CRANFIELD UNIVERSITY

BIRGITTA BEDFORD

LEGIONELLA CONTROL IN WATER SYSTEMS USING COPPER AND SILVER ION GENERATION SYSTEMS

INSTITUTE OF BIOSCIENCE AND TECHNOLOGY

PhD. THESIS

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PhD THESIS
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ABSTRACT

Legionella can cause human disease which can be fatal. Routine monitoring for *Legionella* in water systems is not recommended by UK authorities. Evidence of the efficacy of control modalities against *Legionella* in these water systems is, therefore, not available.

Although studies have been conducted with copper and silver ionization on its efficacy against *Legionella* and on its value in reducing hospital-acquired legionellosis, little evidence of its efficacy is available from routine monitoring data.

This study demonstrates the efficacy of copper and silver ionization against *Legionella* in water systems of 10 hospitals from data obtained from routine monitoring for *Legionella*, copper and silver. The inefficiencies of maintaining temperatures above 50ºC at hot outlets and below 20ºC at cold outlets, as recommended by UK authorities for controlling *Legionella* in water systems, is also demonstrated from the data obtained from routine monitoring for *Legionella* and temperatures. The futility of maintaining hot temperatures above 50ºC and then to reduce them to temperatures that do not present a risk of scalding is also demonstrated from the data obtained.

This efficacy of copper and silver ionization and inefficiency of maintaining temperatures at 50ºC against *Legionella* was demonstrated as well in novel model rigs, built to simulate a typical water system of a small hospital, by data obtained from *Legionella*, copper, and silver analysis, and temperature recordings.

The differences in biofilm formation and *Legionella* growth on the surfaces of copper, polyethylene, and synthetic rubber, which are commonly used plumbing materials, were also examined in the model rigs as well as with a Robbins device. These studies indicated that copper is not as biocidal as previously reported in other studies, and gave similar results to polyethylene, which previously been shown to promote biofilm development. Synthetic rubber, however, showed to promote biofilm production and should not be used as a plumbing material.

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NOTATION

~ = Around

< = Smaller than

> = Greater than

± = Plus or minus

ºC = Degrees Celsius

ЛId = Circumference per diameter

µg/l = micrograms per litre

µm = micro metre

µS/cm^2= Micro Siemens per square centimetre

µWs/cm^2 = Microwatt seconds per square centimetre

ACoP = Approved Code of Practice

Ag = Silver atom

Ag^+ = Silver atom that has lost one electron or silver + ion

BCYE = Buffered charcoal yeast extract

BYCE+ = Buffered charcoal yeast extract with cysteine

BSRIA = Building Services Research Information Association Limited

CFU/l = Colony forming units per litre

CFU/ml = Colony forming units per millilitre

Cu = Copper atom

Cu^+ = (Cuprous) Copper atom that has lost one electron or copper + ion

Cu^{2+} = (Cupric) Copper atom that has lost two electrons or copper +2 ion

DNA = Deoxyribonucleic acid

EC = European Council

GVPV = Glycine Vancomycin Polymyxin and Cyclohexamide selective agent

HSE = Health and Safety Executive

HTM = Health Technical Memorandum

ICPMS = Inductively coupled plasma mass spectrometry

ICPOES = Inductively coupled plasma optical emission spectroscopy

M^2 = Square metre

Mg/l = Milligrams per litre

Ml = Millilitre

Mm^2 = square millimetre

NOAEL = No observed adverse effect level

Nm = Nanometre

O_3 = Ozone

pH = Potential hydrogen

RNA = Ribonucleic acid

TMV = Thermostatically controlled mixing valve

TVC = Total viable count of bacteria

UK = United Kingdom

UKAS = United Kingdom accreditation service

US = United States of America

UV = Ultraviolet

WHO = World Health Organisation

GLOSSARY

Alveolar macrophage = A white blood cell that is specialized for the uptake of particulate material by phagocytosis which is found in the lung.

Amoeba = Free-living, single-celled eukaryote that crawls by changing its shape. A particular genus of protozoa that move in this way.

Analyte = A substance or chemical constituent that is determined in an analytical procedure.

Antibody = Protein produced in response to a foreign molecule or invading oragnsism. Often binds to the foreign molecule or cell extremely tightly, thereby inactivating it or marking it for destruction by phagocytosis or lysis.

Antigen = Molecule that provokes an immune response.

Aspiration = The entry of secretions or foreign material into the trachea and lungs.

Atomization = separating something into fine particles.

ATP = Adenosine triphosphate = The principal carrier of chemical energy in cells.

