



UK Health  
Security  
Agency

# **Examining food, water and environmental samples from healthcare environments**

## **Microbiological guidelines**

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## Amendments

Page	Section	Amendment
All	All	Re-formatted as a UKHSA publication
11	Water sampling	Added consideration of commissioning requirements in water systems intended for patients at high risk of infection (with reference to NETB 2024/3)
18	Legionella sampling	Hot water should reach a temperature of 55°C (rather than 50°C) in healthcare premises
27	Testing parameters and interpretation of microbiology results	Website details updated
28	Testing parameters and interpretation of microbiology results	Explanation added regarding the new version of HTM 03-01, which no longer specifies criteria for microbiological air sampling of operating theatres
31	Table 2c (dried infant formulae)	Testing requirement for salmonella added, and reference made to the need to test for <i>Cronobacter sakazakii</i> if Enterobacteriaceae test is positive
43	Table 11 (operating theatre air quality)	Note added regarding new version of HTM 03-01 (as above)
45	References	Updated

## Executive summary

The microbiological examination of food, water and environmental samples from the healthcare environment has a recognised role in the routine monitoring of decontamination procedures and the demonstration of a safe environment for patients, visitors and staff. Individual guidance documents covering sampling and testing requirements in a range of healthcare environments (such as endoscopy departments, pharmacy sterile suites and hydrotherapy pools) have been published. Although many of these documents are freely available on the internet, they vary in the amount of detail provided. In some cases, particularly with respect to environmental monitoring, no clear guidance has been published.

This document aims to summarise the available legislation and guidance for microbiologists and infection control practitioners working within healthcare settings and to provide additional clarification and guidance on sampling and result interpretation where these are currently lacking.

Since the use of suitable procedures and equipment is essential in order to be able to carry out the appropriate microbiological analyses on a sample and provide a meaningful interpretation of test results, general procedures for collecting food, water, air and environmental samples are also described.

# Introduction

In the 1980s, it was generally held that microbiological sampling of food, water and the environment in hospitals was rarely indicated, except during the investigation of outbreaks. This was, in part, due to a lack of consensus about the best way to proceed and how to interpret results once obtained. Lessons learned from subsequent outbreaks, and issues raised as a result of the increasing complexity of medical service provision have since led to the development of numerous pieces of legislation and expert guidance that address specific aspects of hospital microbiology. The overarching aim is to be able to demonstrate the provision of a safe environment for patients, visitors and staff.

Individual guidance documents covering a range of subjects such as food (Department of Health 1989), pharmacy sterile suites (Beaney, 2016) and swimming and hydrotherapy pools (Pool Water Treatment Advisory Group, 2017) have been published. Although many are freely available on the internet, they vary in the amount of detail provided and this may be confusing for those addressing a problem for the first time. In some cases, particularly with respect to environmental monitoring, no clear guidance is available and expert advice must be sought. While there is, at present, no specific requirement for formal accreditation of laboratories undertaking this type of microbiological investigation, it is advisable, where medico-legal issues may arise, to seek the assistance of a laboratory with appropriate accreditation to perform the specific test and with specialist expertise. These are often available from official laboratories: capabilities fulfilling these criteria may not always be available from a hospital microbiology laboratory.

This document aims to summarise the available legislation and guidance for microbiologists and infection control practitioners working within the healthcare setting and to provide additional clarification and guidance on sampling and result interpretation where these are currently lacking. It is recognised that legislation and guidance are subject to regular review and that advice given here will eventually be superseded by future publications.

Outbreaks and incidents of infection due to food, water or environmental contamination in hospitals and healthcare establishments are of major concern. Hospitals are complex establishments, often with microbiological testing requirements originating from multiple departments and disciplines, sometimes operating independently even from within the same site. Hospital environments can present multiple challenging environmental hazards to which highly susceptible individuals (as well as visitors and staff) may be exposed (Lund and O'Brien, 2009; Dancer, 2014; Robakowska and others, 2021). Whilst this document focuses largely on routine monitoring, the sampling procedures described apply equally to outbreak and incident investigations. However, some additional sampling techniques and targets for microbiological testing may be relevant in these situations, and further advice and guidance should be sought from the local UK Health Security Agency (UKHSA) Food Water and Environmental Microbiology Laboratory as appropriate.

## Sampling procedures

In order to be able to carry out the appropriate microbiological examinations on a sample and provide a meaningful interpretation of test results, it is essential that samples are collected in a suitable manner using the correct equipment. General procedures for the sampling of food, water, the air and the environment are described below. For more unusual sampling requirements, advice on procedures and sampling equipment should be sought from a specialist food, water and environmental microbiology laboratory with appropriate accreditation.

## Health and safety considerations

Collection of food, water and environmental samples in hospitals and other healthcare settings may occur in a variety of locations including wards, operating theatres, equipment decontamination and preparation areas, plant rooms and cooling towers. Each location and reason for sampling will be associated with its own risks. It is important to make an assessment of these risks and put appropriate control measures in place before any sampling is carried out. Examples of hazards include:

- wet floors that present a slip hazard when sampling from swimming and hydrotherapy pools or from kitchen areas
- working at height when ladders or steps are required to reach sampling points
- manual handling when carrying large amounts of sampling equipment to and from the site of sampling
- working in confined spaces when sampling from difficult-to-reach parts of water systems
- exposure to aerosols when sampling from cooling towers and showers – appropriate precautions should be taken to minimise aerosol production, as described in BS 7592:2022 (British Standards Institution, 2022), for example –
  - running taps gently to reduce splashing
  - using a sterile plastic bag with one corner cut off to enclose the shower head and to funnel the water into a sampling container
  - sampling cooling towers from sampling points on the return service of the cooling water to the tower, rather than the tower itself
- lone working in isolated areas such as plant rooms

In addition, some specific safety notes have been included in the sections below.

## General equipment requirements

The following is a list of equipment that may be needed for sampling. The list is not intended to be exhaustive and not all items may be required for all types of sampling:

- sterile food-grade plastic bags, twist-seal bags or honey jars
- tamper evident tags or evidence bags
- labels
- permanent waterproof marker pens and biros
- laboratory sample submittal forms for food, water and environmental samples (usually provided by the laboratory)
- nitrile gloves
- alcohol wipes
- plastic shoe coverings
- cool box with separators, data loggers and 10% by volume of frozen cold packs (see appendix for further guidance) – note that cold packs should not be used for legionella samples
- swabs of varying types (see more information in specific sections below)
- camera (optional)

## Food sampling

There is no requirement for routine sampling of food in healthcare settings. It is, however, essential that food providers, whether subcontracted or in-house, have robust food safety management systems in place, such as HACCP (Hazard Analysis and Critical Control Point), and can demonstrate 'due diligence' (that is, they have done everything possible to ensure the provision of safe and wholesome food). Healthcare providers should ensure that this assurance is extended to include the whole process up to and including the point at which food is served to the patient or client. Many food manufacturers and some hospitals choose to adopt a programme of routine microbiological monitoring as an added measure of quality and this has often been of value in identifying unforeseen problems at an early stage (for example, the presence of *Listeria monocytogenes* in sandwiches supplied to patients). When catering services are contracted out, healthcare providers may require certain levels of microbiological monitoring as part of their service specification; in these situations, it is appropriate to insist that UKAS accredited laboratories are used for microbiological examination, with appropriate methodology and accreditation for the required tests.

