



Public Health
England

Detection and enumeration of bacteria in swabs and other environmental samples

Microbiology Services
Food Water and Environmental
Microbiology
Standard Method

FNES4 [E1]

About Public Health England

Public Health England's mission is to protect and improve the nation's health and to address inequalities through working with national and local government, the NHS, industry and the voluntary and community sector. PHE is an operationally autonomous executive agency of the Department of Health.

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Contents

About Public Health England	2
Contents	3
Status of Microbiology Services Food, Water and Environmental Microbiology Methods	4
Amendment history	5
Introduction	6
Scope	6
Background	6
1.0 Principle	7
2.0 Definitions	8
3.0 Safety considerations	8
3.1 General safety considerations	8
3.2 Specific safety considerations	8
3.3 Laboratory containment	8
4.0 Equipment	9
5.0 Media, reagents and issue of sampling equipment	9
5.1 Commercially prepared dacron swabs with neutralising buffer	9
5.2 Commercially prepared sponge swabs with neutralising buffer	9
5.3 Commercially prepared viscose tipped swabs with Amies charcoal medium	10
5.4 Sampling bags for collection of other environmental samples	10
5.5 Media and Reagents	10
6.0 Sample processing	11
6.1 Sample preparation and dilutions	11
6.2 Inoculation and incubation	13
7.0 Quality control	14
8.0 Calculation of results	14
9.0 Reporting of results	15
9.1 Enumeration	15
9.2 Detection	16
10.0 Reference facilities and referral of cultures	16
11.0 Acknowledgements and contacts	16
References	18
Appendix 1: Swabbing for pathogens and indicator organisms using dacron tipped swabs.	19
Appendix 2: Sponge swabs instructions for use	20
Appendix 3: Detection and enumeration of bacteria in swabs and other environmental samples	21

Status of Microbiology Services Food, Water and Environmental Microbiology Methods

These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology. However, in using Standard Methods, laboratories should take account of local requirements and it may be necessary to undertake additional investigations.

The performance of a standard method depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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Amendment history

Controlled document reference	FNES4 (E1)
Controlled document title	Standard Method for Detection and Enumeration of Bacteria in Swabs and other Environmental Samples.

The amendment history is shown below. On issue of revised or new documents each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Version no. Discarded	Insert version no.	Page	Section(s) involved	Amendment
2/17.12.13	1.0	2	All	All	Updated to reflect move to PHE and document renumbered as FNES4 following the implementation of Q-pulse for Document Management
			5	Introduction	Clarification of the swab types that can be used for testing
			6	1.0 Principle	Clarification of the swab types that can be used for testing
			7	3.0 H&S considerations	Statement related to use of coloured text to highlight hazards and controls.
			9	5.6 Media & reagents	Information note added regarding the use of MRD
			10	6.0 Sample processing	Statement added to clarify reporting of multiple pathogens for single samples. Approximate measurement removed and replaced with gravimetric dilution.
			13	9.0 Reporting	Comment added with regards to reporting when entire sample has not been tested.

Introduction

Scope

This method is applicable to the procedures used for examination of environmental samples including swabs from carcasses in meat processing plants, swabs of food preparation surfaces and other environmental samples such as cloths collected from the food manufacturing environment and bottle rinses.

This support method must be used in conjunction with accredited methods for the detection of bacteria in foods and includes the use of three different types of swab.

Background

The significance of food contact surfaces in food safety is highlighted in the Food Safety Act 1990¹ which states under section 29 (c) that authorised officers can “take a sample from any food source, or a sample of any contact material, which is found by him on or in any such premises”. This implies that environmental samples are to be considered as part of legislation. EU Legislation also states that “Sampling of the production and processing environment can be a useful tool to identify and prevent the presence of pathogenic micro-organisms in foodstuffs², and cleanliness standards have also been laid down for meat premises in European Law³. A recommendation to undertake environmental monitoring of the food preparation environment during the investigation of poor microbiological results is also given in the HPA Guidelines for Assessing the Microbiological Safety of Ready to Eat Foods Placed on the Market⁴.

The environment can be contaminated with a variety of micro-organisms derived from various sources. Estimation of the overall numbers of bacteria (Aerobic Colony Count, *E.coli* and Enterobacteriaceae) present can provide useful information when assessing general hygiene. Environmental monitoring can also be used as part of routine inspections of food premises or in an investigation of a suspected food poisoning outbreak where surfaces are thought to be likely vehicles of cross-contamination or if no food is available for examination.

