Potable Water Test Kit Microbiology Instructions

Lovibond® Water Testing and Colour Measurement

56I681254

Contents

PLEASE KEEP THIS MANUAL SAFE FOR LATER USE

Introduction to Water Microbiology

The Lovibond Potable Water Test Kits for Microbiological analysis have been designed to test the quality of Drinking Water and determine whether there is any faecal contamination.

Microorganisms – also known as microbes – of various types are found naturally in many water sources. They can be advantageous in some processes and are often necessary for the environmental synergy of ecosystems.

There are, however, other types of microbe that can cause disease in both the animal and plant kingdoms. When present in water systems, they can reduce plant and equipment life, potentially affecting the health and safety of operatives and members of the public.

These harmful microbes are introduced into the water through a variety of pathways. Often they are opportunistic pathogens that contribute to the microbial loading of the water and influence the subsequent contamination.

To combat this invasion, chemical and mechanical methods are employed with both systems requiring regular and effective monitoring to ensure they are combating the microbial activity.

Lovibond® Range of Microbiological Testing Equipment

The diversity of the microbes that can affect the water systems is vast. In the majority of cases, the test to determine which microbial species is the causative agent for disease or infection is too complex to be performed on-site.

It is therefore accepted that indicator organisms can be used to monitor the microbiological trends within a water system: using them as the trigger for changing biocide dosing and further investigative work.

The most commonly used indicator organisms tests are:

TVC (Total Viable Count) – tested to indicate the general number of microbes in the system

E.coli/Coliforms – tested to indicate any faecal contamination

Pseudomonas – tested to indicate any non-faecal contamination

The Lovibond® products used in these kits are intended for monitoring these microbes in the field, have been adapted from accepted laboratory methods and are suitable for use by both non-technical and semi-technical members of the team.

Identification of coliform bacteria and Escherichia coli is done according to:

The Microbiology of Drinking Water (2009) - Part 4 - Methods for the isolation and

enumeration of coliform bacteria and Escherichia coli (including E. coli O157:H7)

Methods for the Examination of Waters and Associated Materials

Method A) The enumeration of coliform bacteria and Escherichia coli by a two-membrane filtration technique using membrane lauryl sulphate broth or agar incubated at 37 °C and 44 °C

In the Bluebook of Environment Agency (UK)

Standing Committee of Analysts

Environment Agency (National Laboratory Service)

56 Town Green Street

Rothley

Leicestershire

LE7 7NW

www.environment-agency.gov.uk/nls

The method here is the determination of presumptive coliforme bacteria and presumptive Escherichia coli, it does not cover the additional confirmation testing as described in the method A.

Using the Kit to detect Faecal Contamination

Faecal Contamination is generally accepted as an indicator of the suitability of Water for potability.

The Lovibond®potable water kits contain as standard reagents and equipment for testing Coliforms.

Coliforms are a large group of Gram negative bacteria, and specifically these kits will contain methods for the detection and enumeration of Thermotolerant (faecal) coliforms, Total Coliforms and *Eschericia Coli* (E.Coli).

It should therefore be noted that whilst performing this type of microbiological analysis there are some risks in handling harmful pathogens and particular care should be taken to follow aseptic, hygiene and general procedures detailed in this instructions booklet in order that those risks are minimised.

Membrane Filtration

The standard analytical procedure for isolating and enumerating bacteria from water samples in laboratories.

Those techniques have been adapted for use in the field but form the basic principle from which our methods are derived

Membrane filters are used as they have a known uniform porosity - for microbiology generally 0.45 µm are used.

This size is small enough that it “traps” microorganisms.

A sample, usually 100ml, is passed through the membrane using a filter funnel and vacuum system. Any organisms in the sample are concentrated on the surface of the membrane.

The membrane, with its trapped bacteria, is then placed in a special plate containing a pad saturated with the appropriate medium. This media is specifically formulated for the species of bacteria of interest and it “feeds” the bacteria during incubation so that they form discreet colonies on the upper surface of the filter. These colonies are sometimes “coloured” in order that identification and counting is made simpler.