For in-house catering facilities, a rolling monitoring programme may be agreed locally and will depend upon the resources available and accessibility of laboratory support. It is important to recognise that levels of bacteria which may be acceptable for healthy adults may present an unacceptable risk to those with compromised immunity, and we advise that tolerances should therefore be set very low in the healthcare setting (for example, we



suggest in Table 2b that the detection of *Listeria monocytogenes* at any level should be investigated in ready-to-eat food from the hospital environment, whereas up to 100 cfu/g of this organism may be acceptable in foods for general retail purposes, according to Assimilated Regulation (EC) 2073/2005 [as amended]). It is always advisable to liaise closely with the local environmental health department (EHD) to ensure best practice.

When sampling food, it is essential that the items reach the examining laboratory in a state that is microbiologically unchanged from the time it was sampled; in all cases this will involve the use of sterile containers and in most cases the use of refrigerated storage and transportation.

In the investigation of outbreaks, where legal proceedings may ensue, it is essential to involve the local EHD in the inspection of the premises and the food sampling process. The EHD will always have access to an Official Laboratory with appropriate accreditation for examination of the samples collected as part of an outbreak investigation.

## Equipment required

In addition to the equipment listed under 'General equipment requirements', the following items may be required:

- hair coverings (for example, hair nets or mob caps)
- clean white coat
- sterile sampling utensils (for example, spoons, knives or spatulas)

## Procedure for sampling foods

(Based on Food Standards Agency, 2021)

The sampling procedure may vary depending on the type of food and the reason for sampling. If food-handling practices within a catering unit are being investigated, it may be appropriate to sample the food using the utensils that would normally be used for handling or serving the food. However, if a sub-sample of food is to be examined as supplied by the producer to the hospital catering department, the sample should be collected using sterile utensils.

1. At least 100 grams of food is usually required, unless an alternative quantity has previously been agreed with laboratory staff.
2. Where intact foods are to be examined, the whole sample in its original wrapping is placed inside a food-grade bag.
3. For aseptic sampling of open packs, take a portion of the food using appropriate sterile utensils (see discussion above regarding the need for sterile utensils or otherwise). This will normally be a representative portion of all components but may be a specific portion such as a core sample, surface sample, sandwich or pie

filling and so on. Place the food sample into a sterile food-grade bag or sterile plastic jar, taking care not to allow the sample to touch the outside or top edge of the container. Label the container with the location and sample details, sender's unique reference or identification code, sampling officer and date and time of sampling. When a secure chain of evidence is required, place the container into another food-grade bag and seal with a tamper evident tag.

4. Record the sender's reference code and any relevant information such as the place of sampling, temperature of storage, type of packaging and type of sample on the laboratory sample submittal form.
5. Store samples in a cool box, preferably between 2 and 8°C (taking care to keep raw foods in a separate box from ready-to-eat foods, and hot food separate from cold), and return to the laboratory as soon as possible, preferably on the same day (unless there is a particular reason for a delay such as sampling late in the evening) but always within 24 hours of collection.

If necessary, samples can be left in a cool box overnight, provided that it is properly packed with an adequate number of cold packs (10% of the total cool box volume – see Appendix for further guidance) or transferred to a secure fridge or cold-room, and submitted to the laboratory as early as possible on the following day. A calibrated datalogger should be used to monitor the temperature throughout the storage period.

## Water sampling

The only statutory requirements for water quality, at present, pertain to the quality of drinking water. There are, however, several authoritative guidance documents addressing best practice in the maintenance of water systems in general (for example, the control of legionella; Health and Safety Executive, 2013) and for specific purposes for example control of *Pseudomonas aeruginosa* in augmented care facilities (Department of Health, 2016c), endoscopy rinse water (Department of Health, 2016a), swimming, hydrotherapy and birthing pools (Pool Water Treatment Advisory Group, 2017), renal dialysis water (UK Renal Association, 2016) and dental line supplies (Department of Health, 2013). Bacteria in water systems tend to be few in number due to low nutrient availability and are frequently associated with biofilms which form on the inside surfaces of pipework, valves and so on. Higher counts will be found in water that is stagnant or stationary for long periods, for example tanked supplies, dead legs, infrequently used parts of buildings. It is therefore important to use a risk-based approach to the selection of appropriate sampling points, and to collect sufficient volumes of water to enable adequate assessment of the water quality. All available guidance documents give recommended volumes and methods for sampling. Disinfectants, such as chlorine dioxide, which are used to improve water quality, have residual effects in the sampled water and must be neutralised in order to give an accurate microbiological result. Therefore, appropriate sampling bottles containing neutralising agents must be used and advice on these can be obtained from the testing laboratory prior to sampling.

As with food sampling, the highest standards of water system management and maintenance are essential, including an assessment of temperature control in the water distribution system. Microbiological monitoring is much less important than achieving high engineering standards, but can be valuable during commissioning of new systems and after remedial work has been carried out, as well as during ongoing checks, often revealing unexpected problems or deficiencies in a system. Commissioning of water systems (and associated testing) in clinical facilities intended for patients at high risk of infection is discussed in NHS Estates Technical Bulletin No. 2024/3 (NHS England, 2024). However, testing requirements and the significance of any positive results should be discussed with the testing laboratory and considered as part of the overall Water Safety Plan.

It is important, in any investigation of a water system, to have a thorough knowledge of the supply and the system itself. In this respect, the local estates officers should normally be involved at an early stage. It is usually necessary to sample systematically, working proximally to the problem in order to identify its source. Full temperature profiles are extremely useful in the investigation of raised counts of legionella bacteria.

If all reasonable measures fail to determine the source of a microbiological problem, expert advice should be sought.

## Equipment required

In addition to the equipment listed under 'General equipment requirements', the following items may be required:

- plastic measuring jug or wash bottle
- sodium hypochlorite solution or tablets
- disposable cleaning cloth or wipes
- water bottles (see Table 1)
- food grade plastic bags, sterile scissors and elastic bands (for taking shower samples)
- electronic thermometer with probe
- kit and appropriate consumables for measuring pH and residual disinfectant (may be colorimetric or electronic type)
- timer
- torch

**Table 1. Sample bottles required for the collection of water for different microbiological and chemical analyses**

Test required	Sample bottles
Coliform bacteria, <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , aerobic colony counts, environmental mycobacteria, <i>Staphylococcus aureus</i>	1 × sterile 500ml plastic bottle containing an appropriate neutraliser to neutralise any residual disinfectant in the water.  The most commonly used neutraliser, which is appropriate for chlorinated or brominated water systems and those using ozone or hydrogen peroxide, is sodium thiosulphate. For mains water and hydrotherapy pools, 18mg/L sodium thiosulphate should be added. However, for cooling towers, 180mg/L (that is sufficient to neutralise 50mg chlorine per litre) must be used. If alternative disinfection methods are used, the laboratory should be contacted to obtain the appropriate neutraliser, if one is available.
Legionella	1 × sterile 1 litre bottle Or 2 × sterile 500ml plastic bottles (as above) (See note above regarding neutralisers)
Endotoxin	Designated 'pyrogen-free' containers (for example 30ml universal containers that have been confirmed by the supplier to be pyrogen-free)
Chemical parameters	Specific bottles should be requested from laboratory depending on tests required

## Procedure for sampling tap water

(Based on Standing Committee of Analysts, 2010)

The sampling strategy should determine the sampling technique. If the quality of water as delivered from the tap (that is including any bacteria that are colonising the tap) is of interest, then the tap should not be sanitised and the sample should comprise the first portion of water delivered (that is omit steps 1 to 4 below), preferably immediately after a period of no, or minimal, use. If only bacteria present in the circulating system prior to the tap are sought, the tap should be sanitised and run for 2 to 3 minutes before sampling. When attempting to ascertain the origin of contamination, it may be appropriate to take samples before and after sanitisation and flushing. The following sampling procedure should be followed (with steps 1 to 4 being dependent on sampling strategy):

1. If possible, ensure that the tap is in good condition, with no leaks.
2. Remove any internal and external fittings, such as hosing.

