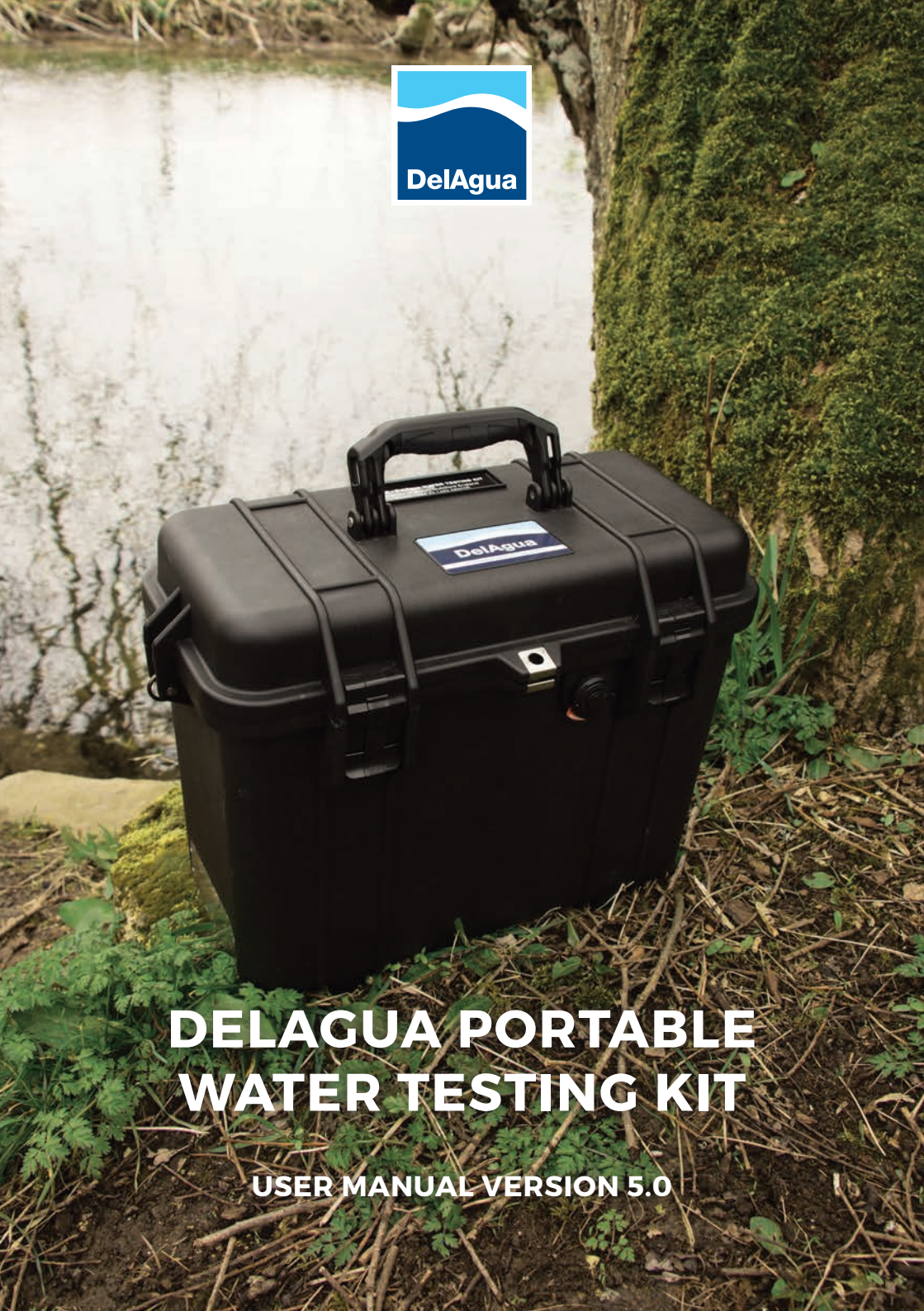

DELAGUA PORTABLE WATER TESTING KIT

USER MANUAL VERSION 5.0

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DelAgua Portable Water Testing Kit User Manual

Revised October, 2015

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 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 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 DelAgua. We can also carry out training via webinar if this is more convenient. The course is **free of charge.**

Alternatively, you can find step-by-step videos of all the key processes on our website **www.delagua.org**.

Please contact us at **info@delagua.org** for more information.

Manual Icon Key

Note: Text with this symbol draws your attention to additional information.

Video Link: Headings with this symbol indicate that there are video sequences which accompany the manual instructions. These videos can be found on our website **www.delagua.org**.

Step No. Numbers in a blue circle indicate the incremental steps of a procedure which should be followed in numerical order.

Caution: Text with this symbol contain information on health and safety. They enforce best practice methods and protect users and others against accidents, injury or hazardous contamination when using the kit.

▶ Indicates the section continues on the next page.

◀ Signifies the end of the section.

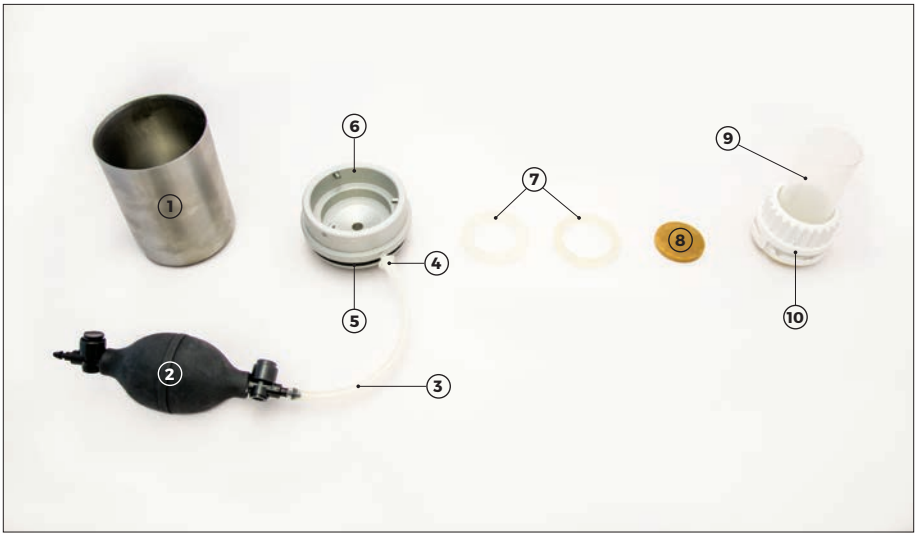
Note: The main sections of this manual describe the use and maintenance of the single incubator water testing kit. The additional procedures for the operation of the dual incubator kit are described in **Appendix J.**