Bioaccumulation = Accumulation of substances, such as pesticides, or other organic chemicals in an organism.

Biodispersant = A liquid or gas used to disperse organic material in a medium.

Biomagnification = Also known as bioamplification or biological magnification, is the increase in concentration of a substance that occurs in a food chain.

Carbon footprint = The amount of carbon dioxide emitted due to the consumption of fossil fuels.

Catabolism = The breakdown of complex molecules in living organisms to form simpler ones, together with the release of energy; destructive metabolism.

Cell lysis = refers to the breaking down of a cell.

Cellulitis = Inflammation of subcutaneous connective tissue.

Cerebellar ataxia = Failure of muscular coordination; irregularity of muscular action caused by a region in the brain that has suffered damage.

Chemostat = A bioreactor to which fresh medium is continuously added, while culture liquid is continuously removed to keep the culture volume constant

Cyanobacteria = A division of micro-organisms (class Cyanophyceae, kingdom Eubacteria) that are related to the bacteria but are capable of photosynthesis. They are prokaryotic and represent the earliest known form of life on the earth. Also called blue-green algae.

Cysteine = A sulfur-containing amino acid, HSCH2CH(NH2)COOH, that occurs in proteins and is a constituent of many enzymes.

Deadend = A capped off end of a pipe.

Deadleg = A length of pipe ending at a fitting through which water flows only when the fitting is opened.

Desolvation = The removal of solvent from a material in solution.

Diabetes mellitus = The commonest form of diabetes, caused by a deficiency of the pancreatic hormone insulin.

Dissolved Oxygen = The amount of oxygen dissolved in water. Important to aquatic ecology, often determines the number and types of organisms living in the water.

DNA = Deoxyribonucleic acid. Polynucleotide formed from covalently linked deoxyribonucleotide units; serves as the carrier of genetic information.

DNA degradation = The random catabolism of DNA accompanying the irreversible damage to tissue which leads to the pathological death of one or more cells.

Endemic = A disease (or condition) regularly found among particular people or in a certain area.

Endocarditis = Inflamation of the the inner lining of the heart muscle, which also covers the heart valves.

Etiologic agent = A viable microorganism, or its toxin, which causes or may cause human disease.

Eukaryote = Living organism composed of one or more cells with a distinct nucleus and cytoplasm. Includes all forms of life except viruses and bacteria (procaryotes).

Exopolymer = A biopolymer that is secreted by an organism into the environment (i.e. external to the organism). These exopolymers include the biofilms produced by bacteria to anchor them and protect them from environmental conditions.

Fatty acid = Compound used as a major source of energy during the metabolism of cells and as a starting point for the synthesis of phospholipids, which are molecules used to construct biological membranes.

Febrile illness = A nonspecific term for an illness of sudden onset accompanied by fever.

Flagella = tail-like projection that protrudes from the cell body of certain bacteria and plays the dual role of locomotion and sense organ, being sensitive to chemicals and temperatures outside the cell.

Genetically homologous = sharing a common ancestor.

Genus = A well deifined group of one or more species that is clearly separate from other genera.

Gram–negative bacteria = Bacteria that do not retain crystal violet dye in the Gram staining protocol.

Gram-staining = A differential staining procedure that divides bacteria into Gram-positive and Gram-negative groups based on their ability to retain crystal violet when decolorized with an organic solvent such as ethanol.

Hemoptysis = The expectoration of blood or of blood-streaked sputum from the larynx, trachea, bronchi, or lungs.

Heterotrophicorganism = An organism that cannot synthesize its own food and is dependent on complex organic substances for nutrition.

Heterotrophic plate count = The reference procedure for estimating the number of live, heterotrophic bacteria in water.

Hydrophillic = Having a tendency to mix with, dissolve in, or be wetted by water.

Hydrophobic = Tending to repel or fail to mix with water.

Inductively Coupled Plasma = (ICP) A type of plasma source in which the energy is supplied by electric currents which are produced by electromagnetic induction. Plasma temperatures are comparable to the surface of the sun. ICP discharges are of relatively high electron density. As a result, the discharges are used to measure the emmission spectrum of chemical elements or chemical compounds.

Inhalation = (also known as inspiration) The movement of air from the external environment, through the air ways, and into the lungs.

In-vitro = Taking place in a test tube, culture dish, or elsewhere outside a living organism.

In-vivo = Taking place in real life.

Isotonic = Having the same concentration of solutes as the blood.

Lymphadenopathy = A disease affecting the lymph nodes.