Determination of the number of aerobic viable micro-organisms and the number of Enterobacteriaceae on a specified area of a surface can provide an indication of the cleanliness and allows monitoring of cleaning procedures over time.

Detection of *Listeria species* in a food processing premises can be used as an indicator of poor cleaning. The presence of foodborne pathogens such as *Listeria monocytogenes*, Salmonella, Campylobacter and *E.coli* O157 in a ready to eat food preparation area or retail setting is of particular concern.

The main value of determining the colony counts on a surface is to assess the cleanliness of that surface. If a swab is taken to demonstrate that procedures used for cleaning are effective, swabbing should be performed after the surface has been cleaned. If it is suspected that cleaning is not being done or the methods used for cleaning are not effective then swabbing can be done as part of a routine hygiene inspection without prior cleaning. A

Detection and enumeration of bacteria in swabs and other environmental samples

high count can be an early warning of inadequate cleaning practices in food premises and may highlight the need for further investigation but careful consideration must be given if the surface is in use at the time of sampling. Cleaning of a surface prior to swabbing during outbreak investigations should be avoided to maximise the possibility of detecting a pathogen.

The method of choice for examination of surfaces is swabbing of a known area (10-100cm²) using a sterile swab that has been moistened in 10mL of neutralising diluent. This semi-quantitative approach enables enumeration of the micro-organisms per cm² and can facilitate interpretation of the results. When qualitative or detection tests only are to be performed the area swabbed need not be known but this may limit the laboratory's ability to interpret the significance of the results obtained. It is assumed that the sample has been taken by a competent sampling officer who has been trained to carry out this procedure.

Microbiological examination of cleaning cloths can also be useful both in monitoring general hygiene and in the investigation of outbreaks, since these cloths are frequently used to wipe food preparation surfaces, and therefore have the potential to spread microbial contamination over the surfaces. Since many cloths are stored wet and may contain food debris, they have the potential to support the growth of high numbers of bacteria if not sanitised appropriately. Both enumeration of hygiene indicators and detection of pathogens can be performed on these samples by rinsing the entire cloth in diluent or a growth medium.

1.0 Principle

Three types of commercially available swabs are issued to customers by PHE laboratories. Two contain a measured volume of commercially prepared neutralising buffer or neutralising diluent while the third has Amies charcoal media. Neutralising buffer/diluent is used to neutralise the effect of disinfectants that have been used to clean surfaces, charcoal containing media has a similar effect. Surfaces are generally swabbed by the customer (with the exception of complex investigations where laboratory staff may be asked to assist with sampling). The laboratory provides the customer with instructions and offers training for use of the swabs but the effectiveness of the swabbing procedure used is often outside the control of the laboratory.

When enumeration tests are required a template enclosing a known surface area (eg 10cm x 10cm) is used. Swabs in diluent are refrigerated at 2-8°C and are submitted to the laboratory as soon as possible to ensure that they are examined on the day of collection or at least within 24 hours of collection. The swabs are vortex mixed or homogenised to aid release of organisms into the diluent. The swab diluent and serial dilutions if necessary are subjected to the same methods as used for examination of food homogenates.

The target count for enumeration tests is in the range of 0 to 300 colony forming units (CFU) per plate. Samples likely to have counts that exceed the countable limit per plate (method dependent) must be diluted.

Detection and enumeration of bacteria in swabs and other environmental samples

Detection of pathogens from a known surface area can be performed by addition of enrichment media to the swab sample. In outbreak situation a large random area can also be sampled to maximise the likelihood of detection.

Enumeration and detection test can be performed on cloths and other environmental samples such as bottle rinses by adding measured volumes of diluent or enrichment media.

2.0 Definitions

For definitions of target organisms refer to the corresponding PHE standard methods for foods for the bacteria of interest.

3.0 Safety considerations

3.1 General safety considerations

Normal laboratory precautions apply⁵⁻⁶.

All laboratory activities associated with this Standard Method must be risk assessed locally to identify hazards⁷⁻⁸. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of local risk assessments to ensure that controls are appropriate and effective.

Information Note: Throughout this method hazards are identified using **red text**. Where a means of controlling a hazard has been identified this is shown in **green text**.