Contents

1.1 The DelAgua Kit

1. Portable Incubator
2. 2 Part Turbidity Tube
3. 48 Petri Dishes in Strap Housing (3 × stacks of 16)
4. Empty Methanol Dispenser
5. Electronic Timer
6. Kit Manual
7. Empty Media Bottles × 10
8. Thermometer
9. Calibration Lid
10. Incubator Lid
11. Filtration Manifold
12. Vacuum Pump
13. Sample Collection Cup
14. 10 Disposable Pipettes
15. Chlorine/pH Comparator Block
16. Phenol Red Tablets (250 Tablets)
17. DPD1 Tablets (250 Tablets)
18. DPD3 Tablets (250 Tablets)
19. Membrane Lauryl Sulphate Broth – 38.1g
20. Membrane Filters
21. Sterile Pads
22. Pad Dispenser

1.2 Filtration Apparatus and Components



1. Vacuum Cup
2. Vacuum Pump
3. Vacuum Pump Connector
4. Vacuum Pump Connection
5. Black Rubber O-Ring
6. Aluminium Gasket
7. Silicone Rings (Pair)
8. Bronze Disc
9. Funnel (marked 10ml, 50ml, 100ml)
10. Plastic Collar

1.3 Contents of the Spares Case

1. Box
2. External Battery Connection Cable
3. Trimmer Tool
4. Tweezers
5. Elastic Strap
6. Steel Sampling Cable
7. Handheld Magnifier
8. Lubrication Grease
9. Fuse
10. Bronze Disc
11. Silicone Rings (Pair)
12. Black Rubber O-Ring

1.4 Additional Materials Needed for Testing

To use the DelAgua water testing kit, the following materials are also required:

For preparation of culture medium:

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

For using the kit in the field:

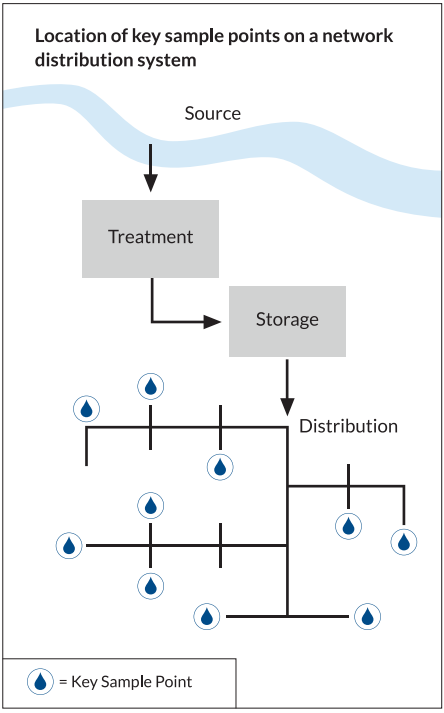
1. Methanol (for alternatives see **Section 3.1**).
2. Paper towels or clean cloths.
3. Wax pencil or marker pen.
4. Report sheets (see **Appendix F**).
5. Lighter, matches or other sources of flame.

Note: It is recommended that you fully recharge the battery in all new DelAgua kits (**Section 6.1**) and check the operating temperature of the incubator on receipt (**Section 7.2**).

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), attention should be devoted to both the main branches and remote points in the network.



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

Recommended Minimum Frequency of Sampling and Analysis of Unpiped/Point Water Supplies:

SOURCE & 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 tube well 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 tube well 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 rain water collection systems	Sanitary protection measures* and testing only if situation demands	Not needed	

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

* For a full, comprehensive description of sanitary protection measures, please refer to the referenced WHO guidelines (above).

Note: 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 Sterilisation of the Filtration Apparatus

The vacuum cup and the filtration apparatus (below) must be sterilised before use and re-sterilised between samples when analysing water from two different sources.

Sterilising equipment in the field presents some practical difficulties. The simplest method is with methanol, which is described below. When methanol is burnt in a low oxygen atmosphere — for example, in the closed vacuum cup — formaldehyde gas is produced as a by-product of combustion. Formaldehyde gas is a very effective disinfectant.

Note: Methanol is expensive to freight and requires special transport conditions. We would recommend that you try to obtain methanol in-country from a pharmaceutical supplier, a local hospital or university laboratory. If necessary, however, methanol can be supplied by DelAgua on request. If methanol is not available, the filtration apparatus and vacuum cup can be sterilised by immersion in boiling water for **10 minutes**.

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

1 Take the plastic collar and secure the filtration funnel in the loose but not free position (see **Section 5.4.3 [pg. 35]**) which will allow the formaldehyde gas to penetrate all areas of the filter head. ▶

2 Pour about **10–15 drops** of methanol into the vacuum cup.

3 Ignite the methanol in the vacuum cup using a cigarette lighter. Place the cup on a flat surface which will not be damaged by heat.

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

> **Caution:** Keep the sample cup turned away from your face and tilted slightly to prevent methanol running onto your hand. Methanol is **extremely flammable** when in contact with a naked flame.

5 Keep the filtration apparatus sealed for at least **15 minutes** before use. ▶

> **Caution:** Filtration apparatus will be hot. Be careful when handling

Note: Sterilise the filtration apparatus immediately after each analysis. 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) *
2. Distilled water. ** 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, approximately 1 litre capacity
6. Pressure cooker, portable steriliser or autoclave ***
7. Heating element, stove or burner if using a pressure cooker or portable steriliser

* The medium is available in 38.1g, pre-weighed amounts from DelAgua

** See **Appendix G** for suggested alternative sources of water

*** A portable steriliser kit is available from DelAgua

Method

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.

