

NATIONAL STANDARD METHOD

GUIDANCE NOTE

# THE MICROBIOLOGICAL EXAMINATION OF WATER SAMPLES

## QSOP 57

Issued by Standards Unit, Evaluations and Standards Laboratory  
**Centre for Infections**



### THE MICROBIOLOGICAL EXAMINATION OF WATER SAMPLES

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# AMENDMENT PROCEDURE

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 06/12/05	1	2	7  12  15  12  21  22  23   24	<b>Section 2.7 Aircraft waters</b> <b>Section 3.2.8 Closing spa pools</b> <b>Appendix 1 Bowser / Tanker</b> <b>Section 3.3</b> <b>Appendix 1 Aircraft waters</b> <b>Appendix 1 Swimming pools</b> <b>Appendix 1 Spa pools</b> <b>Appendix 1 Hydrotherapy pools</b> <b>Examples of comments for reports</b>	Text amended. Reference to <i>clostridium</i> species removed Format of bullet points amended Extra test for consideration deleted Text amended to clarify action levels for total count and Coliform bacteria Action level for Coliform bacteria amended Comments added to Coliform bacteria Action level for <i>Ps. aeruginosa</i> Coliform bacteria and Colony count amended Action level for colony count amended Comments added to <i>E.coli</i> , Coliform bacteria and Colony count <i>Ps. aeruginosa</i> added to the table Note added below the table

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# GUIDANCE NOTE ON THE MICROBIOLOGICAL EXAMINATION OF WATER SAMPLES

## 1 INTRODUCTION

The aim of this document is to provide guidance for the Microbiological testing of water samples across the Health Protection Agency (HPA). The document covers the testing of water samples for human consumption, pool waters and cooling towers.

The results of a laboratory examination of a single water sample are representative only of the water at that particular point at which the sample is taken. Contamination is often intermittent and may not be revealed by the examination of a single sample. The value of microbiological tests is dependent upon their frequent and regular use. Information gained over time through monitoring will provide a comprehensive picture of the overall quality of any particular source of water, any deterioration from which should at once arouse suspicion.

Sampling techniques and sample transportation can influence sample results and good practice is essential. Samples collected from treated waters should have any residual biocide neutralised, wherever possible, at the time of sampling. For oxidising biocides such as chlorine and bromine this is achieved by using sample bottles containing sodium thiosulphate as stated in the MDW 2002<sup>2</sup> Part 2 section 3.1.

## 2 WATER FOR HUMAN CONSUMPTION / FOOD PREPARATION

### 2.1 Mains waters

Mains water supplies are routinely sampled by the water undertakings for compliance with the Water Supply (Water Quality) Regulations 2000<sup>3</sup> which implement the European Drinking Water Directive. For most parameters the water at the consumer's taps has to comply with the regulations. Two kinds of samples are routinely collected, check monitoring samples and audit monitoring samples. **Check monitoring** is performed more frequently and involves a smaller number of parameters and may be carried out by the water utilities laboratories. **Audit monitoring** is less frequent but encompasses a wider range of parameters to ensure that all prescribed values are complied with and may be carried out by the Environmental Health Officers. Since all routine monitoring is performed adequately by the water undertakings and nationally compliance is extremely good, (99.88% of 2.9 million tests carried out in 2003 met all the national and EU drinking water standards) there should be no need for HPA laboratories to undertake routine (check) monitoring tests on public supplies.

Water samples from mains supplies should therefore only normally be examined by HPA or HPA collaborating FWE laboratories in response to a complaint, as part of the investigation of an outbreak or other potential problem and in order to test water in food production premises for compliance with the EC Drinking Water Directive and Food Safety Act, or an investigation of an outbreak / incident or other potential problem (the CCDC / HPU should be involved in decisions in relation to outbreaks / incidents ). In these circumstances the samples will therefore be effectively "audit" samples and examined for the extended range of parameters and any other specific pathogen tests that may be relevant to the investigation. In all these cases water should be examined for Coliforms, *E.coli*, enterococci and possibly examined for aerobic colony count (ACC) at 37°C for 44 ± 4hrs and 22°C for 68 ± 4hrs. If the supply is known to be derived from a surface source or influenced by one (i.e. could be contaminated or diluted with surface water) *Cl. perfringens* may also be included but the European Expert Microbiology Advisory Group has advised the EC that it does not believe there is any public health benefit from the examination of water for *Cl. perfringens* routinely. The ACC is of limited value unless the particular water supply and

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sample point is sampled regularly since one is looking for abnormal changes from the usual pattern. The exception to this would be for samples collected from a new water system in, for example, a newly built hospital or newly modernised part of a hospital where one would expect the ACC to be low and comparable to the incoming main supply if the system had been adequately disinfected and flushed immediately (within 14 days) prior to occupation, or post disinfection samples following maintenance or interruption to service. The ACC may also be useful when investigating complaints since organic contamination or insufficient chlorination may lead to a raised ACC.

