Evaluation of detection methods for *Legionella* species using seeded water samples

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Abstract

South African laboratories are currently using various methods in a non-standardised approach to detect *Legionella* species in environmental samples. In an attempt to provide guidelines for the development of a standard method, a number of currently available detection methods were evaluated, using seeded samples of sterile and non-sterile tap water, cooling water and make-up water.

The samples were seeded with a type strain of *L. pneumophila* serogroup 1 (American Type Culture Collection 33152). The effect of sample concentration by centrifugation and membrane filtration followed by either vortex or sonication for resuspension of organisms was studied. Three currently available culture methods were evaluated: the International standard method (ISO/DIS 11731), the Australian standard method (AS 3896 - 1991) and a locally-developed adaptation of the most probable number method (MPN). In addition, the direct immunofluorescence test and a commercially available latex agglutination test kit were included in the evaluations. The usefulness of treatment with acid or heat prior to culture was also compared.

Our results indicated that concentration by membrane filtration using nitro-cellulose filters with a pore size of 0.45 µm, followed by sonication for 10 min, would be the most appropriate concentration and resuspension method for the samples. In the absence of sample pretreatment with acid or heat, organism recovery from sterile seeded samples on BCYE ranged from 85.9 – 98.7%. However, in the non-sterile samples, these figures dropped to 8.1 – 38.5%. Sample pretreatment resulted in a further loss of at least 50% of organisms in all the samples, regardless of the pretreatment method or culture medium used. In general, the ISO and AS methods were more appropriate than the MPN method for organism recovery from sterile seeded samples. However, for the non-sterile samples, the MPN method yielded better recovery.

Introduction

Large numbers of legionellae in water distribution systems present a potentially serious health risk to workers and the public. Since the first isolation of legionellae in 1976, numerous legionellosis outbreaks have been documented and there has been a steady increase in the incidence of sporadic cases (Lye et al., 1997). For example, the two most recently reported outbreaks, one at a flower show in the Netherlands (Den Boer et al., 2000) and the other in an aquarium in Australia (Tallis et al., 2000), resulted in about 246 confirmed Legionnaires' disease cases. This clearly illustrates the importance of the disease and highlights the need for appropriate detection methods.

Despite new developments in the detection of *Legionella* in environmental sources, it remains problematic. Legionellae were initially isolated by the inoculation of guinea pigs, but with the development of suitable media, these expensive and time-consuming techniques were replaced by culturing. Additional methods like radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), agglutination tests and nucleic acid probes have since been developed and tested in attempts to simplify *Legionella* identification. More recently, a number of polymerase chain reaction-based (PCR) assays have been documented (Mahbubani et al., 1990; Bej et al., 1991; Lye et al., 1997). Although some of these methods were proven to be very successful, culturing remains the method of choice for detection of *Legionella* species from the environment. To improve the recovery of *Legionella* by culturing, the use of certain treatment steps to minimise contamination by non-legionellae, have been introduced (Bopp et al., 1981; Groothuis and Veenendal, 1983). However, despite these developments, no one method has thus far proven to be ideal for all samples in all given circumstances and environments.

Standard culture methods for *Legionella* detection have been formulated in the USA, Britain and Australia, but such standards have not been set for South Africa. Local laboratories have been testing water samples using a variety of culture methods, using a non-standardised approach. Some of these methods are time-consuming, require special reagents and culture media and a high degree of technical skill in their application. The apparent preference of *Legionella* for biofilm conditions and the potential role of protozoa in their multiplication and distribution are not considered in these conventional methodologies. This resulted in contradictory results regarding water quality in South Africa and a lack of confidence in local water testing, specifically for the presence of legionellae.

With this in mind, a research project was launched in 1996 to address some of the controversial issues regarding *Legionella* detection in South Africa. The first stage of the project dealt with the evaluation of a number of isolation and identification methods, using water samples seeded with a type strain of L. *pneumophila* (ATCC 33152). These results are reported here. Three currently available identification methods were evaluated: the Draft International Standard (ISO) method (ISO/DIS 11731, 1996), the

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