1 Measure out **500ml** of distilled water using the measuring cylinder or graduated flask. Decant approximately 400ml of the water into the clean flask or beaker.

2 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. Use the remaining 100ml of water to rinse out the MLSB pot, then add this to the beaker. Stir to thoroughly mix the broth.

The culture medium will be a bright red colour when dissolved. (Below) ►

> ⚠ **Caution:** MLSB is a fine, but non-hazardous powder; avoid creating excess dust which may irritate the nose or upper respiratory tract if inhaled. Spillages can be cleaned up using water and an absorbent cloth.

3 Pour approximately **50ml** (**no less than 40ml**) of culture medium 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.

4 Replace the screw caps on the polypropylene bottles. Ensure the caps are secure but **DO NOT** tighten. Leaving the caps slightly loose prevents the bottles from collapsing during sterilisation.

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

6 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 release from the 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.

7 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) *
2. Distilled, or clean water **
3. Ten polypropylene (60ml)
4. Measuring cylinder or graduated beaker
5. Portable steriliser *** or pressure cooker or cooking pot or pan
6. Heating element, stove or burner

* The medium is available in 38.1g, pre-weighed amounts from DelAgua
** See **Appendix G** for suggested alternative sources of water
*** A portable steriliser kit is available from DelAgua

Method

1 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. Ensure 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 [4–6].

9 If a pressure cooker or portable steriliser is **NOT** available, the medium can be sterilised using a process called *Tyndallisation*.

Tyndallisation procedure on following page. ▶

Tyndallisation: This procedure takes **3 days**.

1. 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.
2. Boil for **20 minutes**.
3. Leave the medium to stand for **24 hours** at room temperature **(20–30°C)** in the dark.
4. On the following day heat the medium in boiling water for a further **20 minutes** and, once again, leave to stand for **24 hours**.
5. On the third day repeat the heat treatment.
6. 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 6°C).
- 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 6°C 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, e.g. cloudiness or yellow colouration (as shown in the image to the right), the contents of the bottle must be discarded. ◄

3.5 Sterilising the Petri Dishes

- Wash the dishes in a solution of mild detergent, rinse thoroughly with clean water and dry.
- Assemble the dishes into batches of 16 in the straps. ►

Options for Sterilising:

- Sterilise the petri dishes in an autoclave, steam steriliser or pressure cooker at **121°C** for **15 minutes** (see **Section 3.2 [5 & 6]**). **OR**
- Place the dishes in a conventional oven at **180°C** for **30 minutes**. **OR**
- 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**
- 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.

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

Caution: 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 must be disposed of safely. **DO NOT** discard contaminated membranes and pads into the environment. After you have completed analysis place the pads and membranes in a biohazard bag and destroy by incineration. Wash the petri dishes with detergent after use, rinse with clean water and dry prior to sterilisation. ◄

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. If the dispenser is lost or damaged, pads may be dispensed in the field using the sterile tweezers (see **Section 5.4.3 [4]** for sterilisation methods). ►

1 Push the tube of pads onto the dispenser.

2 Turn over, as shown. Hold over the petri dish and slowly pull the plastic slider towards yourself to release a pad. Carefully drop onto the petri dish. ◄

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.

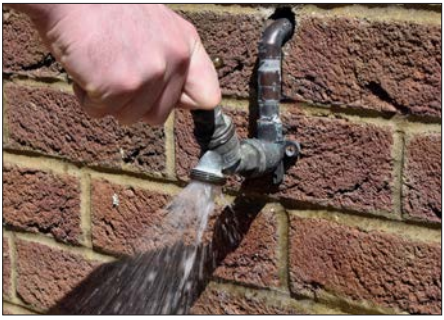
To dispense methanol, lever the dispensing nozzle into the upright position with the tip of the flame sterilised tweezers. Be sure to close the dispensing nozzle after using the kit as the methanol will evaporate.

Caution: 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; e.g. nozzles, pipes, etc. and wipe around the tap with a clean cloth.



2 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.

3 Take a water sample with the non-sterile sample cup (as shown in the image to the right). 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** respectively.

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. ◄

4.2 Sampling from a Lake, Reservoir or Other Surface Water Sources

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

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. **Rinse the cup twice with the sample water before taking the sample.**

2 Submerge the cup about **30cm** below the surface of the water and scoop up the water sample (as shown in the top right image). This scooping action ensures that no external contamination enters the sample cup.

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

3 If it is **dangerous or inconvenient** to take a sample by hand, 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 (as shown above). ◄

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 To fasten the sampling cable to the cup, feed the looped end of the cable through the hole in the side of the cup. Pass the other end of the cable through the loop and pull firmly. *Alternatively*, the clip on the end of the cable can be fastened to the hole.

2 If necessary, increase then length of the cable by attaching a rope or string to the sample cable.

3 Lower the 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.** ◀

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.

Chlorine

pH


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, e.g. the sample cup. Rinse the 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 >0.2mg/litre (0.2ppm)

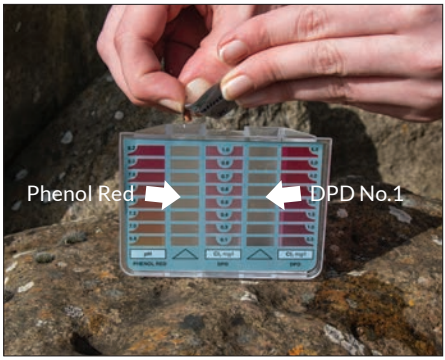
Turbidity <5 NTU.