## 2.2 Bowsers / tankers

These should be tested as mains potable waters at the supply point from the tank / bowser for Coliforms, *E. coli*, enterococci and ACC at 37°C for 44 ± 4hrs and 22°C for 68 ± 4hrs.

## 2.3 Private water supplies (PWS)

Private supplies are tested according to the Private Water Supplies Regulations (SI 1991 no 2790)<sup>4</sup> although they are covered by the same EU directive as mains public supplies but the UK national regulations have not yet been updated in line with the EU directive. In contrast with the situation for mains supplies HPA laboratories may be involved in the check (routine) monitoring of PWS, in particular where it is supplying a service to a hospital with a private supply. Large PWS are sampled more frequently than small supplies and may be subjected to both check and audit monitoring so it could be justified to simply do coliforms and *E. coli* and ACC on routine “check monitoring” samples. For audit monitoring, enterococci should be included as well as coliforms, *E.coli* and ACC. If the water supply is surface derived or influenced, *Cl.perfringens* may also be tested for. *Cl.perfringens* will normally only be tested for following a complaint or failure.

NB These samples should be charged for except for Class F waters.

## 2.4 Bottled Waters, and Spring Waters

For the purposes of this document bottled water is any water in bottles sold for drinking that is not designated as mineral water. The water in many office drinking water dispensers is bottled water. Although the requirements are for testing at the time of bottling, the faecal indicators should be absent at bottling and should not be capable of growth in bottled water during distribution. It is reasonable to expect these to be absent at the point of use. Similarly if *Ps. aeruginosa* is absent at the point of bottling, it should not be present during distribution.

As far as microbiological testing is concerned, in England spring waters are treated in a similar way to bottled waters. The parameters are the same as audit samples for mains water (see section 2.1) with the addition of *Ps. aeruginosa* as a parameter and the sample volume increased from 100 mL to 250mL.

In the UK the Food Standards Agency has implemented into national legislation those provisions of Directive 98/83/EC<sup>5</sup>, which relate to bottled drinking waters and spring waters through The Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2003 (Statutory Instrument 2003 No. 666)<sup>6</sup> as updated to include clauses related to ozone and carbon dioxide by the Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2004 (Statutory Instrument 2004 No. 656). The regulations amend the Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 Statutory Instrument 1540. The original SI 1999 No. 1540 revoked (or replaced) The Natural Mineral Waters Regulations 1985 (SI 73) and The Drinking Water in Containers Regulations 1994 (SI 743). The regulations as amended by Statutory Instruments 2003 SI 666 and 2004 SI 656 therefore cover all drinking water in bottles in England (Scotland, Wales and Northern Ireland have produced separate amending regulations). All bottled waters of any kind are examined using 250mL volumes for *E. coli* and *Ps. aeruginosa* and 1mL volumes for the ACC at 37°C for 44 ± 4hrs and 22°C for 68 ± 4hrs. (NOTE: in table C of the Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2003 (SI 2003: 666) it says 22°C for “72 hours on agar-agar or an agar-gelatine mixture” and 37°C “in 24 hours on agar-

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agar". These conditions are from the old directives. However under the explanatory note (xv) in SI 2003; 666 it says that with effect from 25<sup>th</sup> December 2003, the methods used should be in accordance with Article 7.5 of Directive 98/83/EC. Accordingly we will use the same incubation conditions as used for drinking water and laid down in BS EN ISO 6222 and MDWD 2002<sup>2</sup>.

## 2.5 Mineral waters

The Food Standards Agency has implemented into national legislation those provisions of Directives 80/777/EEC and 96/70/EC in The Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2004 (Statutory Instrument 2004 No. 656) . The regulations amend the Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 Statutory Instrument 1540<sup>9</sup>. The original SI 1540 revoked (or replaced) The Natural Mineral Waters Regulations 1985 (SI 73) and The Drinking Water in Containers Regulations 1994 (SI 743).

