from a pharmaceutical supplier, a local hospital or university laboratory. If necessary, however, methanol can be supplied by the DelAguaon request.
If methanol is not available, the filtration apparatus and sample cup can be sterilised by immersion in boiling water for 10 minutes.

Procedure for sterilising the filtration apparatus using methanol

Note: Methanol is the only alcohol suitable for sterilising the filtration apparatus; there is no substitute.

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 loose but not free position (see Section 5.4.4 [9]) which allows the formaldehyde gas to penetrate all areas of the filter head.



Dry the sample cup

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

Add 20 drops (approximately 1ml) of methanol

4. Carefully ignite the methanol in the sample cup using the cigarette lighter. Place the cup on a flat surface which will not be damaged by heat.

   Caution: Keep the mouth of the sample cup away from your face and the hole uppermost to prevent methanol running onto your hand.

Carefully ignite the methanol

5. Allow the methanol to burn for several seconds and, when almost completely burned up (ie. as the flames are dying down), place the filtration head over the sample cup and push firmly into place to form a good seal.

Replace the filtration head

6. Keep the filtration apparatus sealed for at least 15 minutes before use.

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.

3.2 Preparation of Culture Medium in the Laboratory

You will need the following items:

1. 38.1g of Membrane Lauryl Sulphate Broth (MLSB)(a)
2. Distilled water (b). Check that the pH of the water is between 7.0 and 7.8 using the comparator and phenol red tablets (Section 5.2)
3. Ten polypropylene bottles (60ml)
4. Measuring cylinder or graduated flask
5. Clean flask or beaker, approximately 1 litre capacity
6. Pressure cooker, steriliser or autoclave(c)
7. Heating element, stove or burner

(a) The medium is available in 38.1g, pre-weighed amounts from DelAgua
(b) See Appendix E for suggested alternative sources of water
(c) A portable steriliser kit is available from DelAgua

Method

1. Carefully wash the plastic polypropylene 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 the detergent.

2. Measure out 500ml of distilled water using the measuring cylinder or graduated flask. Decant approximately half of the water into the clean flask or beaker.

3. Add the 38.1g of MLSB powder to the distilled water in the clean flask or beaker and stir until the powder has dissolved. Gentle heat can be applied if the powder is slow to dissolve. Add the remaining volume of distilled water and continue stirring to thoroughly mix the broth.
   The culture medium will be a bright red colour when dissolved.

Note: MLSB is a fine, but non-hazardous powder. However, the dust may irritate the nose or upper respiratory tract if inhaled. Take care to avoid creating excess dust when handling the powder and cover the nose and mouth with a cloth or dust mask to reduce exposure. Spillages can be cleaned up using water and an absorbent cloth.

4. Pour a suitable volume of culture medium (approximately 50ml, but no less than 40ml) into each of the 10 polypropylene bottles. This provides sufficient medium in each bottle to carry out 16 tests; the maximum that can be performed in one day using the Delagua kit.

5. Replace the screw caps on the polypropylene bottles. Make sure the caps are secure but do not tighten. Leaving the caps slightly loose prevents the bottles from collapsing during sterilisation.

6. If an autoclave is available, sterilise the bottles at 121oC (equivalent to 1 bar, or 15 psi steam pressure) for 15 minutes. Tighten the caps carefully once the medium has cooled.

7. If you do not have access to an autoclave, 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 base of the cooker), replace the lid and heat to full pressure (about 1 bar or 15psi).
   Once the cooker has reached full pressure allow steam to issue from the release valve for 5 minutes, then time the 15 minutes sterilisation cycle using a stopwatch or clock. At the end of the 15 minutes, switch off the heat and allow the cooker to cool until it is comfortable to touch. Remove the media bottles and tighten the caps.

8. Label the bottles to indicate sterilised contents and the date and batch of medium.

3.3 Preparation of Culture Medium in the Field

You will need the following items:

1. 38.1g of Membrane Lauryl Sulphate Broth (MLSB)(a)
2. Distilled, or clean water(b)
3. 10, polypropylene bottles (60ml)
4. Measuring cylinder or graduated beaker
5. Portable steriliser(c) or pressure cooker or cooking pot or pan

(a) The medium is available in 38.1g, pre-weighed amounts from DelAgua
(b) See Appendix E for suggested alternative sources of water
(c) A portable steriliser kit is available from DelAgua

Method

1. Carefully wash the plastic polypropylene 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 the detergent.
2. Use distilled water if possible. If this is not available obtain the cleanest water possible. DO NOT use water that has been treated with chlorine or any other chemical disinfectant.
3. Use the comparator and phenol red tablets in the kit to check that the pH of the water is between 7.0 and 7.8. If it is not, it will be necessary to find an alternative source of water.
4. Measure out 500ml of clean water in a beaker.
5. Add 38.1g of the MLSB powder to the 500ml of water in the beaker. Mix to dissolve the powder completely. Apply gentle heat if the powder is slow to dissolve. The culture medium will be clear with a bright red colour when dissolved.
6. Pour a suitable volume of culture medium (approximately 50ml, but no less than 40ml) into each of the 10 polypropylene bottles. This is sufficient medium in each bottle to carry out 16 tests; the maximum that can be performed in one day using the Delagua kit.
7. Replace the screw caps on the polypropylene bottles. Make sure the caps are secure but do not tighten. Leaving the caps slightly loose prevents the bottles from collapsing during sterilisation.
8. If a pressure cooker is available, sterilise the culture medium as described in Section 3.2, paragraph 7.
9. If a pressure cooker or portable steriliser is not available, the medium can be sterilised using a process called Tyndellisation. Note, this

procedure takes 3 days. Place the bottles of culture medium into a cooking pot or pan of boiling water, taking care to ensure that the bottles do not come into contact with the base of the pan (use a rack or stand) or become submerged. Boil for 20 minutes. Leave the medium to stand for 24 hours at room temperature (20-30oC) in the dark. On the following day heat the medium in boiling water for a further 20 minutes and, once again, leave to stand for 24 hours. On the third day repeat the heat treatment. The medium should now be sterile.