Malaise = A general feeling of discomfort, illness, or uneasiness.

Macrophage = A white blood cell that is specialized for the uptake of particulate material by phagocytosis.

Membrane-bound enzyme = Enzyme/catalytic protein that is embeded in the membrane of the enclosed organelle of the cell it belongs to.

Metabolism = The sum total of the chemical processes that take place in living cells.

Morphology = The form and structure of an organism or one of its parts.

Mutagenic = Refers to a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.

Myalgia = Pain in a muscle or group of muscles.

Myocarditis = Infection of the heart, with an inflammatory infiltrate, and damage to the heart muscle.

Nosocomial = Originating in a hospital.

Phagocytosis = Process by which particulate material is 'eaten' by a cell.

Pericarditis = An inflammation of the two layers of the thin, sac-like membrane that surrounds the heart.

Peritonitis = Inflammation of the membrane which lines the inside of the abdomen and all of the internal organs.

pH = Measure of the acidity of a solution. 'p' refers to power of 10, 'H' to hydrogen

Phagosome = A vacuole formed around a particle absorbed by phagocytosis.

Pili = Short, filamentous projections on a bacterial cell, used not for motility but for adhering to other bacterial cell (especially for mating) or to animal cells.

Planktonic organisms = The collection of small or microscopic organisms float or drift.

Postcardiotomy syndrome = Illnes believed to be caused by an autoimmune response against damaged cardiac tissue.

Protozoa = Free-living, single-celled, motile eukaryotic organisms, especially those, such as Amoeba, that live by feeding on other organisms.

Purulent sputum = Mucus that is coughed up from the lower airways, which is off-white, yellow or green, and opaque. It indicates the presence of large numbers of white blood cells.

Organelle = A specialized subunit within a cell that has a specific function.

Prokaryotic cells = Cells that lack a membrane-bound nucleus (from the Greek meaning before nuclei). These cells have few internal structures that are distinguishable under a microscope. Cells in the monera kingdom such as bacteria and cyanobacteria (also known as blue-green algae) are prokaryotes.

Pyelonephritis = Inflammation of the substance of the kidney as a result of bacterial infection.

Quorum sensing = a type of decision-making process used by decentralized groups to coordinate behavior. Many species of bacteria use quorum sensing to coordinate their gene expression according to the local density of their population.

Respiratory epithelial cell = Cells arranged in one or more layers, forming part of a covering or lining of a body surface that take up O_2 and make CO_2.

Respiratory chain = couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O_2) with the transfer of H^+ ions (protons) across a membrane.

Respiratory metabolism = Generating energy by enzyme-mediated electron transport from an electron donor to an external electron acceptor

Ribosome = Particle composed of ribosomal RNAs and ribosomal proteins that associated with messenger RNA and catalyzes the synthesis of protein.

Ringers solution = An aqueous solution of the chlorides of sodium, potassium, and calcium that is isotonic to animal tissue.

RNA = Ribonucleic acid. Polymer formed from covalently linked ribonucleotide monomers.

Septicaemia = Blood poisoning: Invasion of the bloodstream by virulent microorganisms from a focus of infection.

Serogroup = refers to distinct variations within a subspecies of bacteria, classified together based on their cell surface antigens.

Sessile organisms = Organisms that are fixed in one place.

Silastic = Polymeric silicone substances that have the properties of rubber but are biologically inert; used in surgical prostheses.

Sinusitis = Inflammation of a nasal sinus.

Species = Bacterial species are collections of strains that have many stable properties in common and differ significantly from other groups of strains.

Stupor = State of near-unconsciousness or insensibility.

Taxonomy = The practice and science of classification or the result of it.

Teratogenicity = Relating to, or causing malformations of an embryo or foetus.

Thiol group = (sulfhydryl) = Chemical group containing sulphur and hydrogen found in the amino acid cysteine and other molecules.

Total Viable Count = (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mold in a sample. To be specific, the

count actually represents the number of colony forming units (cfu) per ml of the sample.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Legionnaires' disease was first described in the 1970s. Aerobic Gram-negative bacteria, isolated from infected post mortem lung tissue of victims of the unexplained pneumonia outbreak at the 1976 American Legion Convention in Philadelphia were identified as the causative agent. 221 of those attending the Convention became ill with pneumonia. 34 of those affected died (Brenner, 1987). The bacterium was named *Legionella pneumophila,* receiving the name *Legionella* to honour the stricken American legionnaires and *pneumophila* from the Greek word meaning 'lung loving'. The pneumonia contracted was named Legionnaires' disease (Fang *et al.,* 1989).