3. Clean the end of the tap thoroughly with a clean disposable cloth (and detergent if necessary). Disinfect with sodium hypochlorite solution (sufficient to give 1% available chlorine) made up on the day of use, or chlorine dioxide foam. Sanitisation can be carried out by preparing a hypochlorite solution in a measuring jug and suspending it under the tap, such that the end of the tap is immersed in the solution for 2 to 3 minutes. Alternatively, use a wash bottle to spray hypochlorite solution onto the outside and inside of the tap spout. Leave for 2 to 3 minutes before rinsing. (Safety note: sodium hypochlorite is highly corrosive and should be handled with care. Nitrile gloves and goggles should be worn, and if contact with skin, eyes or clothes occurs, wash the affected area immediately with copious amounts of water. Contact with clothes may result in a bleaching effect. If a wash bottle is used, this should produce a directed spray but not a fine mist.)
4. Turn on the tap gently to avoid unnecessary aerosol production and run water to waste for 2 to 3 minutes.
5. Label a sterile bottle (500ml or one litre bottle containing neutraliser – see Table 1) with the location and sample details, sender's reference code, sampling officer and date and time of sampling.
6. Aseptically open the bottle, fill almost to the brim with water, replace and tighten the lid and shake the bottle to distribute the neutraliser.
7. Water samples (except for legionella samples) should be stored and transported between 1 and 8°C. Water samples for legionella testing should be stored and transported at room temperature. All water samples should be submitted to the laboratory to ensure that they are examined promptly, ideally the same day, but always within 24 hours of collection.
8. If both routine testing parameters and legionella are required, then separate one litre and 500ml samples should be collected.

## Procedure for sampling swimming, spa and hydrotherapy pool water

(Based on Pool Water Treatment Advisory Group, 2017)

Normally a single sample of pool water is taken. The most appropriate site for taking a single sample from a pool is where the water velocity is likely to be at its lowest and away from fresh water inlets or outlets. Depending on the size of the pool, it may be advisable to take samples from other sites to establish whether there are 'dead spots' in the water circulation. During investigations of poor water quality, it is recommended that a sample is taken from the balance tank and skimmers, and that swabs are taken from inside or behind any jets and from the lid or cover for the pool if used.

1. Outside shoes should be removed or plastic shoe coverings worn if entering swimming pool areas.
2. Wipe the outside of a sterile bottle (500ml bottle containing neutraliser – see Table 1) with an alcohol wipe (this is not necessary if bottles are individually packed),

- and label with a waterproof marker or biro (indicating the location and sample details, sender's reference code, sampling officer and date and time of sampling).
3. Aseptically open the bottle.
  4. Immerse the bottle, keeping the long axis approximately horizontal but with the neck pointing slightly upwards to avoid loss of the neutralising agent.
  5. Once the bottle is immersed to about 200 to 400mm (8 to 16 inches) below the surface, tilt the bottle to allow it to fill, leaving a small headspace.
  6. On removal from the water, immediately replace the cap and shake the sample to disperse the neutralising agent.
  7. Water samples (except for legionella samples) should be stored and transported between 1 and 8°C. Water samples for legionella testing should be stored and transported at room temperature. All water samples should be submitted to the laboratory to ensure that they are examined promptly, ideally the same day, but always within 24 hours of collection.
  8. If both routine testing parameters and legionella are required, then separate one litre and 500ml samples should be collected.

It is good practice to determine total and combined disinfectant levels and pH value from the same site as the microbiological sample. These should be determined in a separate sample collected in a bottle with no added neutralising agent (for example a sterile plastic universal container) and the tests carried out at the pool-side. These results together with information on the number of users in the pool at the time of sampling should accompany the sample to the laboratory. It is important to also note the type of disinfectant in use in the pool.

## Procedure for sampling water for *Pseudomonas* testing in augmented care areas

(Based on Department of Health, 2016c)

The water outlets to be sampled should be those that:

- supply water that has direct contact with patients
- are used to wash staff hands
- are used to clean or fill equipment that will have contact with patients as determined by local risk assessment

It is recommended that water outlets are tested every 6 months or more frequently if results prove to be unsatisfactory.

Water samples should be taken during a time of low or no use (at least 2 hours or preferably longer without use). The first water delivered from the outlet (that is pre-flush) should be used for routine monitoring, according to the method described in 'Procedure for Sampling Tap Water' (page 14, points 5 to 7). For follow-up samples in response to previous poor

results, pre- and post-flush samples should be collected (that is an initial, pre-flush sample should be taken as described above; the tap should then be run for 2 minutes and a second post-flush sample taken).

## Procedure for sampling water for legionella testing

(Based on British Standards Institution, 2022)

During investigations, sampling must not be carried out in isolation but should be done in conjunction with a review of the risk assessment, up-to-date schematics of the water systems, a review of previous monitoring results (both microbiological and temperature) and a review of current control measures. Sampling must be carried out based on the perceived risk. For example, water should be sampled from the areas where legionella are most likely to multiply, such as the warmest parts of a cold system, the coolest parts of a hot system or areas where there is low usage or stagnation, and areas that pose the greatest risk from exposure. Where there are several floors in the building under investigation, flow and return temperatures should be taken on each floor and to and from the calorifier or other heat source. Further details of appropriate sampling points are given in Approved Code of Practice and Guidance: L8 (Health and Safety Executive, 2013).

For details of appropriate sample bottles to use for legionella sampling, see Table 1. For sampling from taps and swimming or spa pools, see procedures in the relevant sections above (but note that a pre-flush sample is useful from taps, as legionella may flourish in any standing water in the outlet, that is omit steps 1 to 4 in 'Procedure for Sampling Tap Water', pages 12 to 13).

To sample from showers, proceed as described below. Normally, a one-litre sample is taken from each shower head.

1. Label a sterile bottle with the location, sample details, sender's reference code, sampling officer and date and time of sampling.
2. Before turning on the shower, adjust the temperature setting to the midpoint or normal use temperature (35°C to 43°C).
3. Detach the shower head from the hose and, without changing the temperature setting of the shower, place a sample bottle under the end of the hose, turn the shower on gently and fill the bottle almost to the brim.
4. If the shower head is fixed onto the hose, place a sterile food-grade plastic bag over the shower head and secure with a rubber band or adhesive tape. Using clean, disinfected scissors, cut off one of the bottom corners of the bag to form a funnel. Use this funnel to fill the bottle.
5. Replace and tighten the lid and shake the bottle to distribute the neutralising agent.

All water samples for legionella examination should be stored at an ambient temperature (approximately 20°C) in the dark, and returned to the laboratory as soon as possible, preferably the same day but at the latest so that processing can begin within 24 hours of taking the sample.

Safety note: When investigating a legionella case or outbreak, it is essential that an assessment of risks associated with sampling is carried out in discussion with suitably experienced staff before samples are collected and that a sampling plan is drawn up in consultation with other experts for example site engineers and infection control officers (see BS 7592-2022 for more advice).

It is good practice to establish the water temperature at the time of sampling. This is particularly important if an investigation is being carried out to determine the source of legionella in a clinical case or as part of a risk monitoring process. Hot water should reach 55°C within one minute at outlets, whilst cold water should be 20°C or below after running the water for 2 minutes (Health and Safety Commission, 2013). A calibrated stopwatch and calibrated probe thermometer must be used to measure the temperature of the water to ensure conformity with these guidelines. This information should be recorded along with the identity of the site and whether or not the outlet was intended to be hot or cold. For taps with a thermostatic mixer valve (TMV) it will be necessary to take the temperature of the water upstream of the TMV.