3.4 Storage of Culture Medium

Sterile MLSB will be stable for up to 6 months if stored in a refrigerator (between 4 and 6oC). Alternatively, the medium can be stored for up to 3 months in a cool, dark place. If the medium has been stored for several days below 6oC a deposit may form which dissolves when the medium is warmed and gently shaken. The deposit is caused by the lauryl sulphate coming out of solution.

If signs of deterioration are observed, eg. cloudiness or yellow colouration, the contents of the bottle must be discarded.

3.5 Sterilising the petri-dishes

1. Wash the dishes in a solution of mild detergent, rinse thoroughly with clean water and dry.
2. Assemble the dishes into batches of 16 in the straps.
   EITHER
3. Sterilise the petri dishes in an autoclave, steam steriliser or pressure cooker at 121oC for 15 minutes (see section 3.2 paragraphs 6 and 7).
   OR
4. Place the dishes in a conventional oven at 180oC for 30 minutes.
   OR
5. Plunge the bases and lids of the dishes into boiling water for 10 minutes. Pour away the water and assemble the dishes as they dry, but while they are still hot.
   OR
6. Add a few drops of methanol (or ethanol) to a clean cloth and wipe the inside of the lid and the base of each petri-dish. Assemble the petri-dishes and allow the alcohol to evaporate before use.

OR

7. 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.

3.6 Disposal of Contaminated Material

Note: To minimise the risk of infection from contaminated materials, take care not to touch contaminated membranes directly with your hands. Do not eat, drink or smoke while handling contaminated materials. Wash your hands immediately after you have touched any contaminated material and after you have finished your work.

Contaminated material, such as used membranes and pads, MUST be made safe before disposal. DO NOT discard non-sterile membranes and pads into the environment since they pose a major risk to public health.
After you have completed the analysis, stack the petri-dishes in the straps and sterilise the dishes and contents at 121oC for 30 minutes using an autoclave, steriliser or pressure cooker. Alternatively, plunge the petridishes, pads and membranes into boiling water and heat for at least 30 minutes (use a dedicated pan for this procedure. DO NOT use a pan that will subsequently be used for food preparation or other domestic purposes). After sterilisation, the used membranes and pads may be destroyed by incineration.
The petri-dishes must be carefully washed with detergent after use, rinsed with clean water and dried.

3.7 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.
You might find it more convenient to dispense the pads into the petridishes at your base to avoid the need to take the dispenser and pads into 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 (see Section 5.4.4[3] for sterilisation methods). Some kit operators prefer this method to using the dispenser.

3.8 Methanol Dispenser

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. 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 to close the dispensing nozzle after using the kit as the methanol will evaporate.

Note: Methanol is highly flammable. Keep methanol away from naked flames.

4 Sampling Methods

4.1 Sampling from a Tap

1. Remove any attachments from the tap; eg. nozzles, pipes, etc. Check that the tap does not leak and that all seals are in good condition.

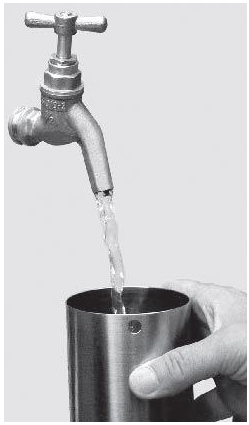
Remove attachments from tap

2. Carefully clean the mouth of the tap with a clean cloth or tissue to remove any dirt or grease. Open the tap and leave water running for at least one minute before taking a sample. Do not adjust the flow of water during this time. This ensures that any deposits in the pipes are washed out and the water sample is representative of the water in the supply pipes.

Leave the tap running for at least one minute

3. Take a water sample with the non-sterile vacuum cup. Rinse the cup twice with the sample water before taking the sample. Analyse the sample for chlorine residual and turbidity using the methods in sections 5.2 and 5.3.

4. If the chlorine residual and turbidity results suggest that there is a risk of microbiological contamination (see Section 5.1), then take a second sample for bacteriological analysis using the sterile sample cup.



Take a sample for bacteriological analysis using the sterile sample cup

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

1. Where there is safe and adequate access to the source it may be possible to take samples by hand.

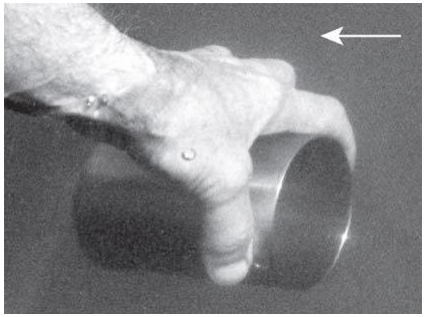
2. Grasp the sample cup firmly, keeping your fingers clear of the top of the cup to avoid contamination, and dip the open mouth of the cup into the water.

3. Submerge the cup about 30cm below the surface of the water and scoop up the water sample. This scooping action ensures that no external contamination enters the sample cup.

Take sample from approximately 30cm below the surface

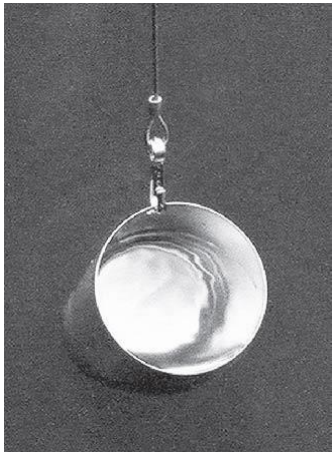
4. Lift the sample cup carefully and place on a clean surface where it cannot be knocked over.

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



In rivers and streams take samples against the current flow (the arrow shows the direction of flow)

6. In many cases it may be inconvenient or dangerous to enter the water. The sample cup can be lowered into the water from a firm area of riverbank or river crossing by fastening the 2m sample cable to the hole in the lip of the sample cup.



Lower the sample cup into the water using the 2m cable

Note: It is important that you obtain a sample which 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 this reason it is often better to sample with the help of the cable supplied with the kit.

4.3 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. Make sure the clip is closed and secure.

Fasten the sampling cable to the sample cup

2. If necessary, increase the length of the cable by attaching a rope or string to the sample cable. Take care not to lose the sample cup.