The original EEC Mineral Water Directive is still in force with amendments and in England the parameters have not been brought in line with other potable waters. In England this is implemented by SI 1999 No.1540 which simply refers to Article 5.2 of 80/777/EEC<sup>7</sup> and the parameters are *E. coli*, coliforms, faecal streptococci and *Ps. aeruginosa* in 250mL and sporulated sulphite-reducing anaerobes in 50 mL and the colony count in 1mL volumes at 37°C for 24 ± 1hrs and 22°C for 68 ± 4hrs. Faecal streptococci is synonymous with enterococci as examined under the new EC drinking Water Directive but sporulated sulphite-reducing anaerobes is not synonymous with *Cl. perfringens* which is currently required for all other waters, when surface derived or influenced.

## 2.6 Ships waters

The HPA in conjunction with the Port Health Authorities has produced guidelines for the examination of water on ships (HPA 2003 *Guidelines for water quality on board merchant ships including passenger vessels*)<sup>10</sup>. During a sanitation inspection of a ship it is recommended that Port Health Officers sample the potable water and the Laboratories test for *E. coli*, enterococci, coliforms, and ACC at 37°C for 44 ± 4hrs and 22°C for 68 ± 4hrs.

## 2.7 Aircraft waters

The PHLS and Port Health Authorities carried out a survey of the quality of supplies of water to aircraft and on board aircraft at UK airports (PHLS 2003 *The Microbiological Quality of Water Onboard Aircraft*)<sup>11</sup>. In this report guidelines for the microbiological testing of water on board aircraft were given that have been adopted by at least one UK airline (BA).

Where microbiological testing is necessary water should be tested for coliforms, *E. coli*, enterococci, the aerobic colony count and *Ps. aeruginosa*. Essentially the quality standards should be the same as those for public water supplies with the addition of *Ps. aeruginosa* and ACC as these are indicators of the efficacy of disinfection. *E. coli* and Enterococci are used as faecal indicators, coliforms are useful as indicators of poor hygiene and the aerobic colony count and *Ps. aeruginosa* counts as checks on disinfection and re-growth within distribution.

# 3 POOL WATERS

## 3.1 Swimming pools

The following guidance was prepared by the Pool Water Treatment Advisory Group in collaboration with the HPA and is incorporated in the BSI 2003 Publicly Available Specification PAS 39 *Management of public swimming pools — Water treatment systems, water treatment plant and heating and ventilation systems* — Code of practice<sup>12</sup>.

### 3.1.1 General

Microbiological contamination of the pool water can result in pathogenic (disease producing) micro-organisms causing infections to bathers. These contaminants can be introduced into

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pool water from bathers, from the pool filters or occasionally from defects in pool engineering (eg that allow the water to be contaminated with sewage).

Tests should be performed monthly to monitor the presence of indicator micro-organisms. These can indicate operational problems that could result in infections. Additionally, tests should be performed:

- a) before a pool is used for the first time
- b) before it is put back into use, after having been shut down for repairs
- c) if there are difficulties with the treatment system
- d) if contamination has been noted
- e) as part of any investigation into possible adverse effects on bathers' health
- f) to validate changes made to the pool treatment regime eg change in biocide, or frequency of addition, or engineering

More frequent sampling will be necessary if there is a problem, or for particularly heavily loaded pools.

Hydrotherapy pools should be tested twice weekly as the people using these pools, are immersed for longer periods (as are staff) and may be more vulnerable to infection.

Microbiological sampling should be performed by trained and competent personnel to prevent sample contamination. Microbiological analysis should be carried out in appropriately accredited laboratories, eg UKAS laboratories. The pH level and the concentration of free and total disinfectant in the pool water should be measured when the microbiological sample is collected. Microbiological samples should be taken at a depth of approximately 30 cms or 12 inches below the surface of the pool.

The results of routine microbiological sampling should always be interpreted in conjunction with:

- chemical tests performed on site and/or in the laboratory at the time of sample collection; and
- a review of the maintenance records for the pool, including records of the pH, residual chlorine levels, mechanical failures and water appearance and other untoward events.

It should be noted that failure to comply with the target levels for one or more of the parameters is often a passing phenomenon.

Subclauses below give the limits for the various tests, and indicate what action should be taken if unsatisfactory results are obtained. Samples should be tested for aerobic colony count, coliforms, *Escherichia coli* and *Pseudomonas aeruginosa*.

If the microbiological results are unsatisfactory the microbiological tests should be repeated immediately.

If the results of the second microbiological tests are still unsatisfactory, an investigation into the management and operating conditions of the pool should be undertaken and a third series of microbiological tests should be made.

NB: The investigation may require the help of the laboratory that does the tests, the district council Environmental Health Department, or an independent consultant.

If results are still unsatisfactory after the investigation and a third series of tests, immediate remedial action is required that may necessitate the pool is closed. See section 3.1.6 Closing Pools.