Since this outbreak, *Legionella pneumophila* have been identified as the causative agents of more outbreaks and cases of disease in humans. Some of these cases have been fatal, especially, in immuno-compromised people, such as patients in hospitals. Most national laws enforce preventing contamination by *Legionella* of anthropogenic water systems (UK Health and Safety at Work Act, UK Control of Substances Hazardous to Health Regulations). Infestation by *Legionella* of water systems in hospitals is, however, common due to complex system layout, poor water use and application of ineffective control methods. Controlling *Legionella* can be difficult because the bacteria survive extreme ranges of conditions and grow and are protected in biofilms and in protozoan cells.

Results of *in-vitro* and *in-vivo* studies demonstrated that *Legionella* was inactivated and that cases of Legionnaires' disease were reduced by the implementation of copper and silver ionization systems (Landeen *et al.,* 1989, Liu *et al.,* 1994, Lin *et al*., 1996, BSRIA TN6/96, Miuetzner *et al.,* 1997, Liu *et al.,* 1998, Stout *et al.,* 1998, Biurrun *et al.,* 1999, Kusnetsov *et al.,* 2001, Stout and Yu, 2003, Chen *et al.,* 2008). These studies were mainly conducted in hospitals in the US. Little data is, however, available on its efficacy in hospitals in the UK, and little is known on its influence on biofilms and on the inactivation of *Legionella* multiplying in biofilms.

This study, therefore, examines the effectiveness of copper and silver ionization against *Legionella* in the water systems of hospitals in the UK. The efficacy of

controlling *Legionella* by applying elevated temperatures, which is generally employed in the UK, compared to copper and silver ionization is also assessed, and the growth of biofilms on plumbing materials, commonly found in UK water distribution systems, is also looked at.

1.2 Microbiology and Taxonomy

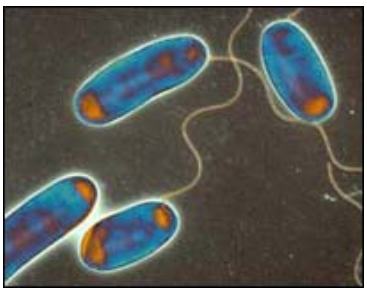


Plate 1.1 *Legionella* bacteria (www.bbc.co.uk)

Legionella appear as small rods, faintly staining Gram-negative. They are unencapsulated and non-spore forming with an average size of 0.5-1.0 µm wide and 1.0-3.0 µm long (Barbaree, 1991). Most species exhibit motility through one or more polar or lateral flagella. *Legionella* cell walls are unique from other Gram-negative bacteria in that they contain significant amounts of branched-chain cellular fatty acids. *Legionella* require low levels of oxygen for growth (Brenner *et al.,* 1985).

Following the initial identification of *L. pneumophila* after the 1976 outbreak, numerous species have been discovered within the *Legionella* genus. Currently, the genus consists of at least 50 species, nine of which can be further divided into serogroups that are genetically homologous but can be differentiated by specific reactivity to antibodies. Currently, 70 serogroups are recognised in the genus, including 16 among *L. pneumophila* (World Health Organisation, 2007).

1.3 Transmission

Legionella exist naturally in water and moist soil. Transmission to humans normally occurs via inhalation or aspiration of droplets of water containing *Legionella* (aerosols). Person-to person transmission does not occur (Dennis and Lee, 1988, Fitzgeorge *et al*., 1993, Fields, 1996, Stout and Yu 1997, Belyi, 1999). Wound infection may be caused by direct entry of *Legionella* into damaged skin, and has been observed after immersion of a wound in contaminated water (Brabender *et al.,* 1983, Lowry *et al.,* 1991).

Transmission hazards have been associated with water distribution systems, cooling towers, humidifiers, whirlpool spas, car wash systems, vegetable water sprayers, and windscreen wiper water. As reviewed by Rathore and Alvarez, (2009), transmission hazards have also been associated with respiratory therapy equipment, nasogastro tubes, dental unit water lines and potting compost (Breiman *et al.,* 1990, Kool *et al.,* 1998, Seenivasan *et al.,* 2005, Kumar *et al.,* 2010, Pravinkumar *et al.,* 2010, www.hcinfo.com, www.nhs.uk).

Disease is mainly caused by the transmitted *Legionella* residing within phagosomes of alveolar macrophages, where they multiply intracellularly until the cell ruptures. Liberated *Legionella* then infect other macrophages (Microbiology Third Edition, 1996). Immuno-compromised people are at increased risk of contracting disease from *Legionella* because their cell-mediated immunity against *Legionella* is impaired (Greenberg *et al.,* 2006).