Increase the length of the cable if necessary using string or rope

3. 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. If possible keep fingers clear of the cable or rope so as not to contaminate the water body.

Lower the sample cup into the well or tank

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

5 Processing of Samples using the Kit

5.1 Introduction

The first tests that should be carried out on a drinking water sample are the determination of chlorine residual, pH and turbidity. The results from these tests will indicate whether or not the water sample is likely to contain living microorganisms and whether it is necessary to carry out analysis for thermotolerant coliform bacteria. The sample must be taken in a clean, but not necessarily sterile cup, eg. the vacuum cup. Rinse the vacuum cup several times with the water that is to be analysed before taking a sample for analysis (see Section 4).

If the results of the analysis are as follows:

- Free chlorine residual greater than 0.2mg/litre (0.2ppm) and
- Turbidity less than 5TU

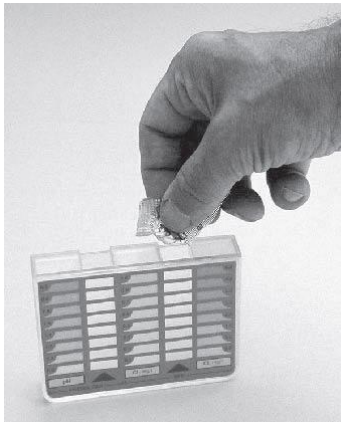
it is unlikely that the sample will contain thermotolerant (faecal) coliform bacteria and therefore it may not be necessary to carry out thermotolerant coliform analysis. If the results do not meet these criteria, it will be necessary to carry out thermotolerant coliform analysis. Under these circumstances, samples for analysis must be taken with the sterile sample cup.

5.2 Analysis of Free Chlorine Residual and pH

1. Wash the comparator cells three times with the water that is to be analysed and finally fill both cells with the sample.

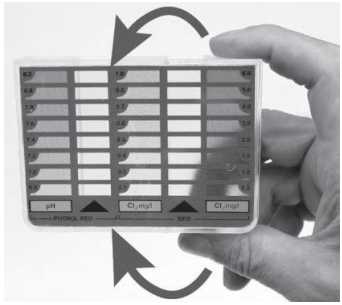
Fill the comparator cells with sample water

2. Drop one DPD No 1 tablet into the right hand cell (C12) and one Phenol Red tablet into the left hand cell (pH).



Add the DPD No 1 and phenol red tablets

3. Replace the lid of the comparator and push down firmly to seal. Invert the comparator several times until the 2 tablets have dissolved completely. If the tablets are slow to dissolve use the plastic paddle from inside the box of tablets to crush and mix the tablets in the sample water. Do not shake the comparator as this will introduce air.



Invert the comparator to dissolve the tablets

4. Immediately read the free chlorine residual (mg/litre) and pH by holding the comparator up to daylight and matching the colour in the cells with the standard colour scales. If the colour falls between 2 standard colours, then it will be necessary to estimate the concentration. Record the result on the daily report sheet (see Appendix D for an example).

5. To test for total chlorine residual, DO NOT discard the liquid in the comparator. Remove the lid and add one DPD No 3 tablet to the right hand cell (C12).

6. Again, invert the comparator several times to dissolve the tablet (use the plastic paddle if the tablet is slow to dissolve). Leave the colour to develop for 10 minutes. Read the total chlorine residual (mg/litre) by matching the colour in the cells with the standard colour scale.

7. Subtract the free chlorine result from the total chlorine result to obtain the combined chlorine concentration:

Summary		
Phenol Red	=	pH
DPD No 1	=	Free chlorine residual
DPD No 1 plus DPD No 3	=	Total chlorine residual
Total - Free chlorine	=	Combined chlorine

5.3 Turbidity Analysis

Note: The turbidity tube covers the range 5 to 2,000 TU

1. Carefully remove the 2 halves of the turbidity tube from their clips in the lid of the case. Push the upper tube (open at both ends) squarely into the lower tube and align the graduation marks up the side. Look through the open end of the tube at the black circle printed on the yellow base of the tube; this is the marker. Ensure that there is good illumination available. Normal daylight is adequate for this purpose.

Assemble the turbidity tube

2. Hold the tube vertically and slowly pour the water sample into the tube until the marker just disappears when viewed from the top of the tube. Avoid creating bubbles, as these may cause false readings. Do not strain to see the black circle as this can sometimes cause biased results.
   Alternatively, the procedure can be carried out in reverse by filling the tube with the sample and then slowly pouring out the water.

Fill the tube until the marker disappears

3. Hold the tube vertically and read the turbidity using the graduations on the side of the tube. The graduations follow a logarithmic scale with the most critical values marked on the side of the tube. The result is the value of the line nearest the water level. This permits a reasonable estimation of the turbidity of the water sample. Alternatively, you can judge the distance of the water level from the 2 nearest graduation marks and calculate a more accurate turbidity value. Remember, however, that you are reading a log scale.

Estimate the turbidity using the graduation marks. The tube is shown horizontal for convenience

5.4 Bacteriological Analysis of Water

Note: Microbiological analysis presents some special risks to the health and safety of yourself and others. It is vitally important that you take great care when handling potentially contaminated materials, such as the petridishes, membranes and pads that have been used for the growth of microorganisms. Although most of the bacteria that will grow on the membrane are relatively harmless, some of the colonies may contain pathogenic bacteria. High standards of hygiene should be applied at all times:

- Never eat drink or smoke when carrying out microbiological tests.
- Do not touch colonies with your fingers or with everyday objects such as pens and pencils that you may use again for other purposes.
- Always wash your hands thoroughly after handling contaminated, or potentially contaminated materials.
- Cover wounds with a waterproof dressing.
- Do not carry out microbiological tests in food preparation areas.
- Do not dispose of contaminated materials into the environment. Always sterilise the items before disposal or cleaning.
- Keep all non-essential personnel, particularly children, away from the work area when handling contaminated materials.
- Always keep your work area clean and tidy.
- Clean and disinfect (methanol, ethanol or a weak solution of domestic bleach can be used or this purpose) the work surfaces after you have finished the analysis.