## Renal unit waters and dialysis fluids

Water used in the preparation of dialysis fluid is tested to determine whether it meets the minimum requirements for microbiological contamination (UK Renal Association, 2016). Samples should be taken from points expected to have the highest bacterial load, such as the end of the distribution loop or the last machine in a dead-end system (EDTNA/ERCA, 2002). If the sample is to be collected from a tap used solely for sampling, ensure that this has been appropriately sanitised as described in 'Procedure for Sampling Tap Water' (point 3).

1. Label a sterile bottle (usually 500ml bottle containing neutraliser for microbiological tests or a pyrogen-free container for endotoxin – see Table 1) with the location and sample details, sender's reference code, sampling officer and date and time of sampling.
2. Aseptically open the bottle and fill almost to the brim with water; replace the lid. Note: If only small volumes of liquid are available for sampling, a smaller sterile plastic container can be used, as neutraliser is not essential for this sample type.
3. Store the water between 1 and 8°C and return to the lab for examination, preferably on the same day but always within 24 hours of collection.



## Endoscopy or washer disinfectors final rinse waters

Guidance on the decontamination of flexible endoscopes is available in 'Decontamination of Flexible Endoscopes (HTM 01-06)' (Department of Health, 2016a). The following paragraph summarises the microbiological information contained therein:

Essentially, the user should define the standard of disinfection required in consultation with an infection control practitioner. For most endoscopic procedures, the final rinse water need only be of potable quality. However, rinse water for endoscopes which enter normally sterile body cavities (for example arthroscopes) will need to be of a higher standard, so it is safest to ensure that final rinse water from automated washer-disinfectors has low microbial counts and does not present a potential hazard to the patient either through infection or by leading to an erroneous diagnosis. It is essential that great care is taken to avoid introducing contamination when obtaining samples. The exact procedure will vary from one model to another, but in general, the machine should be run on a special cycle that allows the cycle to be stopped in the rinse phase and a sample collected via a sterile sampling tube. If this is not feasible, use a sampling point on the machine, disinfect the sampling point with 70% alcohol and run approximately 500ml rinse water to waste before aseptically collecting at least 100ml (and preferably 400ml) in a sterile container.

The sample should be stored between 2 and 5°C and processed as soon as possible, preferably within 24 hours (and always within 48 hours) of collection.

Although the Operational Management manual (Part C) of HTM 01-06, states that endoscopes that are passed into sterile body cavities should be free of endotoxins, this is, in practice, unrealistic and unsupported by evidence; sterile water for injections has allowable limits of 0.25 EU/ml. This statement is also contradicted by a different part of the same document, HTM 01-06 (Part B); Design and Installation manual, which states that 'EWD final rinse-water should not contain more than 30 endotoxin units/ml' and that 'routine endotoxin testing is, therefore, not required unless there is evidence of a major water supply problem', in which case, it would be inadvisable to continue using that supply anyway. Reverse osmosis units will reduce levels of endotoxin in the water supply, but will not eliminate it.

It is essential that microbiological results are monitored sequentially in order to identify normal variation and trends so that early action may be taken if problems arise. During investigations of poor results, collection of water samples prior to the final treatment process (for example supply water and break tank water) should be considered. In addition, check that the correct filters are properly fitted and are included in the daily self-disinfection cycle of the washer or disinfectors, and that a regular schedule of maintenance and replacement of the filters is in place.

## Dental unit water lines

There is no regulation or guidance in the UK regarding the frequency of sample collection from Dental Unit Water Lines (DUWL). However, where monitoring is undertaken, the following procedure should be followed:

1. Label a sterile bottle (usually 100ml or 500ml bottle containing neutraliser – see Table 1) with the location and sample details, sender's reference code, sampling officer and date and time of sampling.
2. Purge the 3:1 and/or high speed outlets of the dental unit for 2 minutes before collecting water samples.
3. Aseptically open the bottle and collect 100ml of water from the 3:1 and/or high speed outlets.
4. Store the water between 1 and 8°C and return to the lab for examination ideally on the same day but always within 24 hours of collection.

## Heater cooler units

Heater cooler units used in cardiopulmonary bypass and ECMO procedures have been the focus of attention since 2015, due to an outbreak of *Mycobacterium chimaera* infections associated with these machines (Public Health England, 2017). Water sampling from these machines for the detection of slow-growing mycobacteria is described in a separate document (Public Health England, 2016), as summarised below:

1. The heater cooler machine should be connected and running for a minimum of 5 minutes before water sampling is performed.
2. Ideally the water sampling should take place just prior to the machine undergoing its disinfection cycle.
3. Bottles containing sodium thiosulphate should be used, as described in Table 1.
4. Water should be sampled from both circuits that is the 'patient' circuit and the 'cardioplegia' circuit via the tubing systems. Ensure that sterile tubing or fittings are available for each machine being tested.

Ensure that samples are submitted to a laboratory that has appropriate validation and accreditation for processing such samples for the detection of slow-growing mycobacteria.

## Environmental surface sampling

Cleaning of the hospital environment is essential to protect patients from hospital acquired infections and must be carried out according to current guidelines. Care facilities must carry out risk assessment of the healthcare environment, document cleaning tasks and monitor the effectiveness of cleaning. NHS guidelines use visual inspection only as a measure of cleanliness (NHS England, 2021). Routine sampling of environmental surfaces in healthcare environments is therefore not usually indicated. It may, however, be required in order to

identify an environmental source of infection or contamination, to demonstrate efficacy of disinfection or cleaning procedures or as a research tool. It is essential that careful thought is given to the nature and purpose of the sampling, whether quantitative or qualitative results are needed and what actions will be taken on sampling outcomes. Diluents and isolation media should be appropriate for the isolation of the specific organisms sought. In some cases, it may be necessary to consider the need for controls or sampling over time to establish a baseline.

Effective sampling of surfaces requires moisture in order for the microorganisms to adhere to the sampling matrix – there may be moisture already present on the surface, or, more frequently, a sterile diluent such as saline or buffered peptone water is used. Appropriate neutralisers (such as TLTR neutralising solution – see ‘Media’ section below) must be used if disinfectant residues are likely to be present on the surface to be sampled.

Sampling may be quantitative that is a known area is swabbed (using a swab or sponge) in a standardised way in order to compare results from different sites, or from the same site but taken at different times. This is most often done using a sterile metal or plastic template. It is also possible to sample using an agar contact plate. These methods are only suitable for relatively flat surfaces. If uneven or complex surfaces are to be sampled, contact plates cannot be used, but if it is possible to estimate the area swabbed, this would enable reporting of a semi-quantitative result. Alternatively, qualitative sampling (to determine the presence or absence of a pathogen) is usually appropriate when investigating the source of an outbreak or a cross-contamination incident. In this case, the larger the area sampled, the better the chance of detecting the pathogen of interest.

For large areas, sponges are often found to be most convenient, while nylon, dacron or cotton-tipped swabs are often more convenient for complex surfaces or areas which are less accessible. However, it should be noted that recovery of micro-organisms may be more efficient with some of these alternatives than others, and the laboratory should undertake verification studies to confirm that they can recover the target organisms from whichever swab type is selected. Whilst contact plates give a lower recovery than either swabs or sponges, they may be useful in some situations where low numbers of organisms are expected.

The procedures below are suitable for the detection of bacterial contamination. Swabbing for norovirus or other viruses is not usually indicated. However, in some situations (for example, verification of cleaning procedures during norovirus outbreaks) it may be useful to carry out surface swabbing. Appropriate procedures, equipment and sample numbers should be discussed with the local UKHSA Food Water and Environmental Microbiology Laboratory and/or Virus Reference Laboratory before undertaking any sampling.

