Methods for the Examination of Waters and Associated Materials

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Bacteriological Swabs

2023

Bacteriological Swabs

This booklet contains a method for the analysis of surfaces using swabs.

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Within this series there are separate booklets, each dealing with different topics concerning the microbiology of drinking water, including:

Microbiology of Drinking Water

- Part 1 – Water quality, epidemiology and public health
- Part 2 – Practices and procedures for sampling
- Part 4 – Methods for the Isolation and enumeration of coliform bacteria and Escherichia coli (including E. coli O157 H7)
- Part 5 – Methods for the isolation and enumeration of enterococci
- Part 6 – Methods for the isolation and enumeration of sulphite-reducing clostridia and Clostridium perfringens by membrane filtration
- Part 7 – Methods for the enumeration of heterotrophic bacteria
- Part 8 – Methods for the Isolation and enumeration of Aeromonas and Pseudomonas aeruginosa
- Part 9 – Methods for the isolation and enumeration of Salmonella and Shigella by selective enrichment, membrane filtration and multiple tube-most probable number techniques
- Part 10 – Methods for the isolation of Yersinia, Vibrio and Campylobacter by selective enrichment
- Part 11 – The Determination of Taste and Odour in Drinking Water
- Part 12 – Methods for the isolation and enumeration of micro-organisms associated with taste, odour and related aesthetic problems
- Part 13 – The isolation and enumeration of aerobic spore-forming bacteria by membrane filtration
- Part 14 – Methods for the isolation identification and enumeration of Cryptosporidium oocysts and Giardia cysts

The Microbiology of Water and Associated Materials

- Practices and Procedures for Laboratories
- The determination of Legionella bacteria in waters and other environmental samples. Part 1 – Rationale of surveying and sampling
- The determination of Legionella bacteria in waters and other environmental samples. Part 2 – The determination of Legionella bacteria in waters and other environmental samples. Culture Methods for their detection and enumeration
- The determination of Legionella bacteria in waters and other environmental samples. Part 3 – Method for their detection and quantification by polymerase chain reaction (qPCR) and protocol for method validation
- The Identification of Microorganisms using MALDI-TOF Mass Spectrometry
- Bacteriological Swabs

The Microbiology of Recreational and Environmental Waters

- Part 1 – Water quality, epidemiology and public health
- Part 2 – Practices and procedures for sampling
- Part 3 – Methods for the isolation and enumeration of Escherichia coli (including E. coli O157:H7)
- Part 4 – Methods for the isolation and enumeration of enterococci
- Part 5 – Methods for the isolation and enumeration of sulphite-reducing clostridia and Clostridium perfringens
- Part 6 – Methods for the isolation and enumeration of Staphylococcus aureus
- Part 7 – Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa
- Part 8 – Methods for the isolation and enumeration of Salmonella and Shigella
- Part 9 – Methods for the isolation of Yersinia, Vibrio and Campylobacter by selective enrichment
- Part 10 – A method for the isolation and enumeration of sorbitol-fermenting bifidobacteria by membrane filtration
- Part 11 – Methods for the isolation and enumeration of somatic and F-specific bacteriophages and bacteriophages infecting Bacteroides fragilis
- Part 12 – Methods for the concentration of enteric viruses and the detection and enumeration of enteroviruses by suspended cell assay
- Part 13 – Methods for the isolation and enumeration of microbial tracers

The Microbiology of Sewage Sludge

- Part 1 – An overview of the treatment and use in agriculture or sewage sludge in relation to its impact on the environment and public health
- Part 2 – Practices and procedures for sampling and sample preparation
- Part 3 – Methods for the isolation and enumeration of Escherichia coli, including verocytotoxigenic Escherichia coli
- Part 4 – Methods for the detection, isolation and enumeration of Salmonellae

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, wastewater and effluents as well as sewage sludges and biota.

In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection - often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series “Methods for the Examination of Waters and Associated Materials” and their continuing revision is the responsibility of the Standing Committee of Analysts (SCA) - Established 1972 by the Department of the Environment.

At present, there are several working groups, each responsible for one section or aspect of water quality analysis:

1. General principles of sampling and accuracy of results
2. Microbiological methods
3. Inorganic and physical methods, metals and metalloids
4. Organic methods
5. Biological, biodegradability and inhibition methods
6. Radiochemistry methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will appear on our website – the library for which serves as a record of the bona fide methods developed and produced by the Standing Committee of Analysts.