5.4.1 Introduction

The analysis of water samples for thermotolerant 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 which feeds coliform bacteria, but inhibits the growth of any other bacteria caught in the filter. To ensure that only thermotolerant coliform bacteria grow, the filter is kept at 44°C in the kit's incubator. During this time the coliform bacteria multiply many times to form colonies that can be seen with the naked eye. Thermotolerant coliforms are recognised by their ability to produce a colour change (from red to yellow) in the culture medium at 44°C. It is normal practice in water microbiology to express the results as colony-forming units per 100ml of water (CFU/100ml).

Thermotolerant coliforms are of sanitary significance when present in drinking water supplies. Users should refer to country-specific water quality standards or guidelines, or to the latest edition of the World Health Organization Guidelines for Drinking Water Quality (available on-line at www.who.int) 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 coliforms, can be used to indicate hygiene problems in large distribution networks. Total coliform analysis is carried out using the same procedure as for thermotolerant 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 in Section 7.3. However, this is not convenient when carrying out both thermotolerant coliform and total coliform analysis on a regular basis. A dual incubator kit (see Appendix F) is available from DelAgua which allows both tests to be carried out simultaneously.

5.4.2 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 which must always be kept clean and sterile. These are as follows:

a) All those areas in direct contact with the water sample, eg. 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, eg. the internal surface of the petri-dishes and the absorbent pads.
c) Parts in contact with the membrane filters, eg. 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 which 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 cigarette lighter. Hold the tips in the flame for five seconds and allow them 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.

5.4.3 Selection of Appropriate Sample Volumes for Coliform Analysis

The most appropriate volume to process is that which allows the most accurate count of the bacterial colonies. It is generally agreed that 100 colonies is the maximum that can be counted reliably on a 47mm membrane. Counts above 100 are considered an estimate. If a large number of colonies develops on the membrane you can either divide the plate into sections, count the colonies in one section and multiply the count by the number of sections, or repeat the analysis with a smaller volume of the sample and then adjust the result to give a count per 100ml of the original sample.
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 sites where this information does not exist, the following paragraphs provide some guidance on sample volumes.

Treated water and water in piped distribution systems
Historically, the microbiological quality of drinking water has been assessed using the number of bacteria present in a standard volume of
100ml. Treated water and water in a piped distribution network are unlikely to contain large numbers of thermotolerant coliform bacteria.
For these waters we would recommend using a 100ml sample.
Suggested sample volumes for thermotolerant coliform analysis by the membrane filtration technique (alternative volumes are shown in brackets).

Source	Sample volume
Waters in treatment plants after partial treatment	50ml (100ml or 10ml)
Waters in treatment plants after full treatment	100ml
Reservoirs, distribution networks and household taps	100ml

Other Water Sources
Recommended sample volumes for each source are shown below.

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

Source	Sample volume
Lakes, ponds and other surface waters	10ml (1ml*)
Protected groundwater, eg. wells and springs	100ml (50ml or 10ml)
Unprotected groundwater, eg. open dug wells and springs	50ml (10ml or 1ml*)

*Note: This volume will require the use of sterile pipettes and dilution water.

Please note, these volumes are only a guide. 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.

5.4.4 Sample Processing for Thermotolerant (Faecal) Coliform Analysis.

1. Using the absorbent pad dispenser, place one pad into each petri-dish (this operation may be done at base before leaving for the field). If the dispenser is damaged, the pads can be dispensed using the sterilised tweezers.

Dispense one pad into each petri dish

2. Allow the medium to warm to ambient temperature before use. Pour enough culture medium onto the absorbent pad in the petri dish to soak the pad and leave a slight excess (approximately 2.5ml). Try not to let the medium run down the side of the bottle onto the pad, and do not allow the bottle neck to come into contact with any external objects. Replace the bottle cap immediately. If too much medium has been added, drain off the excess and wipe up any spillage with an absorbent cloth or tissue. Always ensure that a slight excess remains in the petri-dish to prevent the pad drying out during incubation.

Pour the medium onto the pad

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 for approximately 5 seconds and leave to cool.

Flame the tips of the tweezers

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.

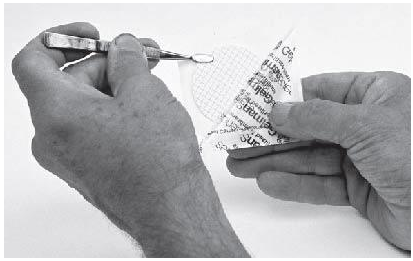
Allow the tips of the tweezers to cool

5. Remove the sterile sample cup from the filtration apparatus. Push the filtration apparatus firmly onto the vacuum cup (if this is difficult, lubricate the black rubber O-ring with silicone grease; see Section 6 for maintenance of the kit). 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.

Assemble the filtration apparatus

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 the packet.
   Hold the membrane only by the edge and do not let the membrane filter touch anything while it is being transferred to the filtration apparatus.



Remove membrane from packet

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 facing upwards) onto the bronze disc filter support. Replace the filter funnel and collar immediately, without allowing them to come into contact with any external objects. Hold the funnel between the thumb and forefinger to ensure that the collar will not slip off and that the fingers do not come into contact with the interior surface of the funnel.

Place the membrane onto the filter support

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

Fit the filter funnel and plastic collar

Note: The plastic collar has 3 adjustment positions:

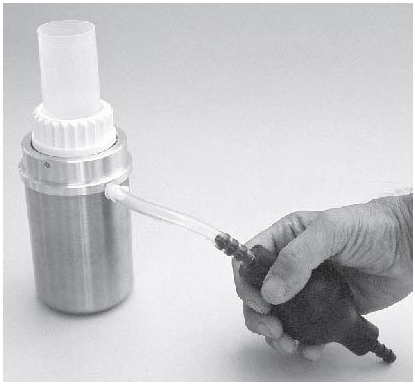
1. Completely free - the apparatus can be dismantled when in this position.
2. Loose but not free - all interior surfaces are exposed to the atmosphere. This is the position used when sterilising the apparatus.
3. Fully tightened - the funnel forms a tight seal between the membrane support and the membrane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

10. Rinse the sterile sample cup once with the water before taking the sample. Take care not to allow external contamination (eg. dirt and debris) to enter the sample cup.

