

## For Detection and Enumeration of Bacteria in Potable, Hot and Recreational Water

### Instructions for Use



#### Important Notice

The test kit should only be used as part of an investigatory process into microbiological contamination in potable, hot, recreational and other waters. The test kit must be used strictly in accordance with these instructions or instructions authorised by ECHA Microbiology Ltd. The results only relate to the portion of sample tested and not necessarily to other water in the system. Although guidance may be sought by the user on sampling and the interpretation of results, the responsibility for carrying out the sampling and test procedures correctly is that of the end-user and not ECHA Microbiology Ltd. The test kit is designed to detect a recognised group of micro-organisms of significance, but it is in the nature of microbiology that there may be micro-organisms present which are not detected by the test procedure. ECHA Microbiology Ltd does not accept any liability for any decision or assessment taken or made as a consequence of the results obtained.

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## WHAT IS THE MICROBMONITOR AQUA TEST?

The **MicrobMonitor AQUA** test is a simple, easy to use test kit which has been specifically developed to quantitatively detect the presence of bacteria that can contaminate water. This is known as the Heterotrophic Plate Count (HPC), also called the Total Viable Count (TVC) or the Aerobic Colony Count (ACC). The test can be used to test samples from potable and hot water systems and from recreational water facilities such as swimming pools and spa baths. The test may also be used to test raw environmental water and other waters (contact ECHA for further details). **MicrobMonitor AQUA** is easy to use and it does not require the use of a laboratory or staff specifically trained as microbiologists.

Each test consists of a screw capped bottle containing a thixotropic, nutritive culture gel. The required volume of sample (usually 0.1 ml) is added to the test bottle using a sterile measuring pipette and is then dispersed in the gel by shaking. The gel then re-sets and the test bottle is incubated under specific conditions of temperature and time, according to the type of sample and the assessment required. Bacteria in the sample reproduce to form 'colonies' which appear as purple spots in the gel. The number of colonies is counted or estimated and is equivalent to the number of bacterial colony forming units (CFU) present in the volume of sample tested. The number of colonies counted is divided by the volume of sample tested to give the Heterotrophic Plate Count as number of CFU per ml.

**MicrobMonitor AQUA** is available in packs of 1 and 10 tests each containing everything that is required to perform the test:

- **MicrobMonitor AQUA** test bottles
- Sterile 0.1 ml dual bulb pipettes\*
- Bottle labels
- This instruction leaflet

\*Sterile single bulb pipettes, for testing larger volumes up to 1 ml are available separately.

**MicrobMonitor** tests have been developed and patented by ECHA Microbiology Ltd. and are marketed worldwide by ECHA and its distributors. ECHA Microbiology Ltd. provides a full technical service to support this product.

## WHAT IS THE MICROBMONITOR AQUA TEST USED FOR?

The **MicrobMonitor AQUA** test can be used as a routine monitoring tool, for providing assurances about water quality, for investigation of incidents and for confirming effectiveness of measures taken to prevent or remediate microbial contamination such as water chlorination.

This instruction leaflet provides general instructions for using **MicrobMonitor AQUA**. The technical leaflets listed below provide further technical instructions on the use of **MicrobMonitor AQUA** in specific applications (downloadable from [www.microbmonitor.com](http://www.microbmonitor.com)):

- **MicrobMonitor AQUA** Sampling Pack, Instructions for use (EP199).
- Monitoring of Potable, Recreational and Hot and Cold Wash Water on Ships with **MicrobMonitor AQUA** to Ensure Microbiological Quality and Safety (EP207).
- Monitoring of Water on Aircraft with **MicrobMonitor AQUA** to Ensure Microbiological Quality and Safety (EP198).

## PREPARATIONS FOR TESTING

### Sampling considerations

Sampling should be conducted in a clean and consistent manner which prevents introduction of contamination into the sample. Samples should be taken in a sterile container (e.g. sealable bag or bottle). Sample containers for testing water systems treated with chlorine or other oxidizing disinfectants should include an appropriate amount of chlorine neutraliser (e.g. thiosulphate). The **MicrobMonitor AQUA** sampling pack is available separately. Further details of water sampling procedures for specific applications are available in the technical documents listed above. Once samples have been taken, the numbers of microbes present can change, dependent on storage and transit conditions. Therefore, it is important to test samples as soon as possible, ideally within 6 hours of collection. Samples will give increasingly less reliable results as they get older. To minimize change within the sample, keep it cool (2 - 8 °C) and in the dark, for example in a clean insulated cool box, but do not freeze it. Samples which are maintained in the dark at 2 - 8 °C may be tested within 24 hours of collection.