Using the Kit

1. Planning your Test

a) WHAT YOU MAY NEED THAT’S NOT IN THE KIT

- Notebook and pen or Log book for recording the results of your test
- Methanol
- Autoclave / pressure cooker
- Cigarette lighter
- Paper Towel
- De-Ionised water
- Ice packs or cool bag to transport samples
- Additional sterilised sample bottles / bags
- Waste disposal bags

b) BEFORE LEAVING TO TEST SITE ENSURE :

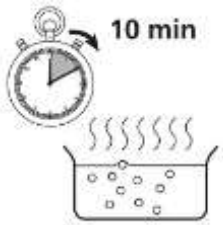
- The equipment is sterilised where possible
- You have enough media pads, media, filters and petri dishes for the number of tests to be carried out
- Prepare the culture media BEFORE leaving (If using pre-prepared media check that this is still bright pink. If Cloudy dispose and prepare new batch)
- Prepare petri dishes with media pads
- Sterilise Filtration device

C) AT THE TEST SITE

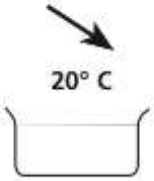
- Find a flat site out of direct sunlight - preferably in the shade
- Label each petri dish with sample I.D
- Collect sample and test within two hours or if that is not possible collect sample, store below 4°C and test within 6 hours
- REMEMBER to switch the incubator on to allow for the 30-minute warm up period.

2. Preparation / Sterilisation - Before Leaving to Site

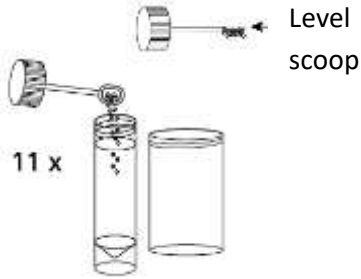
i. MAKING THE MEMBRANE LAURYL SULPHATE BROTH



Sterilise Water - Boil 10min



Allow to cool



Add 11 level scoops of media powder To Media Tube

Add Cooled Sterile water to the Line

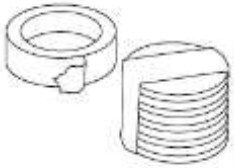
Close lid and shake to dissolve Media will turn pink

Open the cap a little by returning cap until it can be Moved but is still mounted on the container.

ii. PREPARE / STERILISE THE ALUMINIUM PETRI DISHES

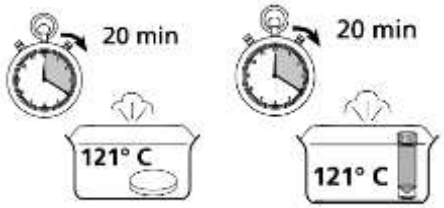
Prepare Petri dishes for Autoclaving

Take new Petri dishes and stack them into pieces of 5

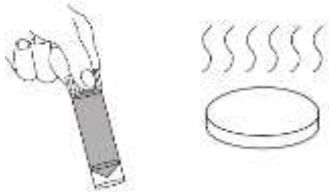


Tape the stack to prevent from falling.

Place loosened tube and petri dish in autoclave



Autoclave Media and petri dishes (They can go in same autoclave)



Close Cap on tube and allow petri dish and tube to cool

iii. Equip Case with the prepared and autoclaved Petri dished and the liquid media

3. At Site - Collect sample (s) and Perform Test Method

IV. PREPARE / STERILISE THE MEMBRANE FILTARTION UNIT

Take out filtration device

Place in filtration flask

Assemble filtration unit

Add some methanol to

The inner parts of the unit

Carefully ignite the

methanol

Close the lid

Feel the unit warms by

the fire. Wait 5 minutes

Before opening the unit

For further use

V. PREPARE PETRI DISHES WITH MEDIA

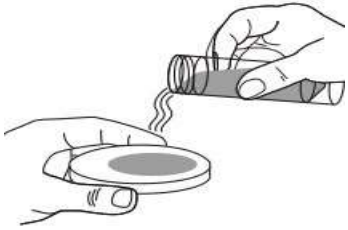
Remove lid from Media pads

Attach media pad dispenser

Remove lid of sterile petri dish

Use gloves/only touch sides

Dispense media pad onto petri dish.



Pour prepared media onto pad

To saturate the pad

Ensure pad is covered and

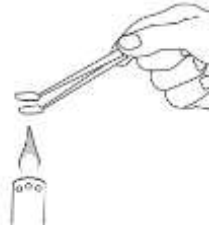
discards any excess medium.