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. To avoid damaging the membrane, tilt the filtration apparatus and carefully pour the first few millilitres of water down the inside of the filter funnel. Return the filtration apparatus to the Upright position and continue adding the sample. Take care not to allow external debris to enter the funnel.

Pour the sample into the filtration funnel

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. Do not allow excess air to be drawn down through the filter once all the water has gone through.



Attach the vacuum pump and create a vacuum to draw the sample through the membrane

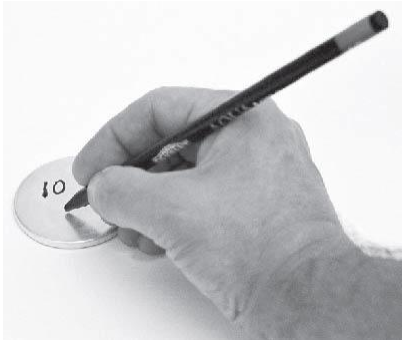
13. Unscrew the collar and remove the funnel and collar with one hand. Using the sterilised tweezers in the other hand, lift the membrane carefully from the filtration base. Hold the membrane by the edge only.

Remove the membrane from the filter head

14. Remove the lid of a prepared petridish and place the membrane, grid side uppermost, onto the absorbent pad soaked in culture medium.
    Start at one edge (it is easier to use the one at a greater distance from the wall of the petri-dish) and lower the membrane on to the pad by 'rolling' so as to avoid trapping air bubbles under the membrane.

Lower the membrane on to an adsorbent pad

15. Replace the lid of the petri-dish and mark the lid with sample information eg. volume filtered, source, time and date; or a code which relates to details on the daily report sheet. A wax pencil or marker pen is suitable for this purpose (the writing needs to be easily removed after the tests are complete).



Replace the lid and label the petri dish

16. Place the petri-dish with the lid uppermost into the carrier (insert the dish at the bottom so that new dishes can be taken from the top of the carrier) and return the carrier to the incubator pot. All 16 petri dishes should be in the rack during incubation. This allows for an even distribution of heat in the incubator. Replace the incubator lid.

17. Resterilise the filtration apparatus (Section 3.1).

Stack the petri-dishes and place in the incubator

5.4.5 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 processing the first and last sample is not more than 3 hours. This restricts the resuscitation time to a maximum of 4 hours. In cold weather resuscitation can be achieved by keeping the samples close to the body (eg. In hands or upright in a pocket but take care to avoid media leaking from the petri-dishes).
Resuscitation time is particularly important for chlorinated waters or marine water where the thermotolerant 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.

5.4.6 Sample Incubation

Incubate the samples for 16 to 18 hours. The incubator is designed to maintain a temperature of 44oC +/- 0.5oC. Always incubate the petri-dishes with the incubator and case lids closed to reduce heat loss and save battery power (note that it is not possible to close the kit lid if you are using the double incubator kit, or if you are powering the kit using the charger or another external power source). Place the kit on a chair or table to prevent heat loss through the floor and avoid incubating samples outdoors during cold weather. In order to maximise battery life, do not leave the incubator on for more than the incubation period, ie. 16 to 18 hours.

There are 3 alternative power sources for the incubator:
1. Mains electricity supply via the charger unit
2. Internal battery
3. External 12v battery (or your vehicle battery)

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 at the same time charge the battery. If the mains electricity fails the internal battery will operate the incubator.

Using mains electricity or generator via the charger unit
When using mains electricity, 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 3-pin plug to the socket in the left hand side of the incubator console.
Plug the battery charger 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.

Connect the kit to the mains power supply via the battery charger

Internal Battery
It is possible to obtain up to 5 incubation cycles from the internal battery when it is new. The number of cycles will reduce as the battery ages. When using the internal battery in this way, do not use the incubator for more than 5 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
If you are planning to work in the field for more than 5 days, or to work in remote areas, it is possible to operate the incubator using an external 12v battery, eg. vehicle battery, using the connection lead provided in the spares case.
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

Connecting the kit to an external battery using the crocodile clips connection

3-pin plug to the left hand side of the incubator console. Switch on the incubator and check that the 'Power On' indicator is lit.
An external battery cannot be used to recharge the internal battery, only to operate the incubator. Very little current is drawn during incubation 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.

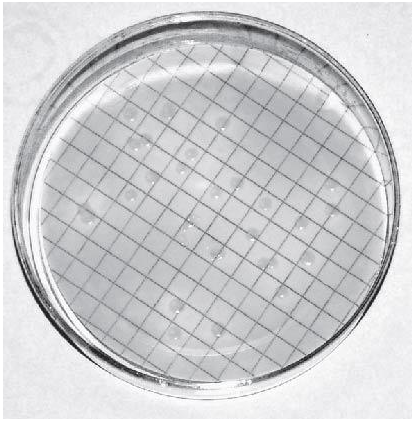
Note: A poorly maintained external battery may cause the internal battery to discharge.

5.4.7 Counting Colonies and Recording Results

Note: It is important that counting is completed as soon as possible after the petri-dishes have been removed from the incubator (certainly within 15 minutes) as the positive colonies will change colour on cooling and standing.

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. If necessary, use a hand lens to examine the colonies.

2. Count all the yellow colonies which have a diameter of between one and 3 millimetres (an example is shown on the front cover).
   Frequently, 2 or more colonies will merge together. Examine the shape of the colony and it is usually clear how many colonies have merged



Count all yellow colonies with a diameter between one and three millimetres

together. Count each of the sub-colonies. Do not count colonies that are transparent, red/pink or blue/grey. These are bacteria which do not ferment lactose and cannot be identified without further study. They are not thermotolerant coliforms.
Colonies may vary considerably in size. Generally, when the membrane contains a large number of colonies, the colonies are smaller in diameter. When 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 up to100 colonies on a membrane. If there are more than 100 colonies on the membrane, the number can be estimated by dividing the membrane into sections and counting the number of colonies in one section. Multiply the result by the number of sections to obtain an estimate of the total number of colonies on the membrane.