### 3.1.2 Aerobic colony count

The aerobic colony count (ACC), sometimes called the total viable count, colony count, or plate count, is a general test that indicates whether the pool disinfectant regime is effective in controlling contamination under operational circumstances. The colony count should be carried out in accordance with BS EN ISO 6222:1999 (BS 6068-4.5:1999) but with incubation at 37°C for 24 ± 1hrs.

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NB: These test conditions are set to isolate the range of organisms that can colonize the mouth and skin of bathers.

The ACC can become increased where there is a higher bather load, reduced chlorine residual or where there are defects in water treatment.

The aerobic colony count should normally be 10 or less colony forming units (cfu) per millilitre of pool water. If a colony count above 10 cfu/mL is the only unsatisfactory microbiological result, and residual chlorine and pH values are within recommended ranges, the water should be retested.

### 3.1.3 Coliform bacteria

Coliforms within swimming pools can be considered as an indication of faecal contamination or poor hygiene (e.g. contamination from shoes or leaves in outdoor pools). Their presence indicates that the treatment has failed to remove this contamination.

NOTE 1 In themselves, coliforms do not usually cause disease.

NOTE 2 The presence of *Escherichia coli* is a better indication of faecal contamination.

Coliforms are sensitive to disinfectant and should be absent in 100mL of pool water. A repeat sample should be taken whenever coliforms have been detected.

A coliform count of up to 10 cfu/100mL is acceptable provided that:

- a) coliforms are not found in the repeat sample
- b) the aerobic colony count is less than 10 cfu/mL
- c) there are no *E. coli* present
- d) the residual disinfectant and pH values are within recommended ranges

### 3.1.4 *Escherichia coli*

*Escherichia coli* is normally present in the faeces of most humans, mammals and birds. It is widely used as a specific indicator of faecal contamination as it is unable to grow within the environment. The presence of *E. coli* in swimming pool water is an indication that faecal material has entered the pool water from the contaminated skin of patients, or from faecal material that has been accidentally or deliberately introduced. It also indicates that the treatment has failed to remove this contamination.

*E. coli* should be absent in a 100mL sample. However, because most bathers will have some faecal contamination of their skin, particularly if they have not showered before bathing, a single positive sample may be the result of recent superficial contamination by a bather that has not yet been decontaminated by the disinfectant residual. A repeat sample should then be taken.

### 3.1.5 *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* can grow within untreated waters and in biofilms. It can cause skin, ear and eye infections when present in large numbers and outbreaks of skin infections have been linked to swimming pools and spa pools.

Well operated pools should not normally contain *Ps. aeruginosa*. If the count is over 10 *Ps. aeruginosa* per 100mL, repeat testing should be undertaken. Where repeated samples contain *Ps. aeruginosa* the filtration and disinfection processes should be examined to determine whether there are areas within the pool circulation where the organism is able to multiply. Where counts exceed fifty, pool closure should be considered.

### 3.1.6 Closing pools

Pools should be closed if following a routine microbiological test:

- a) the result suggests gross contamination (see below); and
- b) there is other chemical or physical evidence that the pool disinfection system is not operating correctly (e.g. if the records show that residual disinfectant levels were

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inadequate or erratic and frequently too low, or the poolwater is of unsatisfactory appearance).

Where there is evidence of gross contamination the pool should be closed to prevent illness in pool users. The local Consultant for Communicable Disease Control (CCDC - via health authority) should be contacted.

The following should be considered as gross contamination:

- a) greater than 10 *E. coli* per 100mL in combination with one or both of the following:
  1. an unsatisfactory aerobic colony count (>10 per mL);
  2. or an unsatisfactory *Ps. aeruginosa* count (>10 per 100mL);
- b) greater than 50 *Ps. aeruginosa* per 100mL in combination with high aerobic colony count (>100 per mL)

### 3.1.7 Additional testing in outbreaks

In the event of an outbreak of illness associated with a swimming pool additional microbiological testing may be considered necessary. This needs to be discussed with the CCDC and the chairman of the outbreak control team. If disinfection is adequate then bacterial and viral tests are unlikely to represent the conditions at the time of the infectious event. *Cryptosporidium* or *Giardia* contamination may still be detectable through examination of backwash water and filter material (routine testing for *Cryptosporidium* and *Giardia* is not considered useful).

For confirmation and typing of *Cryptosporidium* contact the *Cryptosporidium* Reference Unit (CRU), Singleton Hospital, Swansea. Advice on sampling and control can also be obtained from the Water and Environmental Microbiology Reference Unit (WEMRU).