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Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

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Users should ensure they are aware of the most recent version they seek.

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed, and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc. Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety.

These should be consulted and be readily accessible to all analysts. Amongst such resources are:

HSE: Information about health and safety at work

RSC: Laboratory best practices

The Approved List of Biological Agents. (2023) Advisory Committee on Dangerous Pathogens of the Health and Safety Executive (HSE)

1 Introduction

Bacteriological swabs can be used for a variety of routine surface monitoring purposes to show the effectiveness of general cleaning procedures and the disinfection process. Areas that may be tested include internal tap surfaces, laboratory equipment and benches, fridges, sampling points, PPE and people. Routine monitoring should be conducted at sufficient intervals to produce a reliable picture of the level of the resident bacteria of interest within the environment of interest.

Swabs may also be used to test for a wide variety of organisms in aid of investigations into sources of contamination.

2 Scope

Most environments contain a wide variety of microorganisms originating from a variety of sources. These organisms may also be the indicator organisms that are being tested for during the routine biological examination of drinking water, e.g. Coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, *Enterococci spp.*

It is important for laboratories to have assurance that the results achieved from water sample analysis have not been compromised from environmental contamination.

Swabs undertaken on the internal surfaces of customer taps, before any water samples are taken for bacteriological or chemical analysis, may be used to facilitate any investigation of the bacteriological failure of the water sample consequently taken from the tap. Should the swab results show the presence of an organism also found in the water sample, it could indicate that the organism originated from the internal surface of the tap rather than the water supply to the premises.

Swabs taken to monitor the environmental conditions within the laboratory indicate if the conditions associated with the testing may have impacted the results of analysis.

Analysis of surface swabbing can be undertaken to determine the presence of a variety of organisms depending on the nature of the investigation.

3 Principle

A swab type is selected for its desired purpose and an area or object then swabbed to transfer any bacteria onto the swab (Section 6.2). The swab is then used to inoculate a growth medium, e.g., broth or agar, to recover any organisms isolated from the swab.

4 Limitations

The information gleaned from swabs can only be used for qualitative purposes, indicating the presence or absence of the target organism isolated from a surface. This method is suitable for environmental monitoring including the swabbing of hard surfaces, taps and equipment.

It may be appropriate to report a quantitative result as per swab or per the area swabbed (Section 7.2). The numerical result obtained from the analysis may be influenced largely by the area swabbed, the swabbing technique used and the recovery of organisms from the swab.

The type of swab used should be chosen in accordance with its suitability for the particular use required, which should be demonstrable after an appropriate performance assessment.

Alternatives to the use of swabs should also be considered. Contact plates are often used for environmental monitoring of surfaces and remove much of the inherent uncertainty associated with sampling technique and organism recovery experienced with the use of swabs. However, use of contact plates is limited by surface shape. Passive techniques, including settle plates, and active air sampling, are useful for monitoring airborne contamination.

5 Health and Safety

Media, reagents and microorganisms used in this method are covered by the Control of Substances Hazardous to Health Regulations [2] and appropriate risk assessments should be made before adopting this method. Standard laboratory microbiology safety procedures should be followed, and guidance is given elsewhere [1] in this series.

When using the media, reagents and microorganisms required for swab analysis, good laboratory practice and aseptic technique should always be adhered to along with the wearing of appropriate PPE.

6 Apparatus

6.1 Standard laboratory equipment should be used which conforms to the performance criteria outlined elsewhere [1] in this series, e.g. disinfectants, incubators etc.

6.2 Swab Types/Uses

Several swab types are available, but generally fall into the following categories: pre-moistened hygiene swabs; gel transport swabs; liquid transport swabs; dry swabs (polystyrene with break point and wood stick). A summary of swab types, their appropriate uses and advantages / disadvantages are shown in Table 1. This is only for guidance and laboratories should conduct suitable assessments to determine the most appropriate swab for the purpose intended.

