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Sampling for *Legionella* bacteria in water systems — Code of practice

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Summary of pages

This document comprises a front cover, an inside front cover, pages I to IV, pages 1 to 42, an inside back cover and a back cover.

Foreword

Publishing information

This British Standard is published by BSI Standards Limited, under licence from The British Standards Institution, and came into effect on 28 February 2022. It was prepared by Subcommittee EH/3/4, *Microbiological methods*, under the authority of Technical Committee EH/3, *Water quality*. A list of organizations represented on these committees can be obtained on request to the Committee Manager.

Supersession

This British Standard supersedes BS 7592:2008, which is withdrawn.

Information about this document

This is a full revision of the standard, and introduces the following principal changes:

a) a clearer distinction is made between routine and incident sampling;

b) a clearer rationale is provided for routine sampling, including newly recognized sources, risk assessment, surveying and sample points;

c) acceptable transport times have been revised to account for more quantitative results and sampling/transport subcontracting; and

d) it takes account of new standards documents for water sampling and analysis.

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Users may substitute any of the recommendations in this British Standard with practices of equivalent or better outcome. Any user claiming compliance with this British Standard is expected to be able to justify any course of action that deviates from its recommendations.

It has been assumed in the preparation of this British Standard that the execution of its provisions will be entrusted to appropriately qualified and experienced people, for whose use it has been produced.

Presentational conventions

The provisions of this standard are presented in roman (i.e. upright) type. Its recommendations are expressed in sentences in which the principal auxiliary verb is "should".

Commentary, explanation and general informative material is presented in smaller italic type, and does not constitute a normative element.

The word "should" is used to express recommendations of this standard. The word "may" is used in the text to express permissibility, e.g. as an alternative to the primary recommendation of the clause. The word "can" is used to express possibility, e.g. a consequence of an action or an event.

Notes and commentaries are provided throughout the text of this standard. Notes give references and additional information that are important but do not form part of the recommendations. Commentaries give background information.

Where words have alternative spellings, the preferred spelling of the Shorter Oxford English Dictionary is used (e.g. "organization" rather than "organisation").

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In particular attention is drawn to the following statutory regulations:

- Health and Safety at Work etc. Act 1974 [1];
- The Management of Health and Safety at Work Regulations 1999 [2];
- Health and Safety at Work (Northern Ireland) Order 1978 [3];
- The Control of Substances Hazardous to Health Regulations 2002 (as amended) [4];
- Control of Substances Hazardous to Health Regulations (Northern Ireland) 2003 [5].

Introduction

Legionnaires' disease was first recognized in July 1976 and the causative bacterium was later isolated and named *Legionella pneumophila*. Since then, more than 60 species of *Legionella* have been described, of which approximately half have been associated with disease in humans. Legionellae are widespread in the natural aquatic environment and can also be found in damp soils and sediments. Those species that infect humans, particularly *Legionella pneumophila*, grow in warm water, generally within the range of 20 °C to 45 °C, although *Legionella* can be detected in water systems operated outside this range because of the inability to consistently maintain these temperatures in all parts of the systems.

To cause infection, legionellae normally need to be inhaled. Particles of between one and three micrometres (1 µm to 3 µm) are small enough to penetrate down to, and be retained in, the deepest part of the lungs (alveoli) but large enough to contain at least one bacterial cell. These particles are too small to be seen by eye and can remain suspended in air for prolonged periods of time. A suspension of such particles in air is termed an aerosol and is not necessarily visible or even wet.

It is a common misconception that a water spray is an aerosol and that legionellae have to be contained within a wet droplet. A mist of water droplets might constitute an aerosol if the droplets are small enough. Water evaporates from small droplets very rapidly. For particles of less than four micrometres (4 µm), the evaporative process usually takes place in less than one second, although the exact rate of evaporation depends on the prevailing temperature, relative humidity and airflow.

If a water droplet contains a single bacterial cell, the droplet rapidly evaporates to a particle size diameter or droplet nucleus of approximately one micrometre (1 µm). A particle of this size can remain suspended in air for prolonged periods of time and travel over considerable distances. These particles are dry and contain no free moisture. Only bound water is present which represents a small percentage of the total mass. When air is inhaled into lungs, approximately 50% of the particles, which are those of approximately one micrometre (1 µm), are retained in the lungs.

Any mechanical action that causes the surface of a liquid (which contains bacteria) to be broken up might cause the production of small droplets containing bacteria suspended in them. If these droplets are small enough, the water might rapidly evaporate leaving the dry droplet nuclei containing the bacteria, so forming an aerosol. Natural aerosols can be generated by rainfall, waterfalls, bubbles rising through water or via wave formation in constructed water systems or environments. Running taps, showers, fountains, humidifiers, spa pools, whirlpool baths and evaporative cooling towers and certain industrial processes can generate aerosols. Infection is also thought to have resulted from aspiration in certain nosocomial cases, either from drinking contaminated water, or ingesting liquid feeds or ice made with contaminated water, or using contaminated water for purposes such as irrigation or washing wounds.