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. (see **Section 5.4**) ◄

5.2 Analysis of Free & Total Chlorine Residual and pH

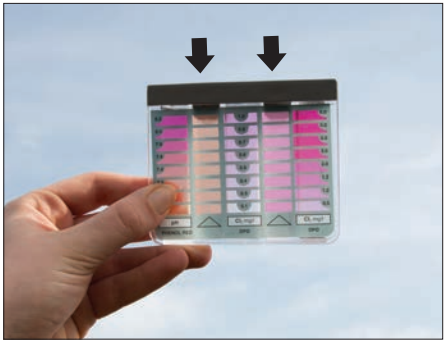
1 Wash the comparator cells **three times** with the water that is to be analysed and then fill both cells with the sample.



2 Drop one DPD No.1 tablet into the right hand cell (Cl_2) and one Phenol Red tablet into the left hand cell (pH).

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.

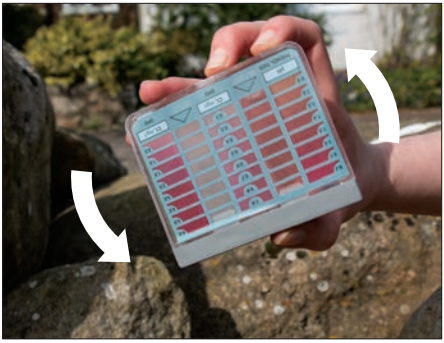
DO NOT shake the comparator as this will introduce air bubbles.



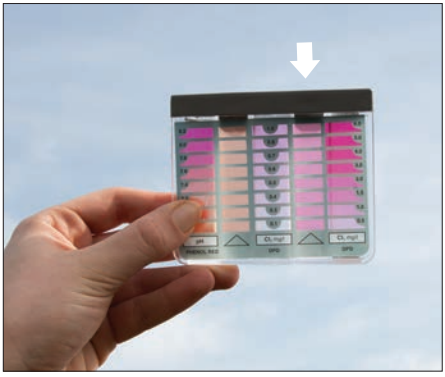
4 Immediately read the free chlorine residual (mg/litre) and pH by holding the comparator up to daylight and matching the colour in the cells with the standard colour scales. If the colour falls between two standard colours, then it will be necessary to estimate the concentration.

Record the result on the daily report sheet (see **Appendix F** 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 (Cl_2).



6 Again, invert the comparator several times to dissolve the tablet. 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 (as shown in the image below).



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

SUMMARY		
Phenol	=	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 NTU

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.

2 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. If possible, hold the turbidity tube over a white surface, this makes visibility of the black circle clearer.

3 Hold the tube vertically and slowly pour the water sample into the tube until the marker disappears when viewed from the top of the tube. Avoid creating bubbles, as this may cause false readings.

DO NOT strain to see the black circle as this can sometimes cause biased results. ▶

4 *Alternatively,* the procedure can be carried out in reverse by filling the tube with the sample and then slowly pouring out the water. Keep pouring until the black marker is visible.

5 Hold the tube vertically and read the turbidity using the graduations on the side of the tube (as shown in the image to the left). The result is the value of the line nearest the water level.

Note: The graduations follow a logarithmic scale with the most critical values marked on the side of the tube, allowing a reasonable estimation. *Alternatively*, you can judge the distance of the water level from the 2 nearest graduation marks to calculate a more accurate value. ◀

5.4 Bacteriological Analysis of Water

Caution: Microbiological analysis presents some risks to the health and safety of yourself and others. It is vitally important that safety guidelines are adhered to when carrying out analysis. (See **Appendix H** for more safety information.)

5.4.1 Introduction

Analysing water samples for thermotolerant coliforms:

- A measured quantity of water is passed through a sterile filter.
- Any bacteria present in the water are caught on the filter.
- The filter is placed on a pad soaked in liquid growth medium which feeds coliform bacteria and inhibits the growth of other bacteria.
- They are incubated at **44°C**; this ensures only thermotolerant bacteria grow.
- During this time coliform bacteria, if present, 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.
- Results are expressed 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 online 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, the only difference being that the filters are incubated at **37°C**.

The DelAgua incubator can be recalibrated to 37°C by following the recalibration procedure in **Section 7.2**. 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 J**) is available from DelAgua which allows both tests to be carried out simultaneously. ◄

5.4.2 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 see Appendix D for guidance on sample volumes. ◄

5.4.3 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).

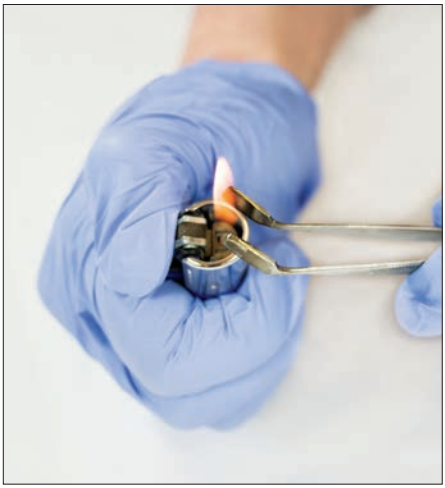
*If the dispenser is damaged, the pads can be dispensed using the sterilised tweezers (see page 33).

2. Allow the medium to warm to ambient temperature before use. Pipette enough culture medium onto the absorbent pad in the petri dish to soak the pad and leave a slight excess (approximately 2.5ml), this prevents the pad drying out during incubation.

Note: During sampling, processing components of the kit should be kept free from dirt and contamination (see Appendix I). ►

3 If bubbles appear on the pad use the pipette to suck these away. Bubbles (as shown in the image to the right) can cause inaccurate results.

> **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.



4 To sterilise the tweezers, flame the tips with a lighter for approximately **5 seconds** and leave to cool.

5 Place the heel of the tweezers into the test kit case (as shown in the image to the right). This ensures that the tips are kept away from all sources of contamination whilst analyses are in progress. ▶

6 Remove the sterile vacuum cup from the filtration apparatus. Push the filtration apparatus firmly onto the vacuum cup (see **Appendix E** for assembling the filtration manifold). 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.

7 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.

8 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. ▶

9 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.