3.2 Specific safety considerations

Refer to individual food methods for specific safety considerations

3.3 Laboratory containment

Swabs, cloths or other environmental samples that are considered to be **“high risk” for the isolation of VTEC O157**, must be **handled in a CL3 Laboratory**.

4.0 Equipment

- Top pan balance capable of weighing to 0.1g
- Stomacher
- Vortex mixer
- Stomacher bags (sterile)
- Other equipment as detailed in PHE, MS, FW&E Standard Methods

5.0 Media, reagents and issue of sampling equipment

5.1 Commercially prepared dacron swabs with neutralising buffer

These are stored at ambient temperature and must be used before the displayed expiry date. Instructions for use and a template are issued to the customer (see Appendix 1).

- 10mL neutralising buffer with dacron tipped swab (eg Technical Service Consultants Ltd, product code TS5-42)
- Sterile templates (eg 10cm x 10cm plastic templates, Technical Service Consultants Ltd, product code TS15-T)

5.2 Commercially prepared sponge swabs with neutralising buffer

These are stored at ambient or under refrigeration (2 – 25°C) and must be used before the displayed expiry date. They should not be used if they appear to have dried out during storage (the chance of swabs drying out during their shelf-life may be reduced by storing between 2 and 8°C). Instructions for use with a template are issued to the customer (see Appendix 2).

- SpongeSicle™ swabs or sponge swabs containing 10mL neutralising buffer (eg 3M, product code SSL-10NB)
- Sterile templates (eg 10cm x 10cm plastic templates, Technical Service Consultants Ltd, product code TS15-T)

Detection and enumeration of bacteria in swabs and other environmental samples

5.3 Commercially prepared viscose tipped swabs with Amies charcoal medium

These swabs are used for the detection of a single pathogen only. They are stored at ambient or under refrigeration (2 – 25°C) and must be used before the displayed expiry date.

- Amies charcoal swabs (eg Technical Service Consultants Ltd, product TS-5 18)

5.4 Sampling bags for collection of other environmental samples

Other environmental samples including cloths or utensils can be collected in sampling bags with wire closures or in a food grade bag. Bottles to be rinsed should be capped (if possible) and placed in a food grade bag. Food grade bags are not provided by the laboratory.

5.5 Media and Reagents

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

Peptone Saline Diluent (PSD)

Peptone	1.0 g
Sodium chloride	8.5 g
Water	1L

pH 7.0 ± 0.2 at 25⁰C

Information note: This media is often referred to as *Maximum Recovery Diluent (MRD)* in the laboratory

Buffered peptone water (ISO formulation)

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate	9.0 g
or anhydrous disodium hydrogen phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g
Water	1 L

pH 7.0 ± 0.2 at 25°C

Detection and enumeration of bacteria in swabs and other environmental samples

Neutralising Diluent (ISO 18593-3)

Sorbitan monooleate (Polysorbate 80)	30.0 g
Saponin	30.0 g
Sodium chloride	8.5 g
Sodium thiosulphate	5.0 g
Lecithin	3.0 g
Peptone	1.0 g
L-histidine	1.0 g
Water	1L

pH 7.0 ± 0.2 at 25⁰C

Refer to individual food test methods for culture media and reagents.

6.0 Sample processing

The diluent used will depend on the tests to be performed. If enumeration tests only are being performed on a swab Peptone Saline Diluent (PSD) can be used as the diluent. If the swab will be subjected to detection of *Salmonella species*, in addition to enumeration tests BPW can be used. Detection of pathogens other than Salmonella from swabs requires the addition of selective enrichment media. Due to this only one pathogen can be sought per swab. Cloths or other environmental samples for detection of a single pathogen are prepared by adding 500mL of the appropriate enrichment medium. If a cloth or other environmental sample is being investigated for more than one pathogen by enrichment or if enumeration tests are being performed in addition to pathogen testing neutralising diluent should be added, the sample homogenised and the resulting liquid aliquoted to perform these tests. The resulting suspension can be mixed with the enrichment media for the pathogens of interest. If this is performed a comment must be added to the report to reflect that the entire sample was not tested (see section 9).

6.1 Sample preparation and dilutions

6.1.1 Preparation of dacron swabs

The sample comprises a swab in a tube of 10 mL neutralising buffer.

Preparation for enumeration tests

Transfer the neutralising buffer and swab tip to a sterile universal container. Vortex mix the universal container for a total of one minute. All vortexed universals must be **allowed to stand for 5 minutes** to allow settling of **aerosols**. This is equivalent to a 10⁰ dilution and gives a lower limit of detection of 10 CFU per swab if 1mL is plated. Further dilutions can be performed if required.