### 3.2 Spa pools (commonly known as Jacuzzi)

The following guidance has been prepared jointly by the HSE and HPA and will be incorporated into the new HSE/HPA Guidance document on Spa Pools. It follows the same pattern as the guidance for swimming pools (Section 3.1 above) and that section should be read for further information on the target organisms. As with swimming pools the collection of routine samples is the responsibility of the owners.

Microbiological samples for indicator organisms should be taken at least once a month as a routine and quarterly for *Legionella* species. More frequent sampling may be required depending on the Risk Assessment, eg if the spa pool is being intensively used and certainly if there are any adverse health effects reported by the bathers. If adverse health effects are suspected the enforcing authority (HSE or the Local Authority) and the microbiologist in the testing laboratory should be informed; as required they will then notify the CCDC within the Local Health Protection Unit. Microbiological sampling should also be done

- when a spa pool is first used or re-commissioned,
- after a report of ill-health following spa pool use,
- if there are problems with the running of the pool or contamination incidents, or
- alterations in the treatment/maintenance regimes.

#### 3.2.1 Microbiological tests

Tests for indicator organisms should include an aerobic colony count (sometimes called the total viable (colony) count or plate count), coliforms and *Escherichia coli*, and *Pseudomonas aeruginosa*. In addition, tests should be carried out quarterly for *Legionella* (note the requirement for sampling for legionellae is not in the current guidance written in 1994 but will be in the new guidance to be published in late 2005 or early 2006). The aerobic colony count (ACC) after 24 hours incubation at 37°C will give an indication of the overall microbiological quality of the spa pool while the continued presence of coliforms and especially *E. coli* will indicate the presence of serious contamination arising as a result of a

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breakdown in the treatment system. The presence of the potential pathogen *Ps. aeruginosa* is also an indication of treatment failure with likely colonisation and biofilm formation of the spa pool filter and within other parts of the system. It is a more sensitive indicator of sustained management problems than the coliforms and may be found in their absence but is usually associated with an elevated ACC.

If there are health problems associated with the use of the spa pool, it may be necessary to test for other organisms, based on epidemiological evidence, such as *Staphylococcus aureus*, *Cryptosporidium*, *Giardia* and perhaps viruses. In these circumstances advice should first be sought from the local Health Protection Unit and the microbiologist.

Routine sampling should be done when the spa pool is in use, preferably when heavily loaded or immediately thereafter.

### 3.2.2 Recommended microbiological standards for spa pools

If these indicator microbiological results are unsatisfactory a review of the records should be undertaken and the microbiological tests repeated immediately. **If the results show gross contamination (see Section 3.2.8) then the spa pool should be taken out of use immediately and any remedial actions implemented before re-sampling.**

If results are still unsatisfactory after the repeat samples and investigation, immediate remedial action is required that may necessitate the spa pool being closed. Note: the investigation may require the help of the laboratory that does the tests, the local council Environmental Health Department, or an independent consultant.

### 3.2.3 Aerobic colony count

The aerobic colony count should normally be 10 or less colony forming units (cfu) per millilitre of spa pool water. If a colony count above 10cfu/mL is the only unsatisfactory microbiological result, and residual disinfectant and pH values are within recommended ranges, the water should be retested.

### 3.2.4 Coliform bacteria (total coliforms)

Coliforms within spa pools can be considered an indication of faecal contamination or poor hygiene (eg contamination from shoes or leaves in outdoor spa pools). Their presence indicates that the treatment has failed to remove this contamination. Coliforms are sensitive to disinfectant and should be absent in 100mL of spa pool water. A repeat sample should be taken whenever coliforms have been detected. A coliform count of up to 10cfu/100mL is acceptable provided that:

- the aerobic colony count is less than 10cfu/mL
- there are no *E. coli* present
- the residual disinfectant and pH values are within recommended ranges

### 3.2.5 *Escherichia coli*

*E. coli* should be absent in a 100mL sample. However, because most bathers will have some faecal contamination of their skin, particularly if they have not showered before bathing, a single positive sample may be the result of recent superficial contamination by a bather that has not yet been decontaminated by the disinfectant residual. A repeat sample should then be taken.

### 3.2.6 *Pseudomonas aeruginosa*

Well-operated spa pools should not normally contain *Ps. aeruginosa*. If the count is over 10 *Ps. aeruginosa* per 100mL, repeat testing should be undertaken. Where repeated samples contain *Ps. aeruginosa* the filtration and disinfection processes should be examined to determine whether there are areas within the spa pool circulation where the organism is able to multiply. There is a risk of an outbreak of folliculitis when the count exceeds

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50cfu/100mL so the spa pool should be closed, remedial action taken and the water re-sampled.