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 Screw the plastic collar down tightly to provide a watertight seal between the filter membrane and the filter funnel. ▶

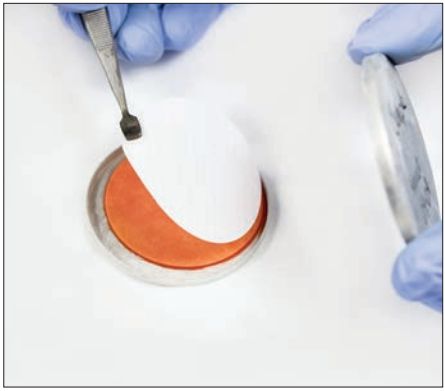
11 Rinse the sample cup once with the water before taking the sample. Take care not to allow external contamination (e.g. dirt and debris) to enter the sample cup.

12 Pour the sample into the filtration funnel up to the appropriate mark (10, 50 or 100ml) engraved on the internal surface of the funnel (as shown in the image to the right). 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.

13 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. ▶

14 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.



15 Remove the lid of a prepared petri dish and place the membrane, grid side up, 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.

16 Replace the lid of the petri dish and mark the lid with sample information e.g. 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). ▶

17 Place the petri dishes into the carrier with the blank sample at the top, followed by the negative (see below for more information on blank and negative samples). Return the carrier to the pre-heated incubator pot.

18 Resterilise the filtration apparatus (Section 3.1). ◄

Note: All 16 petri dishes must be in the rack during incubation. This allows for an even distribution of heat during the incubation cycle.

Note: Each site of testing should have 3 dishes dedicated to it. This includes a negative and two actual samples (duplicates). One "stack blank" plate should be included for each bottle of media used.

The reasons for this stack format are as follows:

Blank plate – One pad soaked in media in a petri dish. This demonstrates effective sterility of the plates and media, which ensures that any positive results are **NOT** originating from contaminated plates and/or media.

Sample Negative – Filter clean water (this does not need to be deionised water, but **MUST** be free from contamination and chlorine) through a membrane following the above process. After incubation this should show no colonies, this demonstrates effective sterility of the filtration manifold at each sample point.

Duplicates – This process ensures that erroneous results due to human error or other means are likely to be picked up due to the chances of exact error repetition being lower.

5.4.4 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 **NO 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 (e.g. 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. ◀

5.4.5 Sample Incubation

- Incubate the samples for **16 to 18 hours**. Only put the stack of petri dishes in the incubator when it reaches temperature. The incubator is designed to maintain a temperature of **44°C +/- 0.5°C.** When the incubator is switched on both lights should turn on ('power' & 'heating'). When the 'heating' light turns off the incubator has reached temperature.
- Always incubate the petri dishes with the incubator lid (without hole) and case lid closed to reduce heat loss and save battery power.

Note: It is not possible to close the kit lid if you are using the dual 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, i.e. 16 to 18 hours.

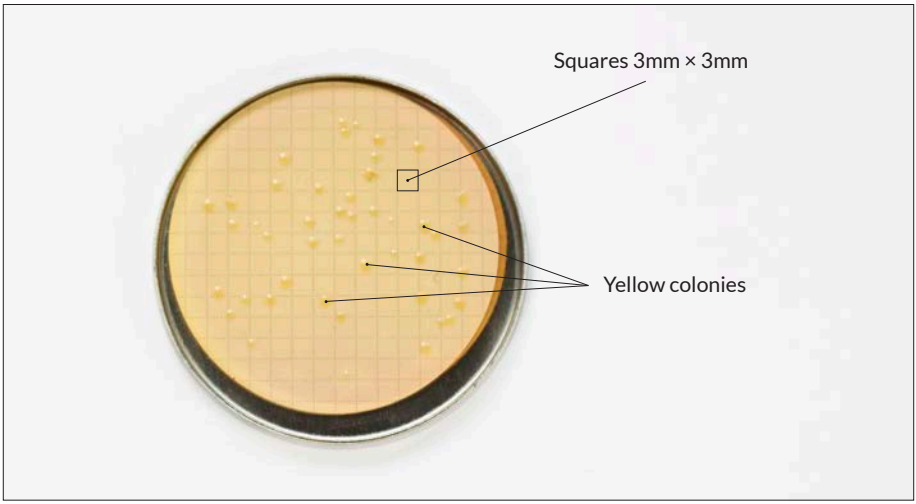
There are 3 power source options for the incubator:

1. Mains electricity supply via the charger unit
2. Internal battery
3. External 12v battery (or your vehicle 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 at the same time charge the battery. If the mains electricity fails the internal battery will operate the incubator. (See **Appendix K** for more information). ◄

5.4.6 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. Carefully lay the plates out, in sample order, on a table to allow effective results comparison.

2 Check the stack blank first. The result of this particular plate will dictate if **ANY** of the other plates can even be considered. If the stack blank is positive then either the media or the plates could be contaminated. A negative stack blank will appear the same colour as the original media, possibly with slight lightening due to a temperature/pH change. A positive stack blank will likely be a yellow colour. The indicator in the media (phenol red) will change its colour from red to yellow as the pH drops due to biological activity. In the event of a yellow stack blank, all other samples should be discarded and the process repeated. ►

3 Once the stack blank is considered as negative, assess the samples one at a time. First assess the sample negative; a positive result will demonstrate ineffective manifold sterility at that site only. If the negative shows positive growth and/or a change in the colour of the media then discard the samples for that site as they cannot be assessed as valid. A negative result for a negative control will have a pink membrane with zero "spots" or "dots" of any colour.

4 Next check the duplicate samples for the site of the negative control. The first observation should be of the colour and condition of the membrane and any liquid remaining in the dish. If the sample appears as per the negative control, this indicates a negative result for that sample site. If the dish is quite dry and the membrane appears dry then it is likely there was insufficient media in the dish at point of processing. This would mean that any growth from that sample could be compromised and it would be good practice to repeat the process for that site. If the dish shows yellowing of the membrane and media then that indicates positive presence of coliforms. If the media and membrane still appears red, but there are spots and bumps on the surface of the membrane, this also indicates a positive result for coliforms in that sample.