1.4 Legionellosis

The disease caused by *Legionella* is called legionellosis. The severity of legionellosis ranges from typical Legionnaires' disease, an acute form of fulminating pneumonia with low attack rate and relatively high fatality rate (a low attack rate means that a small proportion, less than 5%, of those exposed to the bacteria develop the disease), to Pontiac fever, a mild infection with a high attack rate (a high proportion, usually over 80%, of those exposed to the bacteria develop the disease) (Newsome, 2001).

The majority of legionelloses (70-90%) have been caused by *L. pneumophila*. Virulence varies between strains of *L. pneumophila*, for instance some strains could adhere to the respiratory epithelial cells via pili, whereas strains with a mutated gene that encodes for the pili showed reduced adherence *in-vitro* (Stone and Kwaik, 1998).

Legionella pneumophila serogroups 1 (predominant), 6 and 13 are currently considered to be the main causative agent of legionellosis. (Lo Presti *et al.,* 1997, Fields *et al*., 2002, Yu *et al.,* 2002, Faris *et al.,* 2005).

A minority of legionelloses are due to other *Legionella* species, most commonly *L. bozemanii, L. cincinnatiensis, L. dumoffii, L. longbaechae* and *L. micadadei* (Stout and Yu, 1997, Spieker *et al*., 1998, Diederen *et al*., 2005). *L. micadadei and L. dumoffii* are the second and third most common species to cause Legionnaires' disease in children, respectively (Greenberg *et al.,* 2006).

Recognized risk factors for contracting legionellosis include, cigarette smoking, lung disease, immuno-suppression, end-stage renal disease, diabetes mellitus, and advanced and very young age (Rathore and Alvarez, 2009).

Pneumonia is the predominant clinical manifestation of Legionnaires' disease. After an incubation period of 2 to 10 days, patients typically develop fever, weakness, fatigue, malaise, myalgia, chills. Respiratory symptoms may not be present initially but develop as the disease progresses. Almost all patients develop a cough, which is initially dry and non-productive, but may become productive, with purulent sputum and, in rare cases, haemoptysis. Patients may experience chest pain. Neurologic complaints may include headache, lethargy, confusion, cerebellar ataxia, agitation, stupor. Other symptoms include diarrhoea, nausea, vomiting, and abdominal pain

Legionnaires' disease can manifest as septicaemia which is often diagnosed at autopsy. Extra pulmonary legionellosis is rare. The most common site of extra pulmonary infection in adults is the heart. Manifestations of extra pulmonary legionellosis may also include sinusitis, cellulitis, peritonitis, pyelonephritis, pancreatitis, wound infection, lymphadenopathy, prosthetic valve endocarditis, myocarditis, pericarditis, postcardiotomy syndrome (Sopena *et al.,* 1998, and Rathore and Alvarez, 2009).

Middle-aged and older adults have a higher risk of developing Legionnaires' disease than do young adults and children. Among children, more than one third of reported cases have occurred in infants younger than 1 year (Rathore and Alvarez, 2009).

The mortality rate in patients with Legionnaires' disease is 5-80%, depending on certain risk factors. These factors associated with high mortality rates include age (especially those younger than 1 year and elderly patients), predisposing underlying conditions such as chronic lung disease, immunodeficiency, malignancies, end-stage renal disease, diabetes mellitus, nosocomial acquisition, and delayed initiation of specific antimicrobial therapy.

Previous to the Legionnaires' disease outbreak in 1976, in July 1968 an explosive epidemic of acute febrile illness occurred at a county health department facility in Pontiac, Michigan. Illness characterized principally by fever, headache, myalgia, and malaise affected at least 144 persons, including 95 of 100 persons employed in the health department building. The mean incubation period was 36 hours. Illness was self-limited, generally lasting from two to five days. Secondary cases did not occur in family contacts. A defective air-conditioning system was implicated as the source and mechanism of spread of the causative factor. However, extensive laboratory and environmental investigations failed to identify the etiologic agent. Since these investigations a bacterium identical to the bacterium responsible for Legionnaires' disease was isolated from guinea pigs exposed to the Pontiac health department building in 1968 as well as from guinea pigs exposed to water from the evaporative condenser (Glick *et al.,* 1977). The illness, named after the town 'Pontiac', is an influenza-like illness, typically with an abrupt onset. The incubation period is 24 to 48 hours. Prominent symptoms include fever, malaise, myalgia, cough, and headache. Pontiac fever tends to occur in outbreaks and the infection rate is greater than 90%.