Detection and enumeration of bacteria in swabs and other environmental samples

Preparation for detection tests

Transfer the contents of the universal (including the swab tip) to a sterile bag with wire closures. Add the appropriate enrichment medium for the test required to gravimetrically prepare a 10^{-1} dilution.

6.1.2 Preparation of sponge swabs

The sample comprises of a sponge moistened in 10mL of neutralising buffer inside a plastic bag with wire closures. The handle should have been aseptically removed at the time of use. If all, or a portion of the handle is still in place this should be aseptically removed by pushing it up through the bag and snapping it off. It must be noted on the report that the handle was not fully removed.

Preparation for enumeration tests

Add 90 mL of diluent to the bag containing the sponge swab to prepare a 10^{-1} dilution. Place the plastic bag with sponge swab inside a stomacher and homogenise for between 30 seconds and 3 minutes. The homogenisation time required will depend on the manufacturer instructions.

This gives rise to a lower limit of detection of 100 CFU per swab if 1mL is plated. Lower limits of detection can be achieved by reducing the amount of diluent added eg 10 mL can be added equivalent to a 1 in 2 dilution giving a limit of detection of 20 CFU per swab.

Preparation for detection tests

Add 90mL of the required enrichment medium to the swab bag and homogenise for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions.

6.1.3 Preparation of charcoal swabs

The sample comprises a swab in a tube of Amies Charcoal medium.

Preparation for detection tests

Transfer the contents of the tube (including the swab tip) to a sterile bag with wire closures. Add the appropriate enrichment medium for the test required to cover the swab and prepare a 10^{-1} dilution gravimetrically and homogenise for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions of the equipment used.

6.1.4 Preparation of cloths and other environmental samples

The cloth samples that will be submitted are likely to vary considerably in their size and degree of moisture content. It may also be the case that the cloth contains disinfectant and it is important that an attempt to dilute this disinfectant is made. It is therefore recommended that 500mL of diluent is added to the cloth regardless of size and moisture content. The diluent added will depend on the tests to be performed. It is recommended that if enumeration tests only are being performed neutralising diluent is used. The sample should be homogenised for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions and the sample. Where this is not possible the sample should be manipulated manually for at least 2

Detection and enumeration of bacteria in swabs and other environmental samples minutes. This resulting suspension is considered to be the neat or 10^0 dilution. Where enumeration is being performed in addition to the detection of pathogens neutralising diluent should be used and 25mL, aliquoted to 225mL of the appropriate enrichment media.

Other environmental samples including scrubbing brushes, nozzles and utensils are placed in a sampling bag. When enumeration tests are being performed at least 100mL of the appropriate diluent is added. These samples must be manipulated manually for at least 2 minutes, again the resulting suspension is considered to be the neat or 10^0 sample. When carrying out a pathogen detection test, sufficient enrichment media must be added to fully immerse the sample.

6.1.5 Preparation of bottle rinses

The bottle submitted for rinsing is removed from the sample bag aseptically. If the bottle is intended for single use, add 20mL of PSD. If the bottle is re-usable and has been washed add 20mL of neutralising diluent. The internal surfaces of the bottle are thoroughly rinsed by rotating the bottle gently 12 times in one direction. The bottle is allowed to stand for 15-30 minutes. Rinse the internal surfaces of the bottle again by rotating the bottle gently 12 times in one direction⁹. Using a sterile pipette transfer the bottle rinse fluid to a sterile universal container. This is the neat or 10^0 sample. When carrying out pathogen detection tests, add 100 mL of enrichment media to the bottle, rinse the bottle as described above and transfer the broth to a closure bag or sterile container.

6.2 Inoculation and incubation

6.2.1 Enumeration methods

For all samples, dilutions should be selected that will give colony counts within the appropriate range for the test being performed.

Proceed using PHE MS FW&E Standard methods for Foods.

Using a separate sterile pipette for each dilution, aseptically transfer 0.5mL or 1mL volumes (pour plates methods only) of the initial dilution (10^0 or 10^{-1}) of the sample to the appropriate medium. If necessary repeat the procedure with further decimal dilutions prepared in PSD.