5 Count all the yellow colonies (as shown) which have a diameter of between 1mm and 3mm. If necessary use the hand lens provided (See **Appendix L** for more information on counting colonies).

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

VOLUME FILTERED	THERMOTOLERANT COLIFORMS PER 100ml
100ml	Number of colonies × 1
50ml	Number of colonies × 2
10ml	Number of colonies × 10
1ml	Number of colonies × 100

5.4.7 Disposal

Caution: 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

DOs	DON'Ts
✓ **ALWAYS** incubate samples with the incubator lid firmly in place and the kit lid 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. ✓ **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.	× **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. If the kit is in storage, recharge the battery once a month. × **NEVER** operate in cold weather. × **NEVER** leave the incubator switched on for more than 18 consecutive hours.

To Recharge the Battery:

- Connect the charger to the left hand side of the incubator.
- Plug the 3-pin plug from the charger unit into the mains electricity supply and switch on. Check that the incubator is switched off unless it is in use.
- The LED on the charger will be an **orange/amber** colour which will eventually turn **green**. Once it has turned **green** the battery will be usable but will not run for the five full incubation cycles. Ensure the battery is charged for 72 hours after the LED turns green, to be able to run the incubator for five full cycles.
- 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.

> **Note:** 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 3.** ◄

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 and 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 month. For additional information (see **section 7.2.1**) ◄

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. ◄

6.4 Chlorine/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. ◄

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.
- **NEVER** use acids or organic solvents. ◄

6.6 Maintenance

WEEKLY	MONTHLY
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	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 operation of the kit ◀

7. Evaluation and Repair of the Kit

7.1 Troubleshooting Guide for the DelAgua Kit

7.1.1 Problems with Charging the Internal Battery.

Connect the charger to the incubator and plug into the mains. Switch the mains on. The LED on the charger should be an orange/amber colour which will eventually turn green. Once it has turned green the battery will be usable but will not run for the full five incubation cycles. Ensure the battery is charged for 72 hours after the LED turns green, to be able to run the incubator for five full cycles.

If the two lights on top of the incubator **DO NOT** illuminate at all when connected to the mains, the main fuse may have blown. Ensure the charger unit is disconnected from the mains and replace the fuse. Reconnect to the incubator and plug into the mains, if the lights still do not come on then there could be two reasons for this; either the charger is damaged or the battery is completely dead. Replacement chargers and/or batteries are available through DelAgua.

If the LED on the charger **DOES NOT** turn green, this is an indication that the charger is damaged and will require a replacement. Replacement chargers are available from DelAgua. ◄

7.1.2 Attempting to Calibrate the Incubator, but the Incubator is not Reaching or Maintaining the Desired Temperature.

If the battery has been charged and the lights are illuminated, but the incubator is not reaching or maintaining a temperature between 43.5 and 44.5°C the incubator is damaged. A repair kit is available from DelAgua. Contact an electronics technician to carry out repairs or contact DelAgua.

If the lights **DO NOT** illuminate, connect the incubator to the charging unit and connect to mains. If the incubator then reaches and maintains temperature this means there is a fault with the battery and will require replacement.

The incubator should be able to maintain a temperature of between 43.5 and 44.5°C for five 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.

If this is not the case, the battery is damaged or worn out. Battery replacement should be carried out only by qualified electronic technicians. A battery replacement kit is available from DelAgua. ◄

7.1.3 The Charger Unit is Connected to the Incubator and the Mains Electricity is Turned On, but the Lights do not Illuminate.

Disconnect the battery charger from the incubator and connect the incubator to a well charged 12v battery using the lead with crocodile clips supplied with the kit. Switch on the incubator unit. If the lights still do not illuminate then the incubator is damaged. A repair kit is available from DelAgua. Contact an electronics technician to carry out repairs or contact DelAgua.

If the lights do illuminate, there could be a fault with the charger. If the charger is fitted with a 3pin UK style plug, the main fuse may have blown. Ensure the charger unit is disconnected from the mains and replace the fuse. Reconnect to the incubator and plug into the mains, if the lights still do not come on then the charger is damaged and must be replaced or a repair can be carried out through DelAgua. ◄

Note: For any technical assistance relating to the DelAgua Kit please contact us on **(+44) 1672 861 198.** ◄

7.2 Checking & 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. ◄

7.2.1 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.

3 Replace the incubator lid with the calibration lid (with hole) and thermometer assembly. The bulb of the thermometer should be completely immersed in the water, but be careful not to crack the bulb.

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. ◄

7.2.2 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).

3. To increase the temperature, turn the adjustment screw clockwise. To decrease the temperature, turn the adjustment screw anti-clockwise. Make the adjustments in stages, a ¼ turn at a time. After each adjustment, leave the incubator to stabilise for at least **30 minutes**. The complete recalibration procedure may take several hours.

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 it 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 - Spares List

All of the listed spares and consumables are available from DelAgua. Please contact us for a current price list.

Kit Components:

Battery replacement kit containing:
- Battery 12v 9.5Ah
- Silicone sealant

Temperature check kit containing:
- Incubator calibration lid with hole
- Thermometer
- Adjuster/trimmer tool

Electrical repair kit containing:
- Electrical circuit
- Temperature chip
- Sealant
- Foam compound
- Adhesives

Other Spares:
- Filtration apparatus (complete)
- Spares box (specify empty or complete)
- Filter funnel with plastic collar
- Tweezers
- Aluminium Gasket
- Chlorine/pH comparator
- Vacuum cup
- Vacuum pump
- External battery cable
- Sample cup
- Battery charger
- Silicone grease (2g or 100g)
- Sample cable
- Polypropylene bottles 60ml
- Bronze disc
- Plastic methanol dispenser
- Black rubber O-ring
- Petri dishes
- Turbidity tube
- Thermometer

Optional Extras:

- Portable conductivity meter
- Portable steriliser kit

Other items of equipment for the analysis of a range of chemical parameters can be supplied upon request. DelAgua also offer expansion consumables packs allowing for forward planning of testing programmes and high volume analysis.