### Sample type and recommended test volumes

**MicrobMonitor AQUA** can be used to test a wide variety of water samples. The volume of sample which should be tested will depend on the type of sample and corresponding minimum detection level required. The **MicrobMonitor AQUA** kit is supplied with a dual bulb pipette and instructions for testing 0.1 ml, which is the appropriate test volume in most cases. In some guidelines for certain recreational waters, compliance with a more stringent standard of not more than 10 colony forming units (CFU) per ml is specified. In these cases, to improve accuracy, it may be appropriate to test 1ml of sample; an alternative larger measuring pipette (1ml single bulb) is available separately for doing this.

## Preparing the MicrobMonitor AQUA test bottles

If the **MicrobMonitor AQUA** test bottles have been stored refrigerated, allow them to equilibrate to ambient temperature before they are used. Avoid prolonged exposure of the **MicrobMonitor AQUA** test bottles to direct sunlight or other bright light during preparation, incubation and examination of tests.

### Cleanliness during testing

**MicrobMonitor AQUA** tests can be conducted on-site in the field, in an office or in a laboratory. However, precautions should be taken to ensure testing is conducted in a reasonably clean area to avoid contamination of the sample and the test by microorganisms other than those in the test sample. Wash hands before and after testing. During testing wear clean nitrile, vinyl or polythene gloves. Avoid touching areas of the pipette which come into direct contact with the sample and avoid touching the inside of the **MicrobMonitor AQUA** test bottle cap and bottle neck when dispensing sample.

## TEST PROCEDURE

### A Add sample to the MicrobMonitor AQUA test bottle.

**A.1** Immediately prior to testing, shake the sample by rapid inversion several times.



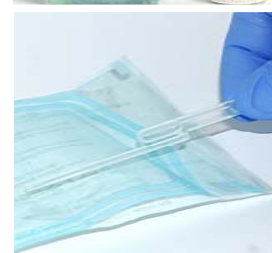
**A.2** Remove the cap of the **MicrobMonitor AQUA** test bottle taking care not to touch the inside and place it on a clean surface facing upwards.



**A.3** Open a peel pack containing a 0.1 ml dual bulb pipette at the bulb end and remove the pipette taking care not to touch the tube section or the lower bulb.

#### Note

Alternatively, for testing larger volumes up to 1 ml, use a sterile single bulb pipette available separately (or other suitable sterile measuring device).



**A.4** Using the pipette, withdraw water from the sample as follows:

**A.4.1** Dip the tube of the pipette approximately 2–3 cm below the surface of the sample.

**A.4.2** Squeeze the top bulb once and then release it.

**A.4.3** Withdraw the pipette from the sample; if there is a drop at the tip of the pipette, touch it against the inside of the sample bottle to remove it.

The tube of the 0.1 ml dual bulb pipette fills with 0.1ml of sample and any excess is captured in the lower bulb. Ensure you squeeze the upper bulb of the pipette only once or you may over fill the lower bulb, this can result in too large a volume of water being dispensed.

#### Note

If using the 1 ml single bulb pipette (available separately), after withdrawing water from the sample as described in A.4.1 to A.4.3, squeeze the bulb to expel excess until the level of water in the pipette corresponds to the required volume mark (e.g. the 1 ml mark, nearest the bulb).



**A.5** Hold the pipette over the neck of the opened **MicrobMonitor AQUA** test bottle and squeeze the top bulb once to discharge the water. It is normal for excess water to remain in the lower bulb of the dual bulb pipette.

**Note**

If using the single bulb pipette (available separately), the bulb should be squeezed fully to ensure the entire measured volume is dispensed.

**A.6** Replace the cap on the **MicrobMonitor AQUA** test bottle and tighten fully. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle.



**B Shake the MicrobMonitor AQUA test to disperse the sample in the gel.**

**B.1** Loosen and break up the gel in the **MicrobMonitor AQUA** bottle containing the dispensed sample, by tapping the bottle firmly in the palm of your hand, or on a rubber bung or **MicrobMonitor "Bumper"** (available separately).