6.2.2 Detection methods

For all samples gravimetrically prepare a 10^{-1} dilution in the appropriate enrichment medium. Enumeration tests can also be performed when samples have been diluted in BPW. If multiple pathogen tests are to be performed sample homogenates can be aliquoted to the appropriated enrichment media to achieve a 10^{-1} dilution.

Proceed using PHE MS FW&E Standard methods for Foods.

7.0 Quality control

Quality control of media and internal quality assurance checks should be performed according to in-house procedures. See PHE MS FW&E Standard methods for Foods for each method.

8.0 Calculation of results

For enumeration tests counts should be calculated, where possible, using dilutions giving 15 to 150 or 300 colonies on the plate. The upper countable limit used is method dependent; please refer to individual food methods.

Calculate count per swab or cloth as follows:

$$\text{Count} = \frac{C}{v(n_1 + 0.1n_2)d} \times n_3$$

where : -

- C is the sum of colonies on all plates counted;
- v is the volume applied to each plate;
- n₁ is the number of plates counted at the first dilution;
- n₂ is the number of plates counted at the second dilution;
- n₃ is the original volume of neat suspension (*ie* 10 for swab, 500 or 100 for other samples);
- d is the dilution from which the first count was obtained *eg* 10⁻² is 0.01;

If the swab is from a measured area the count can be divided by the area swabbed in cm².

eg Sponge swab, area swabbed 100cm², tested for Enterobacteriaceae.

45 colonies confirmed at 10⁻² dilution.

$$\text{Count} = \frac{45}{1(1 + 0)0.01} \times 10$$

$$= 4.5 \times 10^4$$

This count is the count per swab so to calculate the result per cm² it is necessary to divide this by the area swabbed which in this case is 100.

Result per cm² is 4.5x10² CFU per cm².

Detection and enumeration of bacteria in swabs and other environmental samples

eg Dacron swab, area swabbed 100 cm² tested for *E.coli*.

Example: 15 colonies on plate 1 and 18 colonies on plate 2 at 10⁰ dilution (neat).

$$\text{Count} = \frac{33}{0.5(2+0)1} \times 10$$

$$= 3.3 \times 10^2$$

This count is the count per swab so to calculate the result per cm² it is necessary to divide this by the area swabbed which in this case is 100.

Result per cm² is 3.3 CFU per cm²

9.0 Reporting of results

The test report must specify the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

Take as the result the count per swab, per cloth, per item or per cm² expressed as a whole number to two significant figures (if less than 100).

If count is 100 or more, report counts with one figure before and one figure after the decimal point in the form of:

$$\mathbf{a \times 10^b \text{ CFU per swab, cloth, item, or cm}^2}$$

where a is never less than 1.0 or greater than 9.9 and b represents the appropriate power of ten. Round counts up if the last figure is 5 or more, and down if the last figure is 4 or less.

eg : 1920 CFU per swab = 1.9 x 10³ CFU per swab

235,000 CFU per swab = 2.4 x 10⁵ CFU per swab

9.1 Enumeration

9.1.1 Area swabbed not specified

Report the count as CFU per swab.

9.1.2 Area specified

Report as CFU per cm². Include a comment on the report (eg Results expressed per cm² are calculated using the area tested as stated by the customer).

Detection and enumeration of bacteria in swabs and other environmental samples

9.1.3 Cloth or other environmental samples

Report the count as CFU per cloth or per sample.

9.1.4 Bottle rinses

Report the count as CFU per bottle.

9.2 Detection

If a single pathogen test has been carried out only ie no other tests performed on the sample

Report as:-

Not Detected per swab, per cloth or per item.

or

DETECTED per swab, per cloth or per item.

If more than one pathogen test has been carried out (cloths or other samples only) or enumeration tests have been performed in addition to Salmonella (swabs) report as in swab or in item with the comment "Entire sample not tested"

10.0 Reference facilities and referral of cultures

Any pathogen detected in a swab or in other environmental samples, particularly during the investigation of an outbreak must be referred to a reference laboratory for further typing. Please refer to advice provided in individual food methods.

11.0 Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by Microbiology Services, Food, Water and Environmental Microbiology Methods Working Group.

Detection and enumeration of bacteria in swabs and other environmental samples

The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

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Appendix 1: Swabbing for pathogens and indicator organisms using dacron tipped swabs.

This procedure can be used after cleaning to assess cleaning standards it can also be used if no cleaning protocol is in place. During an outbreak investigation swab surfaces prior to cleaning when possible.