### 3.2.7 **Legionella**

Well-operated spa pools should not normally contain *Legionella* species. The microbiological results should not be considered in isolation but in the context of the management records for the spa pool.

### 3.2.8 **Closing spa pools**

The spa pool should be closed following a routine microbiological test if: the result suggests gross contamination (see below); or there is other chemical or physical evidence that the spa pool disinfection system is not operating correctly (eg if the records show that residual disinfectant values were inadequate or erratic and frequently too low, or the spa pool water is of unsatisfactory appearance).

Where there is evidence of gross contamination the spa pool should be closed to prevent illness in users and those working near the spa pool. The appropriate enforcing authority should be contacted.

The following should be considered gross contamination:

1. greater than 10 *E.coli* per 100mL in combination with
  - an unsatisfactory aerobic colony count (>10 per 100mL) and/or
  - an unsatisfactory *Ps. aeruginosa* count (>10 per 100mL) and
2. greater than 50 *Ps. aeruginosa* per 100mL in combination with a high aerobic colony count (>100 per mL)
3. greater than 1000 cfu per litre *Legionella* species

The above guidelines for interpretation of *Legionella* results have been agreed by a number of environmental health departments but note that some departments have a zero tolerance approach on the basis that negative *Legionella* results are achievable.

## 3.3 **Hydrotherapy Pools**

Periodic bacteriological examinations provide valuable information about pool conditions not given by routine chemical and physical tests. Hydrotherapy pool waters should be tested for ACC at 37°C for 24hrs, *Pseudomonas aeruginosa*, *E.coli* and Coliform bacteria<sup>13</sup>.

Total counts of the water of a successfully controlled pool will seldom reach 10cfu/mL. Counts of 11 – 100 cfu/mL are unsatisfactory but require investigating. Counts over 100cfu/mL are unacceptable and require immediate consultation and further investigation as does the presence of *Ps. aeruginosa*. *E.coli* when present indicates faecal pollution, likely to be associated with a major failure of disinfection. Action levels for coliform bacteria is the same as those of pool water (see section 3.2.4).

Testing for the presence of other organisms such as *Cryptosporidium*, *Giardia*, *Legionella* species or *Staphylococcus aureus* may be required when adverse effects have been associated with pool use, but should not be done routinely.

## 4 **COOLING TOWERS**

Cooling towers and evaporative condensers should be routinely monitored to ensure that control of *Legionella* growth is in place, according to the Health and Safety Commission 2000 Approved Code of Practice and Guidance L8. To achieve compliance with L8, it is recommended that systems should be tested on a weekly basis for ACC at 30°C for 49 ± 1hrs (minimum) and quarterly testing for the presence of legionellae (or monthly when commissioning and establishing a treatment programme for new plant). The sampling method should be in accordance with Standing Committee of Analysts (2005)<sup>14</sup>.

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An ACC of not more than 10,000 bacteria per mL and less than 100 *Legionella* per litre indicate that the system is under control. For levels of 10,000 – 100,000 ACC per mL, or 100 – 1,000 *Legionella* per litre, a review of control measures and a risk assessment should be undertaken. Immediate corrective action including disinfection and re-sample should be implemented if levels exceed 1,000,000 ACC per mL or 1,000 *Legionella* per litre.

## 5 ACKNOWLEDGMENTS AND CONTACTS

This Guidance Note has been developed, reviewed and revised by the Water Working Group ([http://www.hpa-standardmethods.org.uk/wg\\_water.asp](http://www.hpa-standardmethods.org.uk/wg_water.asp)). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

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## APPENDIX 1. SUMMARY OF TESTS TO BE PERFORMED ON WATER SAMPLES IN HPA LABORATORIES

### MAINS WATER

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Mains water</b>				Based on Council Directive 98/83/EC		Water Supply (Water Quality) Regulations 2000 (SI 2000 No. 3184)
Enterococci	100mL	0	>0		W3	
E. coli	100mL	0	>0		W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>0		W2 /	
<b>Extra tests for consideration and use when following up failures</b>						
<i>Cl. perfringens</i> including spores	100mL	0	>0	If surface derived or influenced, does not have to be tested at tap if tested at supply point. Only do following failures if appropriate.	W5	
Colony count 22°C for 68 ± 4hrs	1mL	No abnormal change		For one off samples of little value except post disinfection e.g. new water system in a hospital or to investigate complaints about taste / odour.	W4	
Colony count 37°C for 44 ± 4hrs	1mL	No abnormal change		For one off samples of little value except post disinfection e.g. new water system in a hospital or to investigate complaints about taste / odour.	W4	