**B.2** Shake the bottle vigorously for 30 seconds to liquefy the gel and disperse the sample. After shaking, the gel should be slightly viscous but free of lumps and have a uniform consistency and clarity. The presence of bubbles in the gel is normal and will not affect the test.

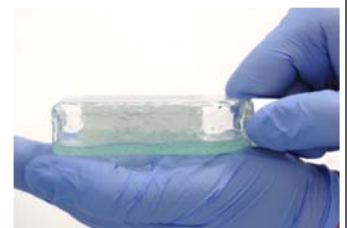


To assist with shaking, the test bottle can alternatively be tapped repeatedly for 30 seconds on a **MicrobMonitor "Bumper"**.

**B.3** Stop shaking the bottle abruptly so that the gel collects in the bottom of the bottle.



**B.4** Immediately tap the bottle in the palm of the hand until the gel forms a flat layer on one of the larger flat bottle sides. Ensure that a uniform layer reaching all corners is obtained.



**C Incubate the MicrobMonitor AQUA Test.**

**C.1** Transfer the **MicrobMonitor AQUA** test bottle to a dark location or incubator for incubation at a temperature appropriate for the assessment being conducted (see below). The gel will set firmly after a few hours. Always incubate the test bottle with the gel on the lower surface. Avoid exposure to light during incubation.



**C.1.1** Potable cold water systems;

Incubate the **MicrobMonitor AQUA** at 22 °C for 3 days. An additional test at 36 °C for 48 hours can also be conducted, as required.

**C.1.2** Hot water and recreational water systems;

Incubate the **MicrobMonitor AQUA** at 36 °C for 48 hours. Examination after 24 hours can, if necessary, provide an early indication of growth.

**Notes on incubation.**

The above recommendations for incubation are consistent with recognised international standards (e.g. ISO 6222 *Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium*).

Various water quality regulations and test standards have different specifications and tolerances for incubation temperature and incubation time. Usually incubation temperatures of 22 °C and/or 36 °C are recommended for the Heterotrophic Plate Count (HPC) (some standards stipulate 37 °C instead of 36 °C). Incubation at 22 °C enables assessment of system microbiological cleanliness and disinfection effectiveness. Incubation at 36 or 37 °C assesses the presence of bacteria which are likely to have been introduced through contamination of the water system and which may potentially pose a health hazard. The test user should adopt the appropriate incubation conditions for their assessment.

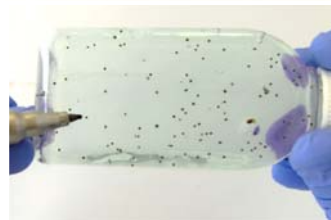
**Notes on requirement for an incubator.**

Where compliance with a specific incubation temperature is needed, a suitable incubator should be used. Typically, this should be capable of maintaining the incubation temperature within  $\pm 2$  °C. Portable incubators are available separately. Prolonged temperature fluctuations outside of the specified range could lead to an under or over estimation of the Heterotrophic Plate Count (HPC) for the designated temperature. Where HPC's are conducted as part of a routine monitoring program to establish trends, consistent incubation conditions should be used.

Where compliance with a specific incubation temperature is NOT needed (e.g. approximate microbiological cleanliness assessments) then it may be acceptable to incubate the **MicrobMonitor AQUA** in any warm, dark location with a reasonably consistent temperature between 20 °C and 30 °C for 3 to 5 days.

**D Examine the MicrobMonitor AQUA Test.**

**D.1** Examine the **MicrobMonitor AQUA** at the end of the specified incubation time. In some cases a preliminary examination (e.g. after 1 or 2 days) may provide an early indication of growth, particularly if heavy contamination is present. To examine the test hold it against a light background and count the number of all visible purple colonies. A hand magnifying lens may help you to identify and count small colonies. All purple colonies in all parts of the test bottle should be counted, including any which are in gel which is not part of the flat layer. It is recommended colonies are marked with a marker pen on the bottle to ensure that they are not counted twice.



**D.2** It is usually possible to count up to about 100 colonies. If the number of colonies is too numerous to count, visually compare the test to the **MicrobMonitor AQUA** Test Results Chart (see page 6). The chart provides an estimate of the colony count.

**D.3** Calculate or estimate the HPC as shown in the **MicrobMonitor AQUA** Test Results Chart (see page 6). Record all results and sample information.