Consumables (supplied by the laboratory on request)

1 x sterile swab with 10ml Neutralising Buffer

1 x sterile 100cm² template

Supplier/product code

TSC Ltd, TS5-42

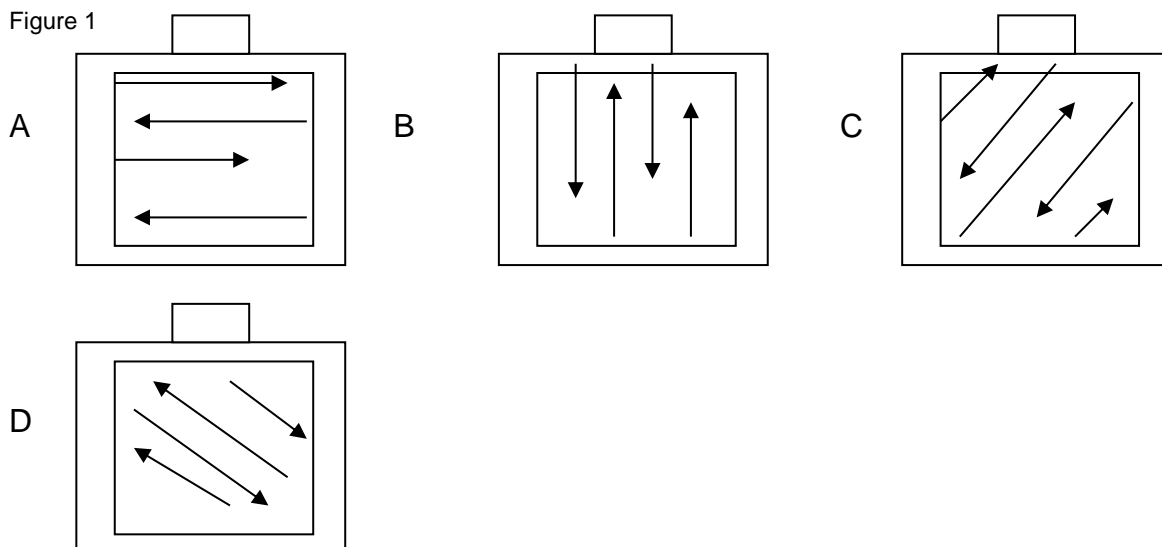
TSC Ltd, TS 15-T

1. Put on a clean overcoat and wash hands thoroughly prior to sampling. Sterile gloves are not necessary unless the sample is likely to lead to legal proceedings.
2. Select the area to be investigated. If the surface is not flat and indicator organisms are being sought estimate the area to be swabbed.
3. If the area is flat remove a template from its bag by the handle and hold it between the thumb and index finger of one hand. Ensure that the template does not come into contact with any other object and place in the selected position.
4. Open the swab pack (do not remove the sterile swab from the pack at this stage).
5. Remove the lid from the tube containing neutralising buffer.
6. Remove the swab from the packaging and insert it into the neutralising buffer to moisten it. Squeeze excess liquid from the swab by pressing the cotton tip against the side of the tube.
7. Replace the lid on the neutralising buffer tube.
8. Using the moistened swab, swab the entire template area for 30 seconds as shown in Figure 1 using firm even pressure and rotation of the swab.
9. Remove the cap of the tube containing neutralising buffer and insert the swab. The top of the swab then becomes the lid of the tube. Push swab firmly in place until it is fully engaged-it should "click".
10. The sample should be labelled appropriately and placed in a suitable cool box in an upright position and transported to the laboratory as soon as possible to ensure it is tested on the same day or at least within 24 hours of collection.