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

Volume filtered	**Thermotolerant coliforms per 100ml**
100ml	Number of colonies x 1
50ml	Number of colonies x 2
10ml	Number of colonies x 10
1ml	Number of colonies x 100

5.4.8 Disposal
All contaminated materials should be sterilised before disposal to avoid creating a risk to the public. DO NOT discard contaminated membranes and filter pads into the environment. Refer to Section 3.6 for recommended procedures to sterilise contaminated materials.

6 Care and Maintenance of the Kit

6.1 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. If the kit is in storage, recharge the battery once a month.

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 3-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. The LED on the battery charger will be an orange/amber colour. Continue charging until the LED light turns green. This process will normally take 3-5 hours with a new battery, but may take longer with an older or fully discharged battery. When the battery is completely charged, switch off the charger, disconnect the charger from the mains electricity supply and the incubator and store in a safe place.

When using the kit in low temperature environments, eg. less than 10°C, the maximum number of 18 hour incubation cycles on one battery charge should not exceed 3.
If any faults or malfunctions are apparent in the kit, refer to the section on 'Fault Finding in the Incubator, Battery and Charger' in Section 7.1.

6.2 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, eg. Every month, as indicated in Section 7.3 'Checking and Recalibrating the Incubator'.

6.3 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.

6.4 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.
Approximately once a month wash the comparator and turbidity tubes in a dilute solution of mild detergent and rinse thoroughly with clean water.
Never use acids or organic solvents.

6.5 Kit Case

The outer case is robust and can withstand a certain amount of harsh treatment. However, try to avoid abrasion and hard impacts. Periodically clean the case with warm water and mild detergent.

6.6 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

Monthly

Weekly maintenance, plus:

1. Check the incubator temperature and recalibrate if necessary
2. Clean all components of the kit, including the case
3. Check all components for damage that may affect the operating of the kit

7 Evaluation and Repair of the Kit

7.1 Fault Finding in the Incubator, Battery and Charger

1. Connect the battery charger to the incubator. Do not switch on the incubator. Connect the charger to the mains electricity supply.
   Does the LED 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 given in Section 6.1.
   How many hours does it take from switching on the charger to the point when the LED light turns green, ie the battery is fully charged?

   If after 48 hours the LED is not green, the battery is 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 DelAgua. Generally, 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' in Section 7.3.
   • 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 for4 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. If the charger is fitted with a 3-pin UK style plug, the main fuse may have blown. Ensure that the charger unit is disconnected from the mains electricity supply. Replace the fuse in the plug. Reconnect the charger to the mains electricity supply.
   Does the LED on the charger light up?

Yes	Go to Step 2
No	The charger is damaged. Replace with a new unit or organise repair through DelAgua. Then go to Step 1

   Note: The battery charger has been selected to deliver the correct output for the internal battery. 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 2 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 DelAgua.

10. Follow the incubator recalibration procedure in the section ‘Checking and Recalibrating the Incubator’ in Section 7.3
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 Delagua. Contact an electronics technician to carry out repairs, or return the kit to DelAgua for repair.

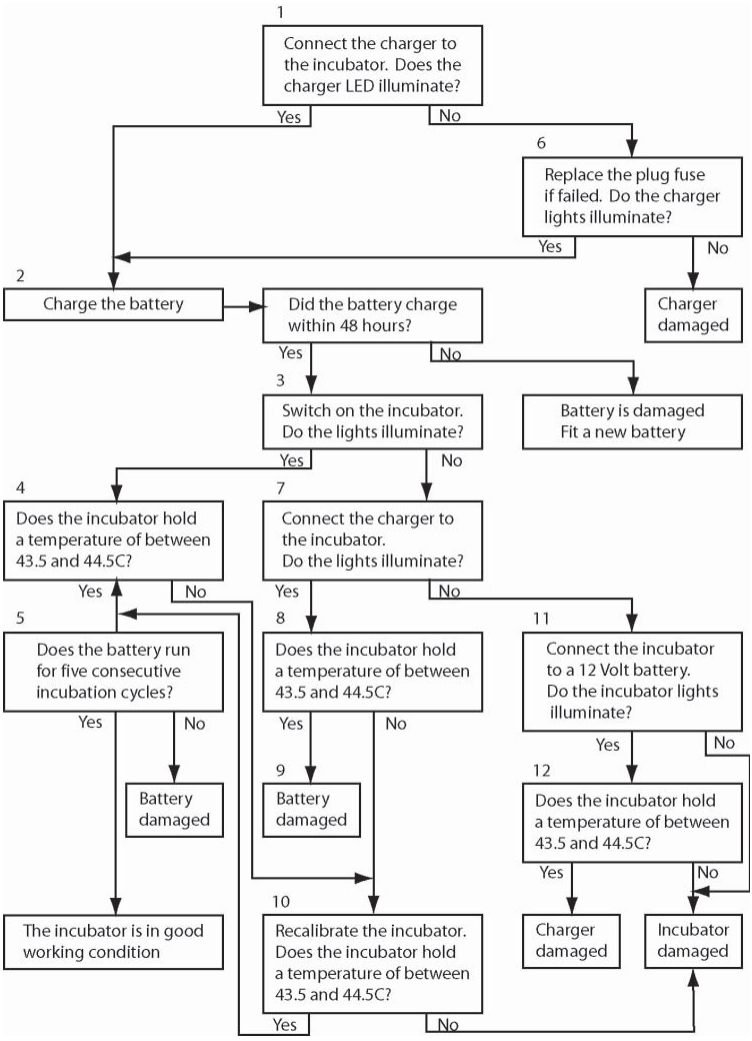
11. Disconnect the battery charger from the incubator unit. Connect the incubator unit to a well charged 12v battery using the lead with crocodile clips supplied with the kit. Switch on the incubator unit.
Do the 2 lights on the incubator console light up brightly?

Yes	Go to Step 12
No	The incubator is damaged. A Repair Kit is available from DelAgua. Contact an electronics technician to carry out repairs, or return the kit to DelAgua 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, then go to Step 1.
No	The incubator is damaged. A Repair Kit is available from DelAgua. Contact an electronics technician to carry out repairs, or return the kit to DelAgua for repair.

7.2 Fault Finding Chart



A damaged or worn out kit can only be repaired by DelAgua if a funding agency will accept the costs involved, ie. repair, freight and insurance.