Legionella infection is therefore usually associated with constructed water systems and associated equipment. To date there is only one case associated with possible person-to-person transmission [6]. As a result, the way to prevent or control outbreaks of Legionnaires' disease is to control the introduction into and growth of legionellae in water systems. In the UK, the control of legionellae falls within the general requirements of the Health and Safety at Work etc. Act [1] and associated regulations, including the Control of Substances Hazardous to Health Regulations [4], [5]. Whereas most infections with legionellae are caused by exposure to water and aerosols derived from it, *Legionella longbeachae* infections are predominantly associated with the use of horticultural potting mixtures made from composted organic waste. However, *Legionella longbeachae* has also been isolated from water sources associated with outbreaks, so the possibility of water as a source of infection with *Legionella longbeachae* cannot be ignored [7].

Legionellae are capable of growth within various protozoa, particularly amoebae and, when grown *in vitro,* have been shown to be supported by some bacteria such as flavobacteria. In addition, legionellae can grow in protozoa which graze biofilm and, when grazed by protozoa, can evade the host defence mechanisms and grow rapidly within them. Growth within protozoa, particularly incorporation within their cysts, provides a mechanism for dissemination and the protection of the bacteria from biocides, heat and drying. This enables the legionellae to survive under conditions that would otherwise be fatal and facilitates their transport within both protozoa and their cysts to more favourable environments where they might subsequently grow. Legionellae can survive but not grow within low-nutrient environments, such as sterile water, but can proliferate when supported by other microorganisms. Growth within biofilms provides them with some protection against adverse environmental conditions, particularly biocides, that would otherwise kill them if they were simply suspended within the water column. Thus, control of microbial colonization and biofilm formation within water systems is of paramount importance for the control of legionellae.

The number of reports of Legionnaires' disease continues to rise, probably due to an increased recognition of the disease using improved diagnostic methods but possibly also resulting from a greater exposure to potential sources, rising global temperatures affecting surface and groundwater temperatures and an increased requirement for evaporative cooling. In addition, an increasing proportion of the human population is elderly and/or immunocompromised as a result of new diseases and drug therapy, and therefore more susceptible. To validate and verify that measures to control legionellae are, and remain, effective there is an increasing need to sample potential sources for the presence of legionellae. Sampling is also required when investigating the sources of infection of cases of Legionnaires' disease, to validate new control processes and to verify that disinfection and commissioning processes have been effective and not introduced legionellae.

This document brings together information on likely sources of Legionnaires' disease, the selection of sampling sites and the methods of sampling for the purposes of routine monitoring, validation, commissioning, investigating a problem, or outbreak investigation. It covers some aspects of risk assessment to facilitate the selection of sampling sites and to minimize the risk to those involved in sampling. However, it does not give exhaustive recommendations for carrying out a risk assessment or for the role of sampling within water safety plans as these are covered in BS 8580-1 and BS 8680.

In the UK, outbreaks of Legionnaires' disease commonly have been associated with evaporative cooling towers and condensers; hot and cold-water systems in buildings such as hospitals and hotels; and spa pools and hot tubs, including those on display. The use of nebulizers or other medical respiratory equipment contaminated with legionellae (usually by filling or washing such items with tap water containing the bacteria) has also been reported to cause infection. Other sources implicated in outbreaks globally include natural warm spas and hot springs, indoor fountains, horticultural potting mixtures, ultrasonic misting devices used for humidification in healthcare and domestic premises and to humidify food display cabinets in shops and restaurants, pressure washers, air scrubbers, effluent treatment plants, ice machines in healthcare premises, etc.

Household plumbing systems have also been implicated as sources of Legionnaires' disease. In one UK study [8], legionellae were isolated from approximately 15% of the homes of patients compared with approximately 5% of homes used for control purposes.

Section 1: General

1 Scope

This British Standard gives recommendations and guidance on the sampling of water and related materials for the investigation of the presence of organisms of the genus *Legionella*. It is applicable to sampling constructed water systems but also gives methods for sampling of biofilms and sediments that might be present. Some of the same sampling principles can be applied to natural water systems.

This British Standard is applicable to the selection of sampling sites and the methods of sampling for the purposes of routine monitoring, validation, commissioning, investigating a problem, or outbreak investigation. Recommendations and guidance on the selection of sampling points are given. The rationale for the selection of sampling points for particular situations is also covered.

This British Standard is intended for use by all those involved in water sampling for legionellae, including those collecting samples on site and those developing water sampling plans and/or directing where samples are taken from, irrespective of the method of analysis adopted.

It is not applicable to the sampling of soils and horticultural potting mixtures.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this British Standard, the following terms and definitions apply.

3.1 aerosol

suspension in a gaseous medium of solid particles, liquid particles or solid and liquid particles invisible to the human eye and having negligible falling velocity

3.2 biocide

substance used to disinfect water systems and components

3.3 biofilm

community of bacteria and other microorganisms and entrained debris embedded in a protective layer at interfaces in water systems

3.4 blind end

length of pipe closed at one end through which no water can pass

NOTE Also known as "dead end".

3.5 calorifier

apparatus used for the transfer of heat to water in a vessel by indirect means and incorporating a source of heat

NOTE 1 See also plate heat exchanger (3.27).

NOTE 2 A "storage calorifier" also stores some of the heated water in the same vessel.