Replace lid to keep sterile

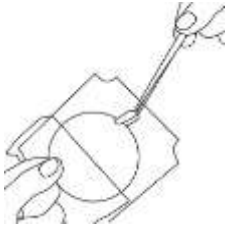
VI. COLLECTION AND FILTRATION OF THE SAMPLE: PLEASE NOTE A SEPARATE FILTRATION WILL BE REQUIRED FOR EACH EST AND EACH RESULT.

Collect 100ml sample using Steel tube or sterile bags.

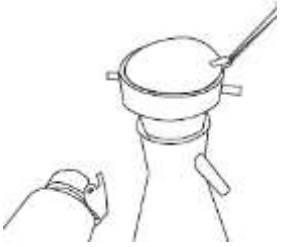
Undo centre part of filtration device



Sterilise forceps using flame. Cool



Using forceps remove white gridded Filter from backing paper (blue)



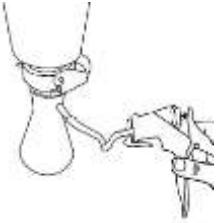
Place filter into filtration device

Lock filter in place by replacing top half of filtration device

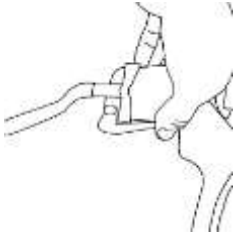
Attach filtration device to vacuum pump using tube

Pour sample into the filtration device

Replace lid of unit



Filter sample using vacuum pump



Vent the system by pressing the bar and Carefully remove Pump and tube from flask

Undo top of filtration device

Using fire-sterilized tweezer

Remove membrane filter

Put membrane filter on

top of the media saturated

Media pad in the petri dish

Put Petri dish lid on

and place upside down

in rack until ready for incubation

4. Incubation

VII. Incubation

Place petri dishes into incubator

Replace incubator lid

Connect incubator to power

(Mains or battery)Turn on Incubator

Total Coliform

Incubate at 30°C for minimum of 4 Hours + 37°C for a minimum of 14hours = R37 Indicator light

E Coli

Incubate at 30°C for minimum of 4 +/- 0.25 hours + 44°C for a minimum of 14hours = R44 Indicator Light

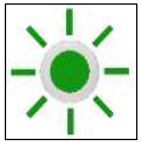
Set Incubation Temperature

R37 or R44

Set Incubation Time

18h

Confirm to start

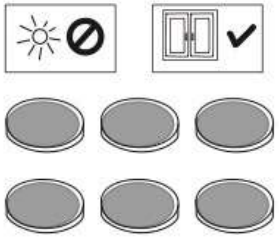


The On / Off Indicator light will flash green once total incubation

Time is completed

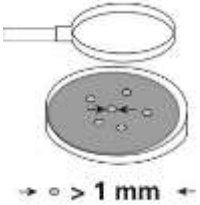
5. Results

After incubation is complete remove Petri dish rack

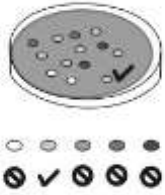


Place on flat surface in good light but away from sunlight

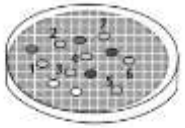
Carefully remove lid of Petri dish



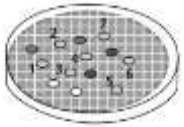
Count YELLOW colonies Using hand lens



DO NOT count clear, red or any other Colour Colonies



Use grid to help counting



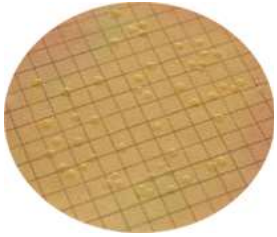
= RESULTS should be logged an expressed as CFU / 100ml

(C.F.U - Colony forming Units)

Number of Colonies on filter incubated at 37°C = Coliform Bacteria

Number of Colonies on filter incubated at 44°C = E Coli Bacteria

Yellow colonies of presumptive Coliform bacteria incubated at 37 °C - do not count the pink colonies



Yellow colonies of presumptive
E. coli incubated at 44 °C

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