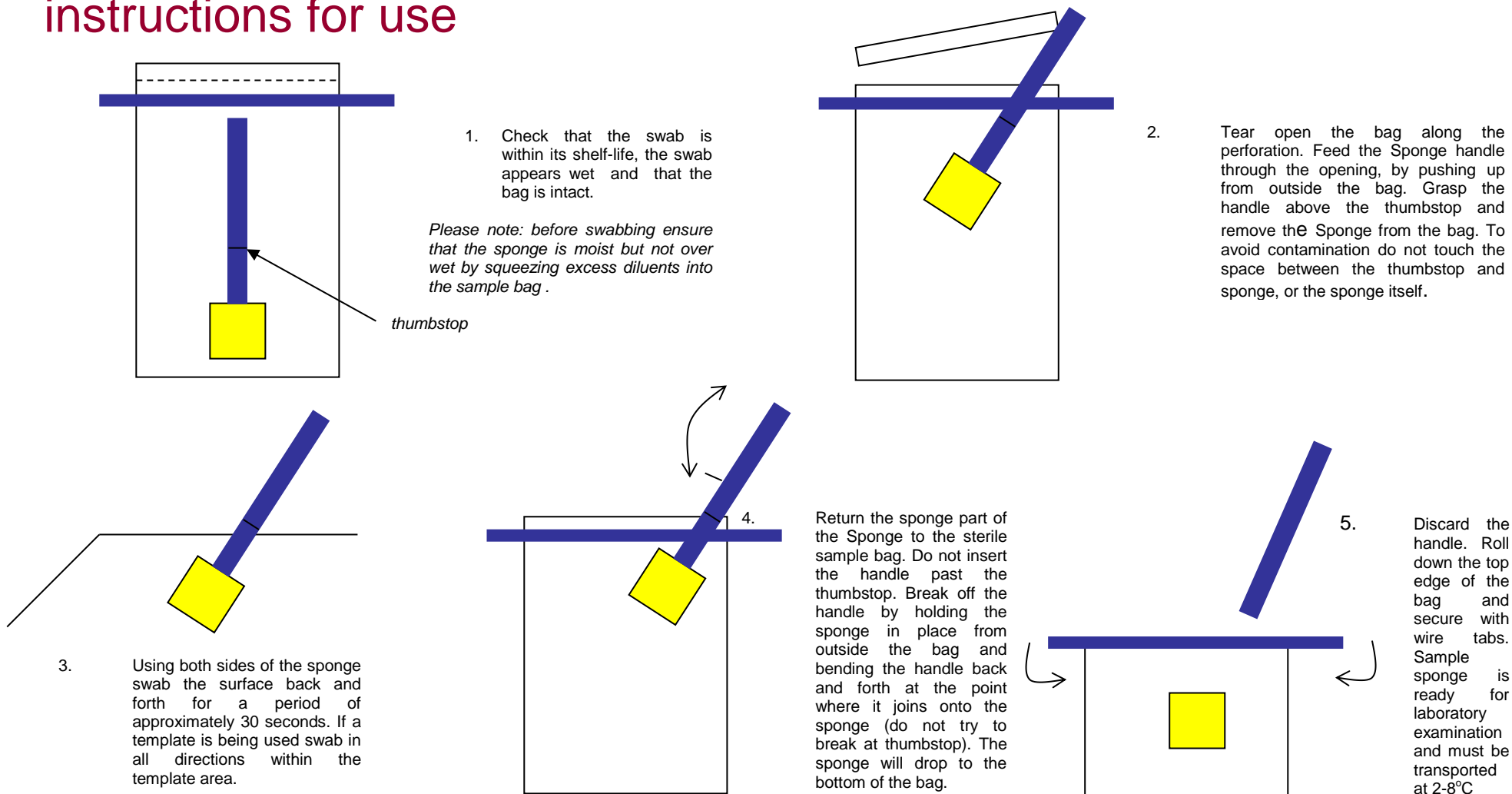
If indicator organisms are being sought the area swabbed in cm² or per 100cm² needs to be known to enable meaningful examination. If pathogens are being sought the area swabbed is less critical in the examination.

It is essential to clearly indicate which investigations are required.

Figure 1



Appendix 2: Sponge swabs instructions for use



Appendix 3: Detection and enumeration of bacteria in swabs and other environmental samples

Enumeration methods

For swabs test neat or prepare a 1:2 or 10^{-1} dilution in PSD or BPW.
For cloths add 500mL of ND



Mix by vortexing or in a stomacher for 30secs to 3mins



Prepare further dilutions in PSD if required



Inoculate appropriate medium for organisms of interest and proceed as for HPA Standard method for food



Count and confirm colonies as for the HPA Standard method for food



Calculate Count per swab, per cm^2 , per cloth or per item

Detection methods

For swabs prepare an approximate 10^{-1} dilution of the sample in the required enrichment medium.
For cloths add 500mL of ND, homogenise and aliquot to the required enrichment media



Mix by vortexing or in a stomacher for 30secs to 3mins



Incubate and proceed as for the HPA Standard method for food



Report as pathogen

DETECTED
or
Not Detected



If required refer isolate to the appropriate reference laboratory