The disease is self-limiting, persisting for approximately 1 week (Rathore and Alvarez, 2009).

Legionellosis is a growing problem not only because *Legionella* are ubiquitous and are present in natural and anthropogenic water systems (Borella *et al.,* 2004), but also because the number of reported infections is on the increase (Montagne *et al.,* 2006).

1.5 Reported cases and outbreaks of Legionnaires' disease

Legionnaires' disease requires to be reported to most national health protection authorities.

Clinical presentation of Legionnaires' disease is however non-specific and many Legionnaires' disease cases are not diagnosed. Many are also not reported. The frequency of Legionnaires' disease is, therefore, underestimated. For instance, although approximately 1000 cases are reported annually to the Center for Disease Control and Prevention (CDC), Atlanta, USA, it has been estimated that over 25000 cases of the illness actually occur (Stout *et al.*, 1982, Marston *et al.,* 1994, World Health Organization 2007, Newsome, 2001).

Between 1995 and 2005 over 32,000 cases of Legionnaires' disease and more than 600 outbreaks were reported to the European Working Group for Legionella Infections (EWGLI). 35 Countries participating in EWGLI reported in the period of 2005 to 2006 a total of 11980 cases, showing a continued increase in reported cases compared with earlier years, see Table 1.5 below. 377 cases of these 11980 reported cases were fatal, giving a case fatality of 6.6%. (EWGLI data – Legionnaires' disease in Europe).

Year	Cases	No. of countries contributing data	Population (millions)	Incidence rate per million population
1993	1242	19	300	4.1
1994	1161	20	346	3.4
1995	1255	24	339	3.7
1996	1563	24	350	4.5
1997	1360	24	351	3.9
1998	1442	28	333	4.3
1999	2136	28	398	5.4
2000	2156	28	400	5.4
2001	3470	29	455	7.6
2002	4696	32	467	10.1
2003	4578	34	468	9.8
2004	4588	35	550	8.3
2005	5700	35	551	10.3
2006	6280	35	563	11.2

Table 1.1 - Total number of reported cases of Legionnaires' disease and incidence rate per million population 1993 -2006 (EWGLI data – Legionnaires' disease in Europe).

The total confirmed cases of Legionnaires' disease in England and Wales reported to the UK Health Protection Agency (HPA) from 1980 to 2009 was 6750 of which 837 were fatal, giving a fatality rate of 12.4% (www.hpa.org.uk).

Reported Legionnaires' disease cases are categorized as either acquired from sources in the community or from sources in healthcare facilities, hospital-acquired, or cases that are associated with travel.

1.5.1 Community acquired Legionnaires' disease

The largest outbreak of community acquired Legionnaires' disease in the world reported to date was in 2001 in Murcia, Spain. More than 800 suspected cases were reported; 449 of these cases were confirmed. Fatality of the reported cases was 1%. (Garcia-Fulgueiras *et al*., 2003).

An outbreak of legionellosis among visitors and participants at a flower show in the Netherlands in 1999 caused 31 deaths and more than 200 cases of the disease (Den Boer *et al.,* 2002).

The total confirmed community acquired cases of Legionnaires' disease in England and Wales reported to the HPA from 1980 to 2009 was 3418 (www.hpa.org.uk).

Britain's worst outbreak of community acquired Legionnaires' disease was in July 2002 when 131 cases were confirmed in Barrow-in-Furness, Cumbria, 4 of these patients died. A further 330 people received hospital treatment for Legionnaires' disease-like symptoms (www.hcinfo.com).

1.5.2 Hospital-acquired Legionnaires' disease

It is envisaged that hospital-acquired Legionnaires' disease cases account for a substantial proportion of cases. Mortality of hospital cases is also higher than that of community acquired Legionnaires' disease cases due to increased mortality risk factors in hospitals (Marston *et al.,* 1994).

The death rate for patients who develop Legionnaires' disease while in the hospital is close to 50%, especially when antibiotics are started late (U.S. National Institute of Health and National Library of Medicine Services).

In hospitals with *Legionella* colonization of the water systems, hospital-acquired legionellosis is frequently endemic, accounting for 10% to 40% of hospital-acquired pneumonias (Muder *et al.,* 1983, Marrie *et al.,* 1991).

The total reported hospital-acquired cases of Legionnaires' disease in England and Wales from 1980 to 2009 was however only 239, with 99 cases of these as suspected cases of Legionnaires' disease (www.hpa.org.uk). This suggests that many of the hospital-acquired cases are either not diagnosed or not reported.