## Equipment required

In addition to the equipment listed in 'General equipment requirements', the following items may be required:

- sterile templates of known area (usually 10 × 10cm)
- sterile sponge swabs (with or without a handle) moistened with neutralising solution (store as indicated by the supplier)
- sterile sponge swabs on sticks with neutralising solution
- sterile cotton-tipped or Dacron swabs with neutralising solution

Note: The laboratory will only hold accreditation for analysis of swab types that have been validated prior to use to ensure that they do not inhibit the target organisms. Therefore, it is advisable to use swabs that are provided by the laboratory.

## Media

### TLTR neutralising solution (Holah, 1999)\*

Sodium thiosulphate	5g
Quarter strength Ringer tablets or Thiosulphate Ringer tablets	2** 2**
Lecithin	3g
Polysorbate 80 (Tween 80)	30g
Maximum Recovery Diluent (0.1% peptone, 0.85% saline)	9.5g
Water	1 litre

pH 7.2 ± 0.2 at 25°C

\*Note: Maximum Recovery Diluent or sterile physiological saline can be used as an alternative to TLTR neutralising solution, but microbiological results may be affected if disinfectant residues are present on the surfaces being sampled.

\*\*Note: quantities given assume that each tablet is suitable for 500ml final solution.

Rodac (contact) plates containing selective or non-selective agar as required.

## Procedure for swabbing flat surfaces

A template may be used to accurately quantify the area to be sampled. Alternatively, the area may be judged by eye, in which case stages 1 to 3 may be omitted.

1. Aseptically open the sterile template pouch allowing access to the template handle. Do not remove the template at this stage.

2. Wash and dry hands thoroughly.
3. Remove the sterile template from its pouch, taking care not to touch the inside surface. Place the template on the surface of interest.
4. Open a sponge swab pack and aseptically take hold of the sponge, either by holding the handle or by using sterile gloves for sponges without a handle. (Refer to the manufacturer's instructions for specific guidance on different types of sponge swab). If not pre-moistened, moisten the sponge by dipping it into an appropriate liquid medium (usually 10ml of TLTR neutralising solution or equivalent, if disinfectant residues are likely to be present on the surface or if an assessment of this cannot be made) and squeezing out excess liquid against the side of the container.
5. Applying a firm pressure, and using up and down movements (taking approximately one second per stroke), swab the entire surface area within the template (up to the inner edge of the template).
6. Hold the sponge at right-angles to the first movement and repeat the process.
7. Aseptically return the sponge to its sterile container. If using a sponge with handle, do not insert the part of the handle you have touched with your hands into the final container; break off the handle according to the manufacturer's instructions.
8. Seal the container and label clearly.
9. Wipe over the area that has been swabbed with an alcohol wipe.
10. Store the sponge at between 1 and 8°C and return to the laboratory as soon as possible to ensure that it is examined on the day of collection or at least within 24 hours of collection.

## Procedure for swabbing objects without a flat surface

A template cannot be used for objects such as door handles, pipework and drains, where no flat surface is available. In this case, follow the procedure described above ('Procedure for swabbing flat surfaces' – points 4 to 10) to swab the desired area. Ensure that a clear record is kept of the exact area swabbed. It is preferable to swab the entire surface of a handle, for example, or an entire utensil, to ensure that a repeat sample of the same surface can be taken in a comparable manner if required. Taking a photograph of the surface sampled can be useful in ensuring reproducibility if resampling is necessary.

## Procedure for environmental monitoring of surfaces using contact plates

Surface contact plates are prepared in specialised plastic dishes, known as Rodac plates, which are filled with a known volume of agar to provide a convex surface that is slightly raised above the top of the dish. Following appropriate quality-control procedures to ensure sterility, they can be used to monitor the cleanliness of surfaces, as follows:

1. Press the agar surface onto the surface to be sampled, rock slightly from side to side then carefully remove from the surface and replace the lid.

2. Use an alcohol wipe to remove any agar debris from the surface that has been sampled.
3. Place the plates in a sterile plastic bag, seal and label clearly.
4. Store the plates at between 1 and 8°C and return to the laboratory as soon as possible to ensure that they are processed on the day of collection or at least within 24 hours of collection.

## Air sampling

The microbiological quality of air varies widely and is dependent upon factors such as temperature, relative humidity, ventilation and exposure to ultraviolet or electromagnetic radiation. In addition, the survival of microorganisms suspended in air will depend upon their susceptibility to these factors and the nature of the particles in which they are carried – fungal spores will remain suspended and survive much longer than vegetative bacteria contained within airborne droplets. Results of air sampling in any particular location will be subject to changes throughout the day and on a seasonal basis so that it is possible to construct a microbiological profile over time. Indoor air is, likewise, subject to the same factors, but in addition, will vary according to the number of people and activity within the room, traffic in and out of the room, basic ventilation such as windows and the performance of any air-handling systems. In order to be meaningful, air sampling must take these factors into account so that results are only compared with those of samples taken under similar, defined conditions.

Sampling may be undertaken to assess air quality in areas such as operating theatres, pharmacy sterile units and sterile supply units. It may also be used as a continuous monitoring system for example in laboratories, in order to assess fluctuations in background counts which may result in contamination of test cultures.

Sampling may be either passive or active. Passive sampling needs no special equipment – agar plates are simply exposed in the area for a defined period of time. Several plates would usually be exposed at the same time in order to assess the average microbial count. This method is time-consuming and needs careful interpretation as air movements and activity may lead to wide fluctuations in results. Active sampling involves the use of mechanical equipment which draws in known volumes of air which then impinge on culture media or filters. Numbers of microbes present per unit volume of air can then be calculated accurately. Before performing any air sampling, special consideration should be given to whether a specific organism or all organisms are to be targeted, the volume of air to be sampled, the need for quantitative or qualitative results and what actions might be taken on the basis of the results obtained.

## Equipment required

In addition to the equipment listed in 'General equipment requirements', the following items may be required:

- timer
- air sampler

## Media

Agar plates containing selective or non-selective media as required (for example Blood Agar or Tryptone Soya Agar for total microbial counts and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) for mould investigations).

Before use, the agar plates should be subjected to appropriate quality control procedures to ensure sterility. Pre-incubation of plates is not recommended for this purpose, as it is likely to dry the agar out and potentially change the composition of the medium.

## Procedure for passive air sampling using settle plates

Settle plates can be used to monitor air quality as follows:

1. Place agar plate (containing selective or non-selective agar, depending on organisms of interest) on a flat surface in the test location, and expose the agar surface by removing the lid.
2. Leave the agar exposed for the agreed period of time (this may vary depending on the likely level of contamination in the test environment, but time periods of at least 30 minutes, and up to 4 hours, are usually recommended). Monitor the exposure time with a timer.
3. Replace the lid, place the plates in a sterile plastic bag, seal and label clearly.
4. Store the plates at between 1 and 8°C and return to the laboratory as soon as possible to ensure that they are processed on the day of collection or at least within 24 hours of collection.

## Active air sampling

Active air sampling of known volumes of air is carried out using specialist equipment, by trained staff, and should be performed according to the manufacturer's instructions for the air sampling equipment used. Further information may be sought from the local food, water and environmental microbiology laboratory or infection control department, and guidance is also provided in HTM 03-01 (Department of Health, 2021).

# Testing parameters and interpretation of microbiology results

Testing requirements and interpretations of results are provided in Tables 2 to 12 for a variety of sample types collected from the healthcare environment. Wherever possible, testing should be carried out by a laboratory that is UKAS-accredited to perform a specific test. It should be noted that for laboratories performing work to the ISO 17025 standard, each test and sample type is accredited separately by UKAS, so it is important to check that the sample types and tests of interest are covered by the laboratory's scope of accreditation. Schedules of accredited tests are available on the UKAS website for each laboratory. Clinical laboratories are usually accredited through UKAS to the ISO 15189 standard, which is for the entire quality system and does not include accreditation for individual tests. As with laboratories accredited to the ISO 17025 standard, the accreditation status of a clinical laboratory accredited to ISO 15189 can be found on the UKAS website.