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## BOWSER /TANKER

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Bowser / tanker</b>				Based on Council Directive 98/83/EC		Water Supply (Water Quality) Regulations 2000 (SI 2000 No. 3184)
Enterococci	100mL	0	>0		W3	
E. coli	100mL	0	>0		W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>0		W2 / W18	
Colony count 22°C for 68 ± 4hrs	1mL	No abnormal change		For one off samples of little value except post disinfection.	W4	
Colony count 37°C for 44 ± 4hrs	1mL	No abnormal change		For one off samples of little value except post disinfection.	W4	

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## PRIVATE WATER SUPPLIES

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
Private water supplies				The parameters here comply with Council Directive 98/83/EC as incorporated into SI 2000 No. 3184 but the Private Water Supplies 1991 Regulations have not been updated.		Private Water Supplies Regulations (SI 1991 No. 2790) <b>NB</b> await updating to be in line with SI 2000 No. 3184
<b>All classes</b>						
<i>Escherichia coli</i>	100mL	0	>0		W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>0		W2 /	
<b>Classes A-E and 1-4 only</b>						
Colony count 22°C for 68 ± 4hrs	1mL	No abnormal change		Only of value if done regularly. For one off samples of little value except post disinfection e.g. new water system in a hospital or to investigate complaints about taste / odour.		
Colony count 37°C for 44 ± 4hrs	1mL	No abnormal change		Only of value if done regularly. For one off samples of little value except post disinfection e.g. new water system in a hospital or to investigate complaints about taste / odour.		
<b>Extra tests for consideration and use when following up failures</b>						
<i>Cl. perfringens</i> including spores	100mL	0	>0	Not recommended for regular testing unless investigating a problem	W5	
Enterococci	100mL	0	>0	Not required by PWS Regs 1991	W3	Will be required for all audit samples when PWS Regs are revised

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## BOTTLED WATER / SPRING WATER

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Bottled water / spring water</b>				Based on Council Directive 98/83/EC	W3	The Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 (Statutory Instrument 1540) as amended by the Natural Mineral Water, Spring Water and Bottled Water (Amendment) (England) Regulations 2003 (SI 2003 No. 666 )and the Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2004 (Statutory Instrument 2004 No. 656).
Enterococci	250mL	0	>0	At bottling	W3	
<i>E. coli</i>	250mL	0	>0	At bottling	W2	
Coliform bacteria (total coliforms)	250mL	0	>0	At bottling	W2	
<i>Ps.aeruginosa</i>	250mL	0	>0	At bottling	W6	
Colony count 22°C for 68 ± 4hrs	1mL	100	>100	Within 12h of bottling		
Colony count 37°C for 44 ± 4hrs	1mL	20	>20	Within 12h of bottling		

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## Mineral Water

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
Mineral Water				Based on Council Directive 80/777/EC* as amended by 96/70/EC & 2003/40/EC		The Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 (Statutory Instrument 1540) as amended by the Natural Mineral Water, Spring Water and Bottled Water (Amendment) (England) Regulations 2003 ( SI 2003 No. 666 )and the Natural Mineral Water, Spring Water and Bottled Drinking Water (amendment) (England) Regulations 2004 (Statutory Instrument 2004 No. 656).
Faecal Streptococci (Enterococci)	250mL	0	>0	At source and during marketing	W3	
<i>Escherichia coli</i>	250mL	0	>0	At source and during marketing	W2	
Coliform bacteria (total coliforms)	250mL	0	>0	At source and during marketing	W2	
Spores of sulphite reducing anaerobes	50mL	0	>0	At source and during marketing		
<i>Ps.aeruginosa</i>	250mL	0	>0	At source and during marketing	W6	
Colony count 22°C for 68 ± 4hrs	1mL	20	>20	At source - conform to normal which should be less than 20/mL		
		that resulting from normal increase		During marketing		<b>CONTINUED ON NEXT PAGE</b>

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## MINERAL WATER ( continued)

Test	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
Colony count 37°C for 24 ± 1hrs	1mL	5	>5	At source - conform to normal which should be less than 5/mL		
		20	>20	within 12h of bottling when maintained at 4°C during this 12h period		
		that resulting from normal increase		During marketing		

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## SHIPS WATERS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Ships waters</b>						HPA 2003 <i>Guidelines for water quality on board merchant ships including passenger vessels</i>
Enterococci	100mL	0	>0		W3	
<i>Escherichia coli</i>	100mL	0	>0		W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>0		W2 / W18	
Colony count 22°C for 68 ± 4hrs	1mL	<100	>1000	For one off samples of little value except post disinfection		
Colony count 37°C for 44 ± 4hrs	1mL	<100	>1000	For one off samples of little value except post disinfection		