In Britain the first reported outbreak of Legionnaires' disease was hospital-acquired at the Stafford District General Hospital in 1984 and 1985. A total of 68 confirmed cases were treated in hospital and 22 of these patients died. A further 35 patients, 14 of

whom were treated at home, were suspected cases of Legionnaires' disease (O'Mahony *et al.,* 1990).

1.5.3 Travel associated Legionnaires' disease

In 2008, the European Surveillance Scheme for Travel Associated Legionnaires' disease (EWGLINET) received reports of 866 cases of travel-associated Legionnaires' disease, which was slightly below the number of cases reported in 2007. 42 of these cases were fatal. Prior to 2007 there had been a steady increase in the number of cases reported to the scheme since its inception in 1987. This was due in part to improved national surveillance and to an increasing number of countries joining the scheme. There is, however, significant under-ascertainment of Legionnaires' disease within Europe. Especially among the newer Member States of the European Union which include countries where surveillance for Legionnaires' disease is less well developed. Therefore, there is potential for case numbers to increase (Ricketts *et al.,* 2010).

The total reported travel-associated cases of Legionnaires' disease in England and Wales from 1980 to 2009 was 3343. 2880 of these cases were associated with travel outside the UK and 463 cases were associated with travel within the UK (www.hpa.org.uk).

1.5.4 Legionnaires' disease in long-term care residents

Pneumonia is the leading cause of death and an important cause of transfer to acute care facilities in long-term care residents (Muder, 1998).

There are a number of epidemiological factors that suggest that long-term care residents might be at particular risk for legionellosis should it be present in the environment. Long-term care residents often have swallowing difficulties, and many receive nasogastric tube feedings, predisposing factors to aspiration which is the primary mode of transmission (Seenivasan *et al.,* 2005).

1.6 *Legionella* sources

Ubiquitously found in nature, *Legionella* exist primarily in environmental waters, although some have been isolated from potting soils and moist soil samples (Fields 1996).

Subsequent to the 1976 outbreak, *Legionella* species were discovered and isolated in lakes, rivers, and naturally warm water such as hot springs in North America (Tison *et al.,* 1983, Campbell *et al.,* 1984) and in Europe (Bornstein *et al.,* 1989). It was noted that significantly more were isolated from waters whose temperatures lay between 36°C and 70°C. In Europe, they have also been isolated from both surface and ground waters (Newsome, 2001).

Legionella are present in all phases of sewage treatment, and population numbers do not decline significantly through the treatment process (Palmer *et al.,* 1993). States *et al.,* 1989 showed the potential for *Legionella* growth within municipal systems, which supports the hypothesis that public water supplies may contaminate the plumbing systems of hospitals and other large buildings (States *et al.,* 1989).

Legionella species have been found in these water systems as well as in drinking water at temperatures below 15ºC (Wullings and van der Kooij, 2006). Rusin *et al.,* (1997) suggested that drinking water could be a significant source of infection with *Legionella* (Rusin *et al.,* 1997). Restrictive values for *Legionella* in drinking water supplies are however not set as *Legionella* are considered pathogenic only when inhaled and not when consumed (UK Water Supply (Water Quality) Regulations 2000).

1.7 Factors that influence *Legionella* growth

Various factors that influence and promote *Legionella* growth have been identified since its initial identification back in 1976.

1.7.1 Physio-chemical factors

Legionella can survive extreme ranges of environmental conditions. It can survive temperatures of 0ºC to 63ºC and has been found in waters at temperatures between 5ºC and 63ºC but significantly more *Legionella* has been isolated from warm waters at temperatures of 30ºC and above. *Legionella* can also survive a wide pH range of 5.0-8.5 and a dissolved oxygen concentration in water of 0.2 to 15 mg/l. *L. pneumophila* grows best at a pH of 5.5 to 6.2 with an oxygen concentration of 6.0 to 6.2 mg/l. However, *Legionella* species were found in an extremely acidic, predominantly eukaryotic algal biofilm community in Yellowstone National Park, US, at a pH as low as 2.7. Small concentrations of salt (NaCl 0.1 to 0.5%) may also enhance the survival of *Legionella* and it has been found in cooling systems using seawater (Fliermans *et al.,* 1981, Nguyen *et al.,*. 1991, Lee and West, 1991, Newsome, 2001, Sheenan *et al.,* 2005).

Metal plumbing components and associated corrosion products are also important factors as these can provide iron and other metals that support the survival and growth of *Legionella* in plumbing systems (States *et al.,* 1985).