In addition to the tests shown in Tables 2 to 12, a range of further microbiological tests may be carried out, and advice given regarding interpretation of results through discussion with the microbiologists at the local laboratory. Advice on the interpretation of results should be sought from a microbiologist with experience of the healthcare environment. Contact details for UKHSA Food Water and Environmental Laboratories and the laboratory for Healthcare-Associated Infections and Antimicrobial Resistance are provided on the UKHSA website ([www.gov.uk/government/organisations/uk-health-security-agency](http://www.gov.uk/government/organisations/uk-health-security-agency)).

Interpretation of microbiology results for water from a mains supply is covered in the Water Supply (Water Quality) Regulations 2016 (Great Britain, 2016a) as amended in 2018 (Great Britain, 2018a), and is not discussed further in this document. Criteria for private water supplies are covered in the Private Water Supplies Regulations 2016 (Great Britain, 2016b) as amended in 2018 (Great Britain, 2018b).

Legionella criteria in water systems, based on Approved Code of Practice and Guidance: L8 (Health and Safety Commission, 2013), are specified in Table 4. However, a legionella specialist should be consulted when interpreting legionella results from a hospital under investigation. More detailed guidance on actions required is available in the Approved Code of Practice: L8 (Health and Safety Commission, 2013).

HTM 03-01 (Department of Health, 2021) states that there is little value in performing microbiological sampling of air quality in an empty operating theatre supplied with ultra clean ventilation (UCV), since other testing such as filter challenge tests and air velocity measurements will have proved that the system is operating satisfactorily. The EPA filters will remove bacteria-sized particles from the air supplied through the ultra clean ventilation (UCV) canopy. Therefore, there will be an insignificant number of bacterial and/or fungal particles present until the theatre is actually used. Moreover, following full validation, in-use



microbiological sampling during a surgical procedure will not be required unless specified by the client's Ventilation Safety Group. However, the summary of acceptance testing in Part A of HTM 03-01 and the annual verification checks in Part B still refer to microbiological sampling, and therefore criteria have been retained in Table 11 for guidance.

**Table 2a. Testing requirements and interpretation of results for cook chill food**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	Minimum requirement of monthly testing of a range of products. A rolling programme of testing to cover all menu items and catering processes is recommended.  Approximately 100g of each item of food to be sampled should be taken prior to reheating or regeneration.	≥ 100,000 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	Department of Health, 1989
		< 100,000 cfu/g	Satisfactory	N/A	
<i>Salmonella</i> species		Detected in 25g	Unacceptable	Withdraw food from use and investigate cause immediately	
		Not detected in 25g	Satisfactory	N/A	
<i>Escherichia coli</i>		≥ 10 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	
		< 10 cfu/g	Satisfactory	Presence of this organism at lower levels may require investigation, depending on local experience and risk assessment	
<i>Staphylococcus aureus</i>		≥ 100 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	
		< 100 cfu/g	Satisfactory	Presence of this organism at lower levels may require investigation, depending on local experience and risk assessment	

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Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
<i>Clostridium perfringens</i>		≥ 100 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	
		< 100 cfu/g	Satisfactory	Presence of this organism at lower levels may require investigation, depending on local experience and risk assessment	
<i>Listeria monocytogenes</i>		Detected in 25g	Unsatisfactory	Investigate cause and put corrective action in place	
		Not detected in 25g	Satisfactory	N/A	

**Table 2b. Testing requirements and interpretation of results for ready-to-eat foods including sandwiches**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
<i>Listeria monocytogenes</i>	As indicated by local risk assessment	>100 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	UKHSA, 2024
Detected in 25g (≤ 100 cfu/g)		Borderline	In foods likely to be served to vulnerable groups, presence of <i>L. monocytogenes</i> at any level may be of significance and should be robustly investigated		
Not detected in 25g		Satisfactory	N/A		
<p>Aerobic colony count; Enterobacteriaceae; <i>Escherichia coli</i>; <i>Staphylococcus aureus</i>; <i>Salmonella</i> species</p> <p><i>Clostridium perfringens</i> (for meat products and those including gravy or stock)</p> <p><i>Bacillus cereus</i> and other <i>Bacillus</i> species (for products including rice or spice ingredients)</p>		Results should be interpreted according to UKHSA Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market.			

**Table 2c. Testing requirements and interpretation of results for dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Bacillus cereus (presumptive)	Criteria apply at end of manufacturing process, but can be used as a guideline during investigations	> 500 cfu/g	Unsatisfactory	Investigate cause and put corrective action in place	Great Britain, Assimilated Regulation (EC) 2073/2005 as amended in Assimilated Regulation (EC) 1441/2007
		≥ 50 - < 500 cfu/g	Borderline (unsatisfactory if present in 2 or more samples in a batch of 5 examined)		
Enterobacteriaceae		< 50 cfu/g	Satisfactory	N/A	
		Presence in 10g	Unsatisfactory	Investigate cause and put corrective action in place; test batch for the presence of <i>Cronobacter sakazakii</i> in 10g	
Salmonella	Criteria apply to products placed on the market during their shelf-life, but can be used as a guideline during investigations	Absence in 10g	Satisfactory	N/A	
		Presence in 25g	Unsatisfactory	Investigate cause and put corrective action in place	
		Absence in 25g	Satisfactory	N/A	

**Table 3a. Testing requirements and interpretation of results for hydrotherapy pool water samples**

Hazard/hygiene Indicator	Timing/frequency of Testing	Result	Interpretation	Action	References
<i>Escherichia coli</i>	Weekly (collect sample while in use)	>0 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	Pool Water Treatment Advisory Group, 2017
		0 cfu in 100ml	Satisfactory	N/A	
Coliform bacteria (total coliforms)		>10 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	
		1 - ≤10 cfu in 100ml	Acceptable*	* This level is considered acceptable provided that Aerobic Colony Count is <10/ml, <i>E. coli</i> is not detected, disinfectant and pH values are acceptable and coliforms are absent in repeat samples	
		0 cfu in 100ml	Satisfactory	N/A	
<i>Pseudomonas aeruginosa</i>		>50 cfu in 100ml	Unacceptable	Close pool and seek advice on remedial actions required	

Hazard/hygiene Indicator	Timing/frequency of Testing	Result	Interpretation	Action	References
		>10 cfu in 100ml	Unsatisfactory	Investigate and take repeat sample	
		1-10 cfu in 100ml	Borderline	Take repeat sample	
		0 cfu in 100ml	Satisfactory	N/A	
Aerobic colony count		> 100 cfu/ml	Unsatisfactory	Immediate investigation required	
		>10 - ≤100 cfu/ml	Borderline	Take repeat sample. Acceptable in the absence of <i>E.coli</i> or coliforms. Repeated raised counts require further investigation.	
		0 - ≤10 cfu/ml	Satisfactory	N/A	
<i>Staphylococcus aureus</i>	As part of wider investigations only – in discussion with local microbiologist	>0 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	Pool Water Treatment Advisory Group, 2017
		0 cfu in 100ml	Satisfactory	N/A	

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Hazard/hygiene Indicator	Timing/frequency of Testing	Result	Interpretation	Action	References
Legionella	Quarterly (depending on risk assessment) and before pool used for first time or after it has been shut down	> 1,000 cfu in 1 litre	Unsatisfactory	Close pool immediately. Shock dose then drain, clean and disinfect. Review risk assessment. Re-test before re-opening.	Pool Water Treatment Advisory Group, 2017
		20 - ≤1000 cfu in 1 litre	Borderline	Take repeat sample. Drain, clean and disinfect pool and review risk assessment and controls.	
		<20 cfu in 1 litre	Satisfactory	N/A	