Swab Type	Tap Swabs	Environmental		Advantages	Disadvantages
		Delayed Processing Following Transportation and /or Storage	Analysis at Time of Sampling		
Pre-moistened hygiene swabs	×	×	✓	Neutralising buffers available. No separate diluent required	Not recommended for storage for the testing of fastidious organisms
Gel transport	✓	✓	✓	Suitable for a wide range of organisms	Potential loss of organism into the storage gel
Liquid transport	✓	✓	✓	Specific medias/ neutralisers available. Covers a wide range of organisms	Potential loss of target organism (depending on processing)
Dry swabs – polystyrene with break point	×	×	✓	Suitable for a wide range of organisms	Separate diluent needed.
Dry swabs – wood stick	×	×	✓	Suitable for wide range of organisms	Separate diluent needed

Table 1 – Swab types and their applications

6.2 Transport Medium Types

Transport mediums (gel or liquid) are utilised to maintain the viability of microorganisms when immediate processing is not possible, without a significant increase in growth. The following transport mediums are examples of what are available:

- Amies transport medium

  Contains salts to maintain osmotic equilibrium, phosphate buffer to maintain pH and sodium thioglycolate to produce a reduced environment necessary for cell function.

- Amies with charcoal medium

  As above with the addition of charcoal which neutralises the metabolic products that are toxic to gonococci and can provide up to 48hr transport survival.

- Stuart medium

  A non-nutritional medium that maintains viability. Calcium chloride maintains the permeability of bacterial cells whilst the sodium glycerophosphate component maintains pH.

- Cary Blair medium

  Has a low nutrient content to prevent replication. Disodium phosphate is incorporated to prevent overgrowth of enteric bacteria.

- Neutralising buffer

  Contains Sodium thiosulphate to neutralise any chlorine absorbed by the swab, i.e., from tap waters.

7 Sampling Methods

7.1 Sampling Techniques

The frequency and method of sampling should be dependent on individual assessments of the area required in line with business needs, e.g., surface evaluations, environmental monitoring, and investigations. Aseptic technique should be applied throughout the processes of sampling and analysis.

7.2 Surface Swabs

Moistened swabs should be employed for all sampling of surfaces. The swab should test a defined area (e.g., 10cm x 10cm) to give consistency of results. This can either be done by swabbing back to front on a bench (Figure 1) or by swabbing the entire area within a defined template (Figure 2). In both circumstances the swab should be rotated to ensure maximum capture of organisms. (These methods are dependent on sampler subjectivity).

Figure 1 – Swabbing of laboratory bench

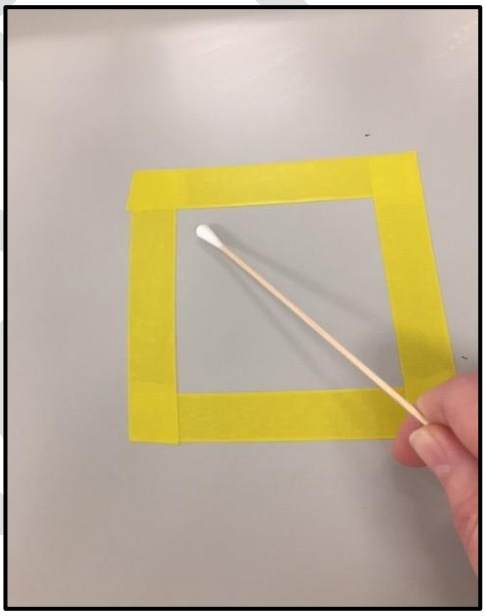

Figure 2 – Swabbing of a defined template

If swabbing surfaces that have been recently cleaned, or it is suspected that cleaning chemicals are present (e.g., when checking cleaning efficacy), a swab with a neutralising buffer that is suitable for the disinfectant should be used.

7.3 Contact Plates

Contact plates pressed against the surface that requires testing are recognised as a useful alternative to surface swabs. The added advantage of this method is that it removes user variability and plates are available in a variety of both selective and non-selective media.

7.4 Tap Swabs

A tap swab is taken to show both the general hygiene of the tap (pre-disinfection) and the efficiency of any disinfection procedures that are applied to the tap (post-disinfection). An assessment of the results obtained from the swab analysis can subsequently be used to inform any appropriate action that may be required to maintain the hygiene of the tap.

To take a swab sample from a tap, a moistened sterile swab should be removed from its packaging and inserted into the tap and moved up and down whilst rotated in a circular motion to ensure maximum contact of all accessible internal surfaces (Figure 3). Where a tap insert is present, the swab should be rubbed over the insert whilst rotating the swab to ensure maximum coverage. The swab should then be aseptically placed back into its container. This container should then be labelled with the appropriate sample details and transported in a cooled environment (5 ± 3 °C) in line with guidance documented elsewhere within this series [1].