Send kits for repair to the address on the back cover of this manual. Before sending your kit, please remove all loose items from the box such as the turbidity tubes, chlorine and pH comparator, filtration apparatus, etc. These items may be lost or damaged in transit, and their additional weight may increase freight costs.

7.3 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 month.

1.3.1 Procedure for checking the incubator temperature

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

1. Remove all contents from the kit and wipe clean the internal surfaces with a clean, damp cloth or paper towel. Pour approximately 50ml of clean water into the incubator pot (giving a depth of approximately 20mm).
2. Push the thermometer through the hole in the testing lid.

Incubator fitted with testing lid and thermometer assembly

3. Replace the incubator lid with the testing lid and thermometer assembly. The bulb of the thermometer should be completely immersed in the water.
4. Ensure 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.
5. 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.
6. Once the incubator has stabilised, if the temperature is between 43.5 and 44.5°C, then recalibration is not necessary and the water may be removed with tissue or a cloth. If the temperature is not within these limits, follow the recalibration procedure below.

7.3.2 Procedure for Recalibrating the Incubator

1. Leave the testing lid and thermometer assembly in place and keep the incubator switched on.
2. Insert the trimmer tool into the hole on the side of the unit and locate the tool in the calibration screw (blue trim).

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

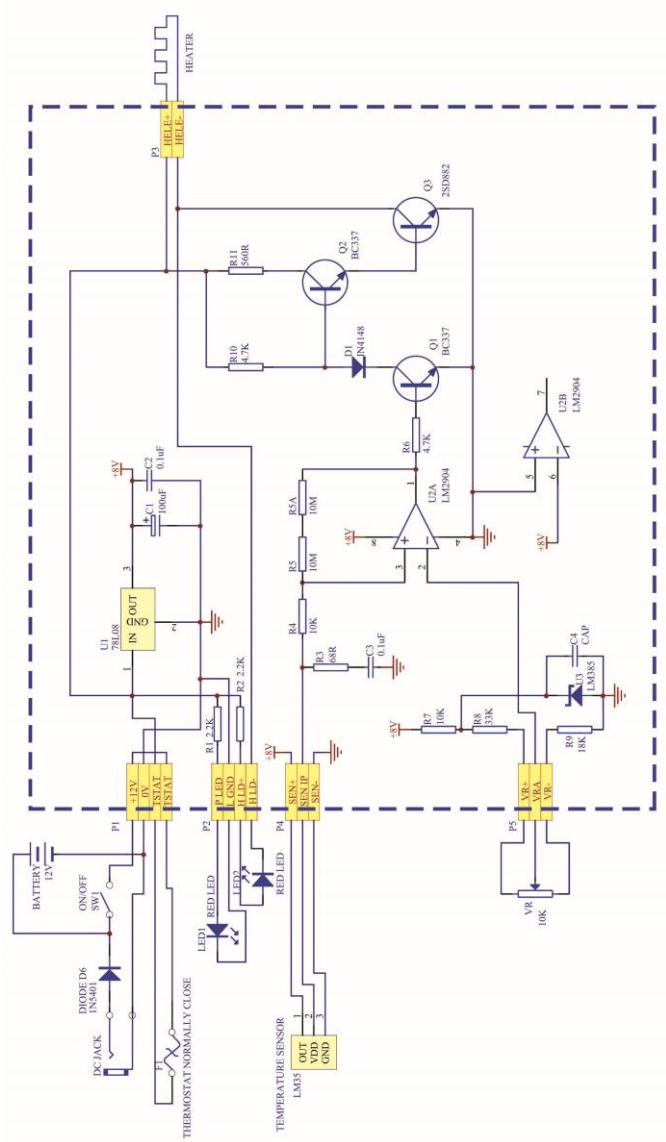
3. To increase the temperature, turn the adjustment screw anti-clockwise. To decrease the temperature, turn the adjustment screw clockwise. Make the adjustments in stages, a little at a time. After each adjustment, leave the incubator to stabilise for at least 30 minutes. The complete recalibration procedure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

4. 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.
5. Switch off the incubator and leave to cool. Do not disconnect the incubator from the mains electricity supply.
6. 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 recalibration process detailed in Steps (1) to (4).
7. Dismantle the temperature checking equipment and store in a safe place. Empty the water out of the incubator and dry the inner surfaces.

Note: The above procedure guarantees an average incubator temperature of 44°C +/- 0.5°C. After reaching the set temperature, the temperature in the incubator may vary within +/- 0.5 °C during incubation.

APPENDICES

Appendix A
Incubator electronic circuit diagram.



Appendix B
Field Checklist
Before leaving for the field, check that you have the following items:

Kit

- Filtration apparatus
- Sample cup
- Vacuum cup
- Sample cable
- Vacuum pump
- Turbidity tubes (pair)
- Chlorine/pH comparator
- Tweezers
- Petri dishes
- Spares box (complete)
- Lighter

Consumables

- Culture medium (one bottle per day and a spare)
- Membrane filters
- Absorbent pads and dispenser
- DPD No 1 tablets
- DPD No 3 tablets
- Phenol red tablets
- Methanol
- Daily report sheets (Appendix D)
- Paper towels or clean cloth
- Some clean water to rinse the equipment after use
- A wax pencil or marker pen

Appendix C
Spares list
The following spares and consumables are available from DelAgua. Please telephone, fax or email for a current price list.
Alternatively, visit our web site at www.delagua.org

Components

Battery replacement kit containing:	Battery 12v 9.5 Ah Silicone sealant
Temperature check kit containing:	Incubator testing lid with hole Thermometer Adjuster/trimmer tool
Electrical repair kit containing:	Electrical circuit Temperature chip Sealant Foam compound Adhesives
Filtration apparatus (complete)	Spares box (specify empty or complete)
Filter funnel with plastic collar	Tweezers
Filtration base	Chlorine/pH comparator
Vacuum cup	External battery cable
Sample cup	Battery charger
Vacuum pump	Silicone grease (2g or 100g)
Sample cable	Polypropylene bottles 60ml (x10)
Bronze disc with sealing gasket set	Methanol dispenser plastic
Black rubber O-ring	Petri-dishes
Turbidity tubes (pair)	

Consumables
Membrane filters and absorbent pads (x200)
Pad dispenser
Culture medium: 38.1g tub for 500ml of growth medium (sufficient for 200 tests)
Culture medium: 500g tub for 6.5 litres of growth medium (sufficient for 2,600 tests)
DPD No 1 Tablets (x250)
DPD No 3 Tablets (x250)
Phenol red Tablets (x250)
Consumables for 200 tests

Optional Extras

Portable conductivity meter
Portable steriliser kit

Other items of equipment and the consumables for the analysis of a range of chemical parameters can be supplied upon request.