**Notes on examining tests.**





Avoid excessive agitation or prolonged tilting of the bottle during examination. If the bottle is agitated or held vertically or on its side for more than a few minutes, the gel can move and any colonies which have formed may be disrupted. If tests have been incubated at >30 °C, allow them to cool for a few minutes before examination so that the gel becomes firmer.

Colonies are usually circular but may have irregular edges. It is the number of colonies that is important, not their size or shape. Different types of microorganisms can grow at different rates in the gel culture medium and therefore the colonies may be of different sizes. Generally, the more colonies there are in the test bottle, the smaller the colonies will be.

Colonies will tend to become visible more quickly in samples with a higher microbial content. Ignore any microbial colonies which develop after the specified incubation period is complete. If incubation is continued beyond the recommended time the test may change appearance but this should be disregarded.

Some bacteria can spread through the gel producing a large, irregularly shaped colony or a streak or patch of purple colour in the gel. The centre of each streak or patch should be counted as a single colony. These bacteria usually grow quickly and thus if tests are examined earlier than the specified incubation time, while the colonies are still small, they are more easily counted. Continue incubation for the specified time and count any new colonies which develop.

MICROBMONITOR AQUA TEST RESULTS CHART

Appearance after incubation	No of colonies counted or estimated	Heterotrophic Plate Count (HPC also called ACC or TVC)	
		Calculated HPC	Estimated HPC (based on 0.1 ml tested*)
	None	Calculate the HPC in cfu per ml =  $\frac{\text{Number of colonies counted}}{\text{Volume of sample tested (ml)}}$ If no colonies are present or if the number of colonies is too numerous to count, use the estimated HPC.	<10 cfu per ml
	Count exact number of colonies (10 shown)		100 cfu per ml
	If possible count exact number of colonies (100 shown)		1000 cfu per ml
	Results similar to chart; estimated count ≥1000 colonies		Colonies too numerous for an accurate count; use estimated HPC

\* If using the pipette supplied, the volume of sample tested is 0.1ml. If 1ml of sample is tested, the estimated HPC will be 10 times less than that shown.

DISPOSAL

MicrobMonitor AQUA test bottles showing microbial growth should be disinfected before disposal. Wear gloves and avoid touching the inside of the bottle or cap. Wash hands after handling any MicrobMonitor AQUA test bottles showing microbial growth. Disinfect tests showing microbial growth by incineration or by immersing the bottles with the cap removed, in a strong solution of household bleach (e.g. 1 in 10 dilution of 5% sodium hypochlorite) or alternative disinfectant for 24 hours. Alternatively, chlorine release tablets are available (supplied separately) for decontaminating tests.

Disinfected tests, unused tests or those showing no microbial growth can be disposed of as normal waste in accordance with local waste regulations.

## STORAGE AND SHELF LIFE

The shelf life of **MicrobMonitor AQUA** is 1 year from the date of production. The expiry date is printed on the batch label of each **MicrobMonitor AQUA** box. Store **MicrobMonitor AQUA** tests between 2 to 22 °C, in the dark. The expiry date applies only to product stored as stipulated. Do not store **MicrobMonitor AQUA** frozen. Brief exposure to freezing temperatures during transit will not affect the performance of the test. Avoid prolonged exposure of **MicrobMonitor AQUA** tests to light during storage and use. A slightly darker discolouration in the **MicrobMonitor AQUA** gel may develop over time during storage but this will not affect the performance of the test.

## INTERPRETATION

The chemical and microbiological quality of supplies of potable and recreational water is normally regulated by national authorities. In determining health risks, these regulations will typically require compliance with standards for specific microorganisms, for example, *Escherichia coli* which is an indicator of faecal pollution. However, as the tests for these organisms should only be conducted by suitably trained personnel in an appropriate facility, they cannot always be conducted safely on board ships, offshore facilities and in locations which are distant from a suitable laboratory. Because **MicrobMonitor AQUA** enables Heterotrophic Plate Counts (HPC) to be conducted easily and safely in the field, it provides a useful tool for routine operational monitoring of potable and recreational water quality.

Various regulatory and industry specific guidance specifies the use of HPC in monitoring water quality. HPC will not directly correlate with a microbiological health hazard as most of the microorganisms detected are likely to be harmless. However, the number of bacteria present in a water sample, as indicated by the HPC, can provide an overall indication of the microbiological cleanliness of potable and recreational water systems. HPC can be used as an operational indicator of the effectiveness of water disinfection. HPC can also be used to determine trends. An increase in HPC during water distribution or with time indicates post treatment contamination or re-growth of microorganisms within the water distribution system. This will not happen if there is enough residual disinfectant in the water. A sudden increase in HPC above historical baseline values should trigger actions to investigate and if necessary remediate the situation, for example by hyperchlorination. Increases of HPC at 36 or 37 °C can provide an early indication of contamination by bacteria which may potentially pose a health hazard.