**Table 3b. Testing requirements and interpretation of results for birthing pool water samples**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
<i>Escherichia coli</i>	Weekly (collect sample while in use)	>0 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	Pool Water Treatment Advisory Group, 2017
		0 cfu in 100ml	Satisfactory	N/A	
Coliform bacteria (total coliforms)		>10 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	
		1 - ≤10 cfu in 100ml	Acceptable	This level is considered acceptable provided that Aerobic Colony Count is <10/ml, <i>E. coli</i> is not detected, disinfectant and pH values are acceptable and coliforms are absent in repeat samples	
		0 cfu in 100ml	Satisfactory	N/A	
<i>Pseudomonas aeruginosa</i>		>50 cfu in 100ml	Unacceptable	Close pool and seek advice on remedial actions required	
	>10 cfu in 100ml	Unsatisfactory	Investigate and take repeat sample		

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Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
		1-10 cfu in 100ml	Borderline	Take repeat sample	
		0 cfu in 100ml	Satisfactory	N/A	
<i>Legionella</i>	Quarterly (depending on risk assessment) and before pool used for first time or after it has been shut down	> 1,000 cfu in 1 litre	Unsatisfactory	Close pool immediately. Shock dose then drain, clean and disinfect. Review risk assessment. Re-test before re-opening.	
		20 - ≤1,000 cfu in 1 litre	Borderline	Take repeat sample. Drain, clean and disinfect pool and review risk assessment and controls.	
		<20 cfu in 1 litre	Satisfactory	N/A	

**Table 4. Testing requirements and interpretation of results for hot and cold water systems**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Legionella	As indicated by risk assessment	≥1000 cfu in 1 litre	Unsatisfactory	The system should be re-sampled and an immediate review of the control measures and risk assessment carried out to identify any remedial actions, including possible disinfection of the system.	Health and Safety Executive, 2013
		≥100 - <1000 cfu in 1 litre	Undesirable	(a) If only one or 2 samples are positive, system should be resampled. If a similar count is found again, a review of the control measures and risk assessment should be carried out to identify any remedial actions  (b) If the majority of samples are positive, the system may be colonised, albeit at a low level, with legionella. Disinfection of the system should be considered but an immediate review of control measures and risk assessment should be carried	

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Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
		<100 cfu in 1 litre	Satisfactory	No action; system under control	
<i>Pseudomonas aeruginosa</i> *	In augmented care wards – every 6 months or as indicated by risk assessment (sample to be collected without pre-flushing)	<p>&gt;10 cfu in 100ml</p> <p>1-10 cfu in 100ml</p> <p>0 cfu in 100ml</p>	<p>Unsatisfactory</p> <p>Undesirable</p> <p>Satisfactory</p>	<p>Investigate cause and put corrective actions in place. Re-sample after 3 days and re-check at 2 and 4 weeks after remediation.</p> <p>Re-test and refer back to those responsible for the Water Safety Plan to determine what actions may be required.</p> <p>No action; system under control</p>	Department of Health, 2016c

\*Investigation of water supplies for other *Pseudomonas* species may be required during outbreak investigations.

**Table 5. Testing requirements and interpretation of results for renal dialysis fluid and water used for the preparation of dialysis fluid**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	Monthly (or more frequently if necessary)	>100 cfu/ml	Unsatisfactory	Take out of use until corrective action implemented	UK Renal Association, 2016
		>50 - ≤100 cfu/ml	Borderline	Investigate cause and put corrective action in place	
		0 - ≤50 cfu/ml	Satisfactory	N/A	
Endotoxin /ml		>0.25 EU/ml	Unsatisfactory	Take out of use until corrective action implemented	
		>0.125 - ≤0.25 EU/ml	Borderline	Investigate cause and put corrective action in place	
		<0.125 EU/ml	Satisfactory	N/A	

Note: The production of ultrapure dialysis fluid is generally achieved by the use of additional filters which form part of the dialysis machine hydraulic pathway. ISO 23500-1:2024 states there is no requirement to test for bacterial growth or endotoxins when the haemodialysis system is fitted with a filter of appropriate capacity to retain bacteria and endotoxin that is validated by the manufacturer and operated according to the manufacturer's instructions, unless the manufacturer requires such tests to be performed.

**Table 6. Testing requirements and interpretation of results for endoscopy final rinse water**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	Weekly	>100 cfu in 100ml	Unacceptable	Discuss with Infection Control Team (ICT); complete risk assessment; consider taking washer or disinfectant out of use (particularly for endoscopes used for sterile sites such as ERCP and bronchoscopes).	Department of Health, 2016a
		10 - ≤100 cfu in 100ml	Unsatisfactory	Discuss with ICT; complete risk assessment to investigate potential problems; super-chlorinate or repeat self-disinfect cycle	
		1-9 cfu in 100ml (on a regular basis)	Acceptable	Acceptable provided that <i>Pseudomonas aeruginosa</i> is not detected	
		<1 cfu in 100ml	Satisfactory	N/A	
Environmental mycobacteria	Quarterly	> 0 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	
		0 cfu in 100ml	Satisfactory	N/A	

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Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
<i>Pseudomonas aeruginosa</i>	Quarterly	> 0 cfu in 100ml	Unsatisfactory	Investigate immediately and take repeat sample	
		0 cfu in 100ml	Satisfactory	N/A	
Endotoxin	Not routinely required	≤ 30 EU/ml	Satisfactory	Risk low even above this level but would usually be associated with high microbial counts and subject to remedial action	Department of Health, 2016a

**Table 7. Testing requirements and interpretation of results for final rinse water in surgical instrument washer disinfectors**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count (final rinse water – where products are rinsed after the disinfection stage)	Weekly	≥1 cfu in 100ml	Unsatisfactory	Discuss with Infection Control Team (ICT); complete risk assessment; super-chlorinate or repeat self-disinfect cycle; consider taking washer or disinfectant out of use.	Department of Health, 2016b
		<1 cfu in 100ml	Satisfactory	N/A	
Aerobic colony count (other water services supplied to washer or disinfectant)	Not specified	≥100 cfu in 100ml	Unsatisfactory	Discuss with ICT; complete risk assessment; super-chlorinate or repeat self-disinfect cycle.	
		<100 cfu in 100ml	Satisfactory	N/A	
Endotoxin (for washer disinfectants that are used for surgically invasive items or those that come into contact with parenteral solutions)	Annually	>0.25 EU/ml	Unsatisfactory	Investigate immediately and take repeat sample	
		≤0.25 EU/ml	Satisfactory	N/A	



**Table 8. Testing requirements and interpretation of results for dental unit water lines**

<b>Hazard/hygiene indicator</b>	<b>Timing/frequency of testing</b>	<b>Result</b>	<b>Interpretation</b>	<b>Action</b>	<b>References</b>
Aerobic colony count at 22°C	As required	>200 cfu/ml	Undesirable	Discuss with microbiologist; investigate cause and put corrective action in place	Department of Health, 2013
		100-200 cfu/ml	Acceptable	Ensure appropriate controls are in place	
		<100 cfu/ml	Satisfactory	N/A	

**Table 9. Heater cooler unit waters**

<b>Hazard/hygiene indicator</b>	<b>Timing/frequency of testing</b>	<b>Result</b>	<b>Interpretation</b>	<b>Action</b>	<b>References</b>
Environmental mycobacteria	Quarterly	Detected in 100ml	Unsatisfactory	Take out of use, disinfect and retest	Public Health England, 2017
		Not detected in 100ml	Satisfactory	N/A	
Legionella	Monthly	≥1000 cfu/l	Unsatisfactory	Any detection of legionella should be investigated and, if necessary, the system resampled to aid interpretation of the results in line with the monitoring strategy and risk assessment	Health and Safety Executive, 2013
		Up to 1000 cfu/l	Undesirable		
		Not detected	Satisfactory	N/A	

**Table 10a. Testing requirements and interpretation of results from pharmacy contact plates**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony counts	Weekly	<50 cfu	Satisfactory for Grade D	Area considered clean but activity should be restricted to low risk activities and an investigation into the source of contamination considered <sup>#</sup>	European Commission, 2022
		<25 cfu	Satisfactory for Grade C		
		<5 cfu	Satisfactory for Grade B	Area can be used for aseptic preparation and filling <sup>**</sup>	
		No growth	Satisfactory for Grade A	Area can be used for high risk operations <sup>*</sup>	

Note: Where counts exceed the specified limits, action should be taken on the basis of trend analysis and characteristics, significance and source of isolates.