Appendix D
Daily report sheet

Daily Report Sheet

Health Region_____ Authority_____

Date___/___/___ Code_____

Province_____ District_____

Community_____ Analyst_____

Comments

Source of sample	Time	Colour	Odour	Turbidity (TU)	Chlorine (mg/L)			pH	Thermotolerant coliforms (TTC)		
					Free (DPD No 1)	Total (DPD No 3)	Combined (total - free)		vol. filtered (ml)	No. of colonies	TTC per 100ml

Appendix E
Alternative sources of water for media preparation

We would strongly recommend that you try to find a source of distilled water to prepare the medium. However, we appreciate that this may not always be possible and would suggest the following as suitable alternatives:

- A high quality bottled water that has not been treated with chlorine or any other disinfectant that has a residual activity.

- Rainwater. Collect a sufficient volume of rainwater in a clean container and leave it to stand overnight to allow any suspended matter to settle out. Carefully pour off the water into a separate clean container. Alternatively, the rainwater can be clarified by filtration through a membrane filter or, more quickly, through one of the membrane pads (see the relevant paragraphs of Section 5.4.4).

- Water from a well protected groundwater source. Groundwater from a well protected source is usually suitable for preparing the medium. However, check the pH of the water before use to be sure that it is not too acidic or alkaline (see Section 3.2).

Appendix F
Additional instructions for operating the dual incubator kit
The dual incubator kit allows you to double the number of tests carried out at one temperature or test the same sample at 2 temperatures, typically:

- 37°C - Total coliform count (TC).
- 44°C - Thermotolerant coliform count (TTC).
-

The analytical procedures for TC and TTC using MLSB are identical except for the incubation temperature. Similarly, the colony characteristics of TC are the same as TTC, although you may find a more varied colony size and differences in the intensity of the yellow colour.
Many water samples, particularly untreated water samples, will contain bacteria other than coliform bacteria that can grow on MLSB at 37°C. The colony characteristics of these background organisms will vary: only the coliform bacteria will produce yellow colonies. However, be prepared for high background counts when performing TC analysis.
There are 2 additional procedures that you should be aware of when operating the dual incubator kit.

Charging and connecting the battery

The dual incubator kit is powered by an external 12V battery that is contained inside the separate battery pack. On the front of the battery pack, the lead connects to the dual incubator and the 3-pin socket is used to plug the charger into.

- To charge the battery, connect the battery charger to the 3-pin socket and charge for 12 hours
- You must use the integral lead on the external battery pack to connect to the dual incubator. Connect. Note: you must leave the lid of the kit open when the battery pack is connected to avoid damaging the battery lead.
- The battery pack can be connected to the battery charger and to the kit at the same time.

Temperature calibration

The procedure for checking and calibrating the temperature of the 2 incubators is the same as described in Section 7.3 for the single incubator kit. However, the corresponding temperature adjuster screws are located underneath the labels showing the incubator temperatures.

The temperature adjuster screws are located underneath the temperature labels

Appendix G
Alternative types of media that can be used with the Delagua kit for the isolation of coliform bacteria

The list is not exhaustive, but includes the most readily available culture media that may be used with the Delagua kit. New media are being developed all the time and some may be suitable for use with the kit. The important characteristic to look for in a medium is that it should be a broth (liquid) medium that can be used with the membrane filtration technique. Some broth media are intended for use with the multiple tube method of analysis (most probable number) and will not work using the membrane filtration technique. Check the instructions on the product information sheets. Agar-based (solid) media also are not appropriate for use with the kit due to the shallow design of the petri dishes. Some media require the use of supplements to improve the yield of the target organism: check the instruction sheet carefully. Always read the safety data sheets for the medium before use and check the colony characteristics for the target organism.

Medium	Uses	Incubation temperature	Characteristics of positive colonies	Characteristics of negative colonies
Membrane lauryl sulphate broth (MLSB)	Total and thermotolerant coliforms	37°C - total coliforms 44°C - thermotolerant coliforms Incubation time - 18 hours	Yellow colonies. Colour density may vary.	Red, pink, colourless, occasionally blue-grey.
m Endo broth	Total coliforms	35°C for 24 hours.	Red to red-black colonies with a golden-green metallic sheen	Light red or colourless.
M-FC medium	Thermotolerant coliforms	44.5°C for 24 hours.	Blue colonies.	Generally pale brown, cream, colourless.
m-colibIue 24 broth	Total coliforms and *E.coli*	35°C for 24 hours.	Coliforms produce red colonies; *E.coli* produces blue colonies.	Possible range of colours, but not red or blue
MI broth (you will need a UV light to examine the colonies)	Total coliforms and *E.coli*	35°C for 24 hours	Coliforms produce colourless, cream or pale yellow colonies and fluoresce blue/white under UV light. E.coli produces blue colonies that fluoresce under UV light	Colonies do not fluoresce under UV light.
Teepol NutriDisk (available as membrane pad impregnated with the medium. The medium is not readily available in any other form.)	Total coliforms and thermotolerant coliforms	37°C total coliforms 44°C thermotolerant coliforms Incubation time - 24 hours.	Yellow colonies. Colour density may vary.	Red, pink, colourless, occasionally blue-grey.
Tergitol - TTC (available as membrane pad impregnated with the medium. The powdered agar medium is not suitable for use with the kit.)	Total coliforms and thermotolerant coliforms	37°C total coliforms 44°C thermotolerant coliforms Incubation time - 24 hours.	Yellow-orange colonies with a yellow-orange halo under the colony.	Colourless, pink or red.