The levels of HPC which can be deemed acceptable will depend on the system tested, whether the sample is taken before or after disinfection, the industry sector and any relevant regulation. Regulatory guidance and standards may vary in different geographical regions and for different industry sectors. For potable waters, regulations commonly cite the World Health Organisation (WHO) *Guidelines for Drinking Water Quality 4<sup>th</sup> Edn., 2011 (GDWQ)* as the standard reference for ensuring water quality. For recreational waters, (WHO) *Guidelines for Safe Recreational Water Environments, Volume 2, Swimming Pools and Similar Environments, 2006* are an appropriate reference. Additional guidance specific to industry sectors is available from WHO and other organisations including Port Health Authorities (PHA).

The following table shows typical standards for HPC of potable water, hot water and recreational water facilities based on guidelines used by UK PHA's (Data Source; UK Health Protection Agency *Guidance for Water Quality on board Merchant Ships including Passenger Vessels, 2003*).

Sample	HPC TEST TEMPERATURE	HPC STANDARD PER ML	
		ACCEPTABLE	ACTION LEVEL (treatment required)
POTABLE WATER	22 °C	<100	>1000
HOT WATER	36 or 37 ° C	<100	>1000
SWIMMING POOL	36 or 37 ° C	<10 *	>100
SPA BATHS	36 or 37 ° C	<100	>100

\* WHO *Guidelines for safe recreational water environments, 2006* recommend that operational levels should be <200 per ml.

Further information on specific industry applications can be found in the **MicrobMonitor AQUA** Technical Guidance documents listed on page 2.

## ALSO AVAILABLE

### Optional Products for use with MicrobMonitor AQUA

- **MicrobMonitor AQUA** Water Sampling Packs (ECHA16/MMAWSP/010) - containing sterile plastic sample bottles or bags (suitable for sampling from chlorinated or un-chlorinated systems), alcohol wipes, chlorine release tablets, sterile swabs (for sample point decontamination) and gloves.
- **Incubator Cultura** (ECHA14/IN230V or ECHA14/INV110V) - small portable incubator which enables optimal, consistent incubation temperature of **MicrobMonitor** tests; holds about 15 **MicrobMonitor** AQUA test bottles; mains operated (110 or 230 Volt).
- **MicrobMonitor** Bumpers (ECHA16/TB/001)- soft, rubber-like, hemispheres which stick on to a bench top and enable easy breaking and shaking of **MicrobMonitor** AQUA gel.
- Sterile 1 ml single bulb pipettes (ECHA07/PP/1ML/010) - sterile measuring device graduated at 0.25 ml for testing sample volumes greater than 0.1 ml.
- Sterile swabs (ECHA15/SW/03) - swabs for sampling surface contamination (e.g. tank surfaces, filter cartridges etc.) and also for cleaning tap outlets before sampling from them.
- Chlorine release tablets (ECHA21/CP/10) - for disinfection of used **MicrobMonitor** AQUA tests.

### Other Test Kits & Ancillary Items

Other test kits and ancillary items are available from ECHA Microbiology Ltd. For our full range of test kits and ancillaries see [www.microbmonitor.com](http://www.microbmonitor.com). ECHA also offers laboratory testing, training courses, consultancy including on-site attendance for audit and investigation of microbial contamination and corrosion in systems, products and facilities.

- **MicrobMonitor<sup>2</sup>** — a simple and easy to use test kit which enables the quantitative assessment of the viable microbial content in fuels, oils and associated water in accordance with IP Standard Method IP613 and ASTM D7978.
- **Sig Sulphide<sup>®</sup>** — simple test kit for Sulphate Reducing Bacteria (SRB) which can cause serious corrosion and sulphide spoilage in water systems and other industrial systems.
- **Sig Nitrite<sup>®</sup>** — simple test kit for Nitrite Reducing Bacteria (NRB) which can cause depletion of corrosion inhibitors in closed water systems.
- Other testing ancillaries, indicator papers and sampling and monitoring equipment.