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## AIRCRAFT WATERS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Aircraft waters</b>						PHLS 2003 <i>The Microbiological Quality of Water Onboard Aircraft</i>
Enterococci	100mL	0	>0		W3	
<i>Escherichia coli</i>	100mL	0	>0		W2 / W18	
Coliform bacteria (total)	100mL	0	>0		W2 / W18	
<i>Ps.aeruginosa</i>	100mL	0	≥100		W6	
Colony count 22°C for 68 ± 4hrs	1mL	<100	≥1000		W4	
Colony count 37°C for 44 ± 4hrs	1mL	<100	≥1000		W4	

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## SWIMMING POOLS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Swimming pools</b>					<b>Yes</b>	BSI 2003 PAS 39 <i>Management of public swimming pools — Water treatment systems, water treatment plant and heating and ventilation systems</i> — Code of practice
<i>Escherichia coli</i>	100mL	0	>0	See section 3.1.4	W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>10	See section 3.1.3	W2 / W18	
<i>Ps.aeruginosa</i>	100mL	0	>10	See section 3.1.5	W6	
Colony count 37°C 24 ± 1hrs	1mL	≤10	>10	See sections 3.12	W4	

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## SPA POOLS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
Spa Pools ("Jacuzzis")						PHLS 1994 Hygiene for Spa pools ISBN 0901144371 (Scheduled to be replaced in 2005 by HSE/HPA 2005 <i>Management of Spa Pools:- Controlling The Risks of Infection</i> currently in press)
<i>Escherichia coli</i>	100mL	0	>0	If >10 and colony count >10 or <i>Ps. aeruginosa</i> >10 close pool	W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>10	See section 3.2.4	W2 / W18	
<i>Ps.aeruginosa</i>	100mL	0	>10	See section 3.2.8	W6	
Colony count 37°C for 24 ± 1hrs	1mL	≤10	>10	See section 3.2.3	W4	
<i>Legionella</i>	1000mL	<100	≥100	If >1000 close pool	W12 / W13 / W14	Currently not required but recommend quarterly in the new HSE/HPA guidance <i>Management of Spa Pools:- Controlling The Risks of Infection</i> currently in press)

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## HYDROTHERAPY POOLS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
<b>Hydrotherapy Pools</b>					Yes	PHLS 1999 Hygiene for Hydrotherapy Pools 2 <sup>nd</sup> Edition
<i>Escherichia coli</i>	100mL	0	>0	If >0 immediate consultation and investigation required	W2 / W18	
Coliform bacteria (total coliforms)	100mL	0	>10	See section 3.1.3	W2 / W18	
<i>Ps.aeruginosa</i>	100mL	0	>0	If >0 immediate consultation and investigation	W6	
Colony count 37°C 24 ± 1hrs	1mL	10	>100	If > 10 report as unsatisfactory If > 100 immediate consultation and investigation required	W4	

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## COOLING TOWERS

	Volume to be tested	Acceptable level	Action level	Comments	HPA Method	UK Legislation / Guidance
Cooling Tower Water					Yes	Health and Safety Commission 2000 Approved Code of Practice and Guidance L8 <i>Legionnaires' disease – the control of legionella bacteria in water systems</i> . HSE Books, PO Box 1999, Sudbury, Suffolk, CO10 2WA. ISBN 0-7176-1772-6
<i>Legionella</i>	1000mL	<100/L	≥100	If >1000/L immediately resample and then disinfect	W12 / W13 / W14	
Colony count 30°C for 49 ± 1hrs	1mL	≤ 10,000	>10,000	If > 100,000.mL immediately resample and then disinfect	W4	

Mineral Water \*The microbiological requirements have not changed since the original directive; tests in unshaded boxes are required on all samples; tests in shaded boxes are only required for some samples temperature tolerances are those in Microbiology of Drinking water 2002; requested by the CCDC; or to support adverse results of inspections by EHO's subject to local agreement with the laboratory.

### EXAMPLES OF COMMENTS FOR REPORTS

COMMENT	EXPLANATION
<b>Satisfactory:</b>	target organism not detected in the volume examined
<b>Acceptable:</b>	target organism detected but at counts below the threshold level
<b>Unsatisfactory:</b>	target organism detected at counts between the acceptable level and action level
<b>Unacceptable:</b>	target organisms detected above the recommended action level requiring remedial action

Note: The above comments for reports cannot cover all circumstances but is a guide to consistency of reporting.

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