To obtain the sample stability storage time, the swab type used should be validated against each target organism.

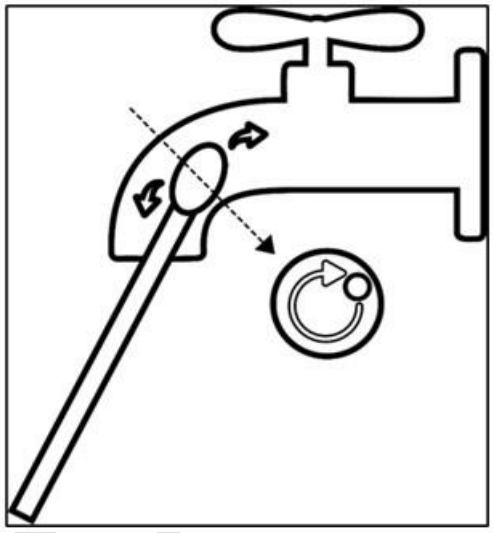

Figure 3 – Swabbing of a tap

8 Analytical Methods

8.1 Technique

The most appropriate processing method for the swab should be determined by routine testing requirements and the intended purpose of the results. The media used for the analysis is dependent on the target organism and will give a presence / absence result. Non-selective growth media can be used to give an indication of the general microbial ecology of a specific location.

Standard confirmation and identification tests can be carried out if required.

8.2 Streaking Method

The swab should be streaked directly onto the surface of the chosen media. The swab should be rotated constantly throughout the streaking to ensure that all surfaces of the swab, including the tip, come into contact with the surface of the agar. The areas of the surface streaked should not overlap to prevent the removal of organisms (Figure 4).

Figure 4 – Streaking pattern

8.3 Resuspension

Aseptically place the swab into a suitable diluent, such as Maximum Recovery Diluent, and re-suspend the cells by vortex or using a sonic bath. The duration and intensity of the use of such equipment should be validated to optimise recoveries. Swabs with an alginate tip should be allowed to fully dissolve before processing. If the swab is stored or transported in a diluent, this should be used as re-suspension fluid, adding other suitable diluents to make up to a larger volume as required.

8.3.1 Filtration

The suspension can be filtered onto a sterile membrane filter and then processed onto appropriate media, such as MLGA [4] or S&B [5], as documented in procedures elsewhere in this series. Laboratories are advised to check their chosen swab type prior to sampling, to ensure the transport medium does not impede filtration.

8.4 Alternative Testing

In addition to filtration, as mentioned above, alternative defined substrate media techniques are available to use for the isolation of coliform bacteria and *E. coli* or *Enterococci spp.*. Further information about these can be found in Part's 4 and 5 of the Microbiology of Drinking Water series.

These types of methods can be used as a presence/absence test or quantitatively depending on the end users' requirements. The medium should be made in line with manufacturer's instructions.

The swab is aseptically added into the medium, resuspended (Section 8.3) and incubated according to the manufacturer's instructions. If also transferring the transport medium into the defined substrate medium, users should validate any impact this may have on the test

system. Samples can either be compared to a comparator issued by the manufacturer (the same volume of which must be aliquoted into the same sample container used to analyse the sample, to ensure consistency when reading the results) or to AQC standards.

AQC standards would comprise of controlled appropriate reference cultures for positive and negative reactions and would be processed alongside the samples.

9 References

1. Standing Committee of Analysts, The Microbiology of Water and Associated Materials (2017). Water Quality and Public Health. Practices and Procedures for Laboratories, in this series, Environment Agency.

2. The Control of Substances Hazardous to Health Regulations 2002, Statutory Instrument 2002 No. 2677.

4 Standing Committee of Analysts, The Microbiology of Drinking Water (2016). Part 4 – Methods for the Isolation and enumeration of coliform bacteria and Escherichia coli (including E. coli O157 H7).

5 Standing Committee of Analysts, The Microbiology of Drinking Water (2012). Part 5 - Isolation and enumeration of Enterococci.

Members assisting with these methods.

Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

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Grateful acknowledgement is made to those individuals and companies who provided colour photographs and diagrams.

Correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts:

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Amendment History

Bacteriological Swabs is a new book. Therefore, there isn't an amendment history in this occasion.

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