\* Grade A: The local zone for high risk operations, for example filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station.

\*\* Grade B: For aseptic preparation and filling, this is the background environment for the grade A zone.

# Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

**Table 10b. Testing requirements and interpretation of results from pharmacy glove prints**

<b>Hazard/hygiene indicator</b>	<b>Timing/frequency of testing</b>	<b>Result</b>	<b>Interpretation</b>	<b>Action</b>	<b>References</b>
Aerobic colony counts	Sessional (for Grade A and B areas only)	<5 cfu/glove	Satisfactory for Grade B	Action should be taken on the basis of trend analysis and characteristics, significance and source of isolates.	European Commission, 2022
	Including 5 fingers on both hands	No growth	Satisfactory for Grade A	N/A	

**Table 10c. Testing requirements and interpretation of results from pharmacy passive and active air sampling**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	Sessional, weekly and quarterly  (Passive sampling using 90mm settle plate with 4hr exposure)	<100 cfu	Satisfactory for Grade D	Area considered clean but activity should be restricted to low risk activities and an investigation into the source of contamination considered#  Area can be used for aseptic preparation and filling**  Area can be used for high risk operations*	European Commission, 2022
		<50 cfu	Satisfactory for Grade C		
		<5 cfu	Satisfactory for Grade B		
		No growth	Satisfactory for Grade A		
	Sessional, weekly and quarterly  (Active sampling)	<200 cfu/m <sup>3</sup>	Satisfactory for Grade D	Area considered clean but activity should be restricted to low risk activities and an investigation into the source of contamination considered#  Area can be used for aseptic preparation and filling**  Area can be used for high risk operations*	
		<100 cfu/m <sup>3</sup>	Satisfactory for Grade C		
		<10 cfu/m <sup>3</sup>	Satisfactory for Grade B		
		No growth	Satisfactory for Grade A		

Note: Where counts exceed the specified limits, investigation should be undertaken and action should be taken on the basis of trend analysis and characteristics, significance and source of isolates.

\* Grade A: The local zone for high risk operations, for example filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station.

\*\* Grade B: For aseptic preparation and filling, this is the background environment for the grade A zone.

# Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

**Table 10d. Testing requirements and interpretation of results from broths for process validation of material transfer techniques in specialised tissue labs**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	Sporadic – usually when training new staff	Growth	Unsatisfactory	Investigate cause and put corrective action in place	Beaney, 2016
		No growth	Satisfactory	N/A	

**Table 11. Testing requirements and interpretation of results for operating theatre air quality (as determined by active air sampling)**

Hazard/hygiene indicator	Timing/frequency of testing	Result	Interpretation	Action	References
Aerobic colony count	On commissioning or following any work that may affect the nature of the air supply or its distribution (this does not include routine filter changes); in empty theatre after ventilation system has achieved steady state*	> 10 cfu/m <sup>3</sup>	Unsatisfactory	Do not bring theatre into use. Check that the sampling technique has not led to erroneous results. Ensure with local estates department that airflows and rates are as specified in guidance; ensure that air handling unit is constructed as in guidance with filters to specification and fitted such that air cannot bypass filtration. Repeat sampling. If still unsatisfactory, seek external advice.	Department of Health, 2007
		0 - ≤ 10 cfu/m <sup>3</sup>	Satisfactory	If counts near or above the acceptable level contain a preponderance of fungi, check that the final filter is of adequate grade (F7 or greater) or that air is not bypassing poorly fitted or missing filters.	
	During a surgical operation*	> 180 cfu/m <sup>3</sup> (averaged over 5-minute period)	Unsatisfactory	Investigate and re-test	
	0 - ≤ 180 cfu/m <sup>3</sup>	Satisfactory	N/A		

Note: There are no criteria included for microbiological air sampling in the most recent version of HTM 03-01 (Department of Health, 2021), but the document still states that air sampling may be undertaken where required. Therefore, criteria from the 2007 edition have been retained here for guidance.

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**Table 12. Testing requirements and interpretation of results for bioburden testing of medical instruments**

<b>Hazard/hygiene indicator</b>	<b>Timing/frequency of testing</b>	<b>Result</b>	<b>Interpretation</b>	<b>Action</b>	<b>References</b>
Aerobic colony count	Prior to autoclaving	Monitor trends – acceptable limits to be determined based on previously generated data		Adverse trends should prompt an investigation of the cause, and implementation of corrective actions	International Organisation for Standardisation, 2018



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# Appendix

## Guidance on refrigerated transport of food, water and environmental samples using cool boxes

### Scope

This guidance relates specifically to the use of cool boxes for refrigerated transport of samples. It is the responsibility of the person collecting the samples to ensure that the cool box is clean prior to use and that it has been packed properly.

### Safety considerations

Fully loaded cool boxes present a potential manual handling hazard and it is recommended that those involved in sampling and transport receive manual handling training. Cool boxes must not be over loaded and it is recommended that a maximum full weight of 15kg be observed.

### Cool box requirements

Good quality cool box that has been tested in accordance with EN 12546-2:2000 and has been shown to hold a temperature of between 5°C and 15°C for a minimum of 24 hours.

A minimum of 10% of the total cool box volume of frozen cold packs that have been frozen at –18°C for a minimum of 24 hours, for example 6 × 500ml cold packs (or equivalent) in a 30 litre box. The cold packs must be evenly distributed within the cool box to achieve the necessary cooling of samples.

Use of sample separators is recommended to prevent direct contact of the samples with the frozen cold packs and facilitate air circulation inside the cool box. Alternatively cover the ice packs with a non insulating layer before adding samples. Further advice can be obtained from your laboratory.

### Method for packing a cool box

1. Insert frozen (minimum –18°C for 24 hours) cold packs to cover the base (and, if possible, sides) of the cool box.
2. Ensure that the samples are not in direct contact with the cold packs by placing a separating (non-insulating) layer over the cold packs.
3. Place the samples inside the cool box to allow adequate air circulation between samples. Do not overload the cool box. Place the datalogger (if using) alongside the samples.
4. Place another separating layer over the samples and datalogger, and add the remaining frozen cold packs over the top of this layer.

5. Place sample paperwork (request forms complete with sample details) in a plastic wallet and place inside the cool box.
6. Securely close the cool box.

## Special considerations

Environmental and ready to eat food samples can be transported in the same cool box.

Water samples must be transported in a separate cool box to food and environmental samples.

Hot and cold samples must be transported in separate cool boxes and increasing the volume of cold packs used to 15% should be considered when collecting hot food or water.

Legionella water samples should be transported at ambient temperature and protected from daylight. Frozen cold packs should not be placed in the box.

## Reference

BS EN 12546-2:2000. Materials and articles in contact with foodstuffs. Insulated containers for domestic use. Specification for insulated bags and boxes.

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