1.7.2 Microbial factors

The growth and survival of *Legionella* is affected by the presence of other organisms. Studies have demonstrated that *Legionella* were incapable of growing in sterile water because it required nutrients supplied by other micro-organisms, including bacteria (Wadowsky and Yee, 1983), protozoa (Rowbottom, 1980), and *cyano*bacteria (Tison *et al.,* 1980).

Protozoa play an important role in the increase and spread of *Legionella* in anthropogenic water systems and therefore in the occurrence of Legionnaires' disease (DeClerck *et al.,* 2009*). Legionella* can infect and grow, similar as with macrophages,

inside protozoa to such an extent that the protozoan bursts releasing live *Legionella* into water systems (Wadowsky *et al.,* 1991, Nahapetian *et al.,* 1991, Cirillo *et al.,* 1994, Kuiper *et al.,* 2004, Berk *et al.,* 2005).

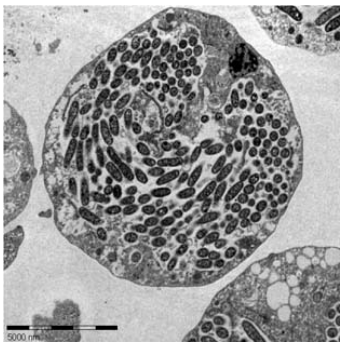


Plate 1.2 *Hartmannella vermiformis* amoeba filled with *L. pneumophila*. (Holland/Özel, Robert Koch Institut)

Kuiper *et al.,* (2004) demonstrated the intracellular growth of *L. pneumophila* in amoebae, with 25.9% (± 10.5%) of the amoeba, *Hartmannella vermiformis*, containing *L. pneumophila* on day 10 and more than 90% containing *L. pneumophila* on day 14.

Hartmannella vermiformis containing *Legionella* were also isolated from hospital hot water systems. The amoeba entered these systems through the mains water distribution, open tanks and during maintenance (Rowbottom, 1986, Kilvington and Price, 1990, Barker *et al.,* 1992, Abu Kwaik *et al.,* 1997, Rohr *et al.,* 1998). The protozoan *Tetrahymena pyriformis* containing *L. pneumophila* was found in tap water at 35ºC (Fields *et al.,* 1984), and *Legionella* were also observed multiplying within *Acanthamoeba* species isolated from drinking water (Michel *et al,* 1998). *Tetrahymena pyriformis* was also found to be a habitat for *L. longbeachae* found in potting soil mixes (Steele and McLennan, 1996).

Protozoa provide not only nutrients for *Legionella* to grow but also protect *Legionella* against adverse extracellular or environmental conditions such as high temperatures, drying, chlorine and other biocides. With 1mg/l of chlorine, various bacteria including *Legionella*, when contained within *Tetrahymena* and *Acanthamoeba,* required 60 to 200 times more contact time to kill 99% of the bacterial cells than if they were freely suspended in water (Newsome, 2001), and *L. pneumophila* was recovered from *Acanthamoeba* after exposure to 50mg/l of free chlorine for 19 hours (Kilvington and Price, 1990).

Cirillo *et al.,* (1994) showed that growth in protozoa also enhanced the ability of *L. pneumophila* to enter human cells, and Brieland *et al.,* (1997) found that parasitic *L. pneumophila* were more pathogenic than free living *L. pneumophila*.

Micro-organisms, including *Legionella,* become attached to surfaces submerged in water and form layers of microbial cells, called biofilms. Biofilms play an important role in the spread of *Legionella* as they also provide nutrients for their growth and a protective environment enabling them to survive water treatment processes (Kuchta *et al.,* 1993, Lin *et al.,* 1998, Barbeau *et al.,* 1998, Atlas, 1999, Kuiper *et al.,* 2004, Newsome, 2001).

A study carried out in 1994 showed that *Legionella* were more easily detected from swab samples of biofilm than from flowing water, which suggested that the majority of *Legionella* were essentially biofilm associated (Rogers *et al.,* 1994).

Legionella cells persisting in biofilms (sessile *Legionella*) are also much more resistant to disinfectants than the planktonic, free-living, cells (Cargill *et al.,* 1992, Stewart and Costerton, 2001, Tachikawa *et al.,* 2005).

1.8 Biofilms

Biofilms are widespread in nature and can be found on the surfaces of pipes in any anthropogenic water system. As well as through attachment to surfaces, biofilms may also form suspended in water, also referred to as 'floating biofilms' (Declerck