Consumables:

Membrane filters and absorbent pads

- Pad dispenser
- Culture medium: 38.1g tub for 500ml of growth medium (sufficient for 200 plates)
- Culture medium: 500g tub for 6.5 litres of growth medium (sufficient for 2,600 plates)
- DPD No.1 Tablets
- DPD No.3 Tablets
- Phenol Red Tablets

Alternatively, visit our website at **www.delagua.org** for more information.

APPENDIX B - Field Checklist

Before leaving for the field, check that you have the following items:

Kit

- ☐ DelAgua incubator in pelican case
- ☐ Filtration apparatus
- ☐ Sample cup
- ☐ Sample cable
- ☐ Turbidity tubes (pair)
- ☐ Chlorine/pH comparator
- ☐ Tweezers
- ☐ Petri dishes in strapped housing
- ☐ Spares box (complete)
- ☐ Incubator lids
- ☐ Thermometer

Other Items

- ☐ 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 in dispenser
- ☐ Daily report sheets (**Appendix F**)
- ☐ Paper towels or clean cloth
- ☐ Some clean water to rinse the equipment after use
- ☐ A wax pencil or marker pen
- ☐ Lighter/matches
- ☐ Gloves
- ☐ Pasteur pipettes
- ☐ Instruction manual

APPENDIX C - Alternative Types of Media that can be Used with the DelAgua Kit for the Isolation of Coliform Bacteria.

Note: 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 characteristics to look for in a medium are 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 with 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 (MLSB)	Total & Thermotolerant Coliforms	37°C - Total Coliforms 44°C - Thermotolerant Coliforms Incubation Time - 18hrs	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-F C Medium	Thermotolerant Coliforms	44.5°C for 24 hours	Blue colonies	Generally pale brown, cream, colourless
m-ColiBlue 24 Broth	Total Coliforms & *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)	Total Coliforms & *E.Coli*	35°C for 24 hours	Coliforms produce colourless, cream or pale yellow colonies and fluoresce under UV light	Colonies do not fluoresce under UV light
Teepol NutriDisk (Available as membrane pad impregnated with the medium. Not readily available in any other form)	Total & Thermotolerant Coliforms	37°C - Total Coliforms 44°C - Thermotolerant Coliforms Incubation Time - 24hrs	Yellow colonies. Colour density may vary	Red, pink, colourless, occasionally blue-grey
Tergitol - TTC (Available as membrane pad impregnated with the medium. The powered agar medium is not suitable for use with the kit)	Total & Thermotolerant Coliforms	37°C - Total Coliforms 44°C - Thermotolerant Coliforms Incubation Time - 24hrs	Yellow-orange colonies with a yellow-orange halo under the colony.	Colourless, pink or red.

APPENDIX D - Appropriate Sample Volumes for Coliform Analysis

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

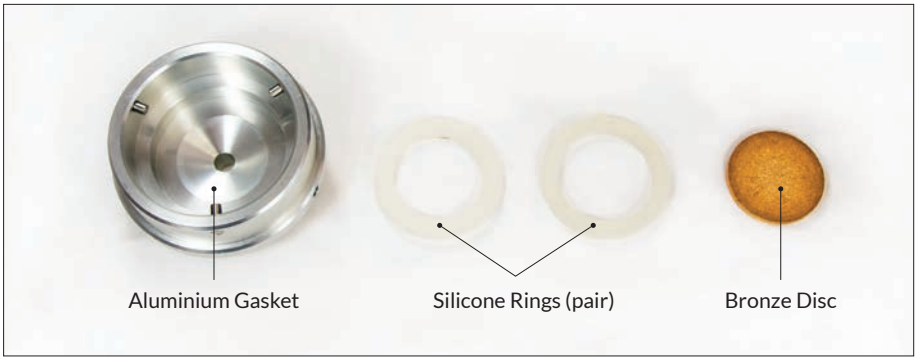
Recommended sample volumes for each source are shown below.

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, e.g. wells and springs	100ml (50ml or 10ml)
Unprotected groundwater, e.g. open dug wells and springs	50ml (10ml or 1ml*)
***Note:** This volume will require the use of sterile pipettes and distilled or dechlorinated water.	

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.

APPENDIX E - Assembly of the Filtration Manifold



1 Place the large silicone ring into the circular space in the gasket.

2 Then place the smaller silicone ring on top.

3 Push the bronze disc into the middle. The large ring acts a platform for the disc to be placed onto. The disc should be smooth side up.

4 Screw the plastic collar and filter funnel down tightly.

5 Push the filtration apparatus firmly onto the vacuum cup. If this is difficult, lubricate the black rubber O-ring with silicone grease.

Note: When storing the filtration manifold it is advisable to lubricate the black O-ring with silicone grease.

APPENDIX F - Daily Report Sheet

Health Region: ..... Authority: .....

Date: ... / ... / ..... Code: .....

Province: ..... District: .....

Community: ..... Analyst: .....

Comments:

Source of sample	Time	Colour	Odour	Turbidity (NTU)	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 G - Alternative Sources of Water for Media Preparation

Note: We would strongly recommend that you try to find a source of distilled water to prepare the medium, with a pH between 7.0 and 7.8. 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.
- 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.
- Sodium Thiosulphate tablets can be used as a dechlorinating agent. Add two tablets to 500ml water that may contain chlorine and shake until they are completely dissolved. The water will then be suitable to use for medium preparation. These tablets are available from DelAgua.
- De-ionisation packs can be used in situations where distilled or de-ionised water is not readily available. DelAgua are able to supply a portable de-ionisation pack. This is simply filled with around 500ml of clean water, and shaken for approximately 5 minutes. Each pack will produce approximately 4-5L of de-ionised water before being depleted and responsibly discarded. This is indicated by a simple colour change of the beads in the pack, from Blue to Yellow/White.

APPENDIX H - Safety Guidelines

It is vitally important that you take great care when handling potentially contaminated materials, such as the petri dishes, 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.
- × **DO NOT** carry out microbiological tests in food preparation areas.
- × **DO NOT** dispose of contaminated materials into the environment.
- ✓ **ALWAYS** wash your hands thoroughly after handling contaminated or potentially contaminated materials.
- ✓ **ALWAYS** cover wounds with a waterproof dressing.
- ✓ **ALWAYS** keep all non-essential personnel, particularly children, away from the work area when handling contaminated materials.
- ✓ **ALWAYS** keep your work area clean and tidy.
- ✓ **ALWAYS** clean and disinfect (methanol, ethanol or a weak solution of domestic bleach can be used for this purpose) the work surfaces after you have finished the analysis.

APPENDIX I - 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:

- All those areas in direct contact with the water sample, e.g. the internal surface of the vacuum cup, the internal surface of the filter funnel, the upper part of the filtration base and the surface of the bronze disc.
- Surfaces in contact with the culture medium, e.g. the internal surface of the petri dishes and the absorbent pads.
- 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 which may contaminate them and interfere with the bacterial count.

APPENDIX J - 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 two additional procedures that you should be aware of when operating the dual incubator kit:

1 Charging and Connecting the Battery

The dual incubator kit is powered by an external 12V battery that is contained inside the separate battery pack. Inside the battery pack, there are two connections; one lead connects to the dual incubator and the other connects to the 2-step charger. This in turn ends in a 3-pin plug to connect the whole system into the mains supply.

- To charge the battery, connect the battery charger to the 3-pin socket and plug into the mains supply. Switch on to charge (For charging times see **Section 6.1**).
- The battery pack can be connected to the battery charger and to the kit at the same time.

> **Note:** You must leave the lid of the kit open when the battery pack is connected to avoid damaging the battery lead.

2 Temperature Calibration

The procedure for checking and calibrating the temperature of the two incubators is the same as described in **Section 7.2** for the single incubator kit. However, the corresponding temperature adjuster screws are located underneath the labels showing the incubator temperatures.

Note: Each incubator has a temperature label located above it. The temperature adjuster screws are located to the side of the incubator (as shown above) and are also labelled.

APPENDIX K - Three Power Sources for 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.

Internal Battery

It is possible to obtain up to five incubation cycles from the internal battery. 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 **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

If you are planning to work in the field for more than five days, or to work 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.

To operate 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 '−') and connect to the left hand side of the incubator console.

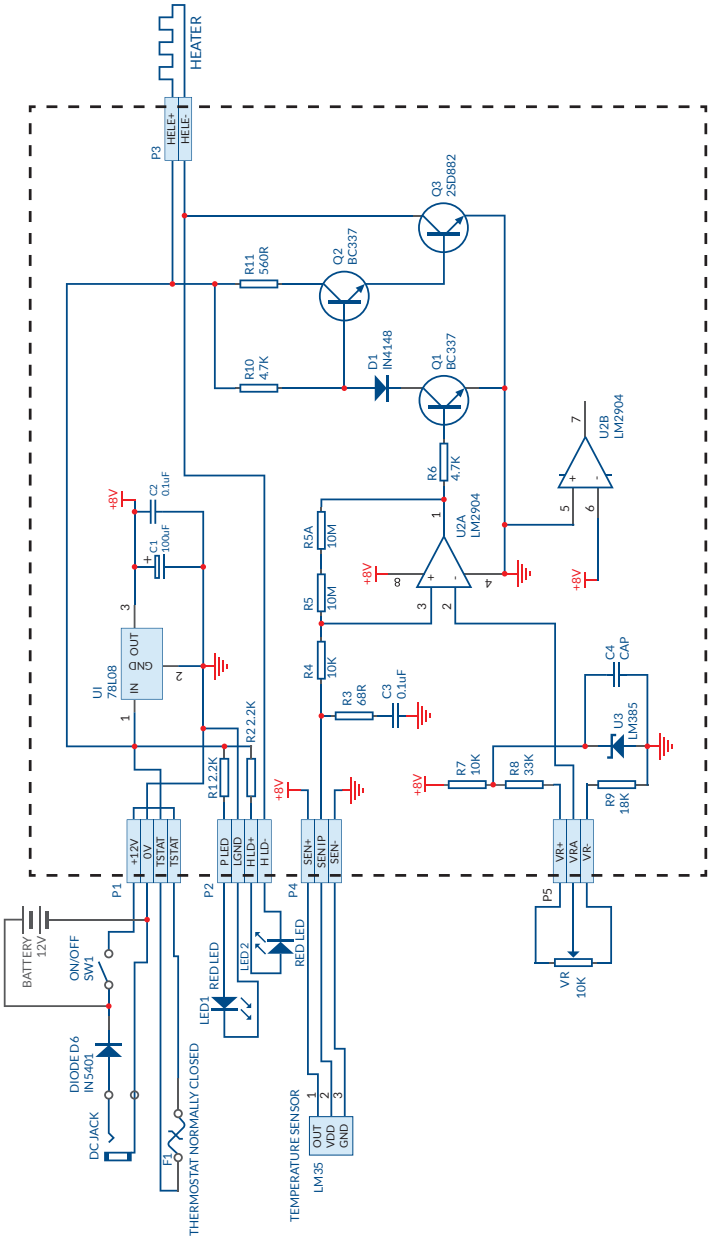
Switch on the incubator and check that the 'Power On' indicator is lit. An external battery cannot be used to recharge the internal battery, only to operate the incubator. Very little current is drawn during incubation and it is usually safe to operate from a vehicle battery for one incubation cycle without risk of discharging the vehicle battery excessively. Only run from a car battery if the vehicle **IS** being driven regularly.

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

APPENDIX L - Counting Colonies

- Frequently, 2 or more colonies will merge together. It is usually clear how many have merged, count each of these.
- **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 to 100 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.

APPENDIX M - Incubator Electronic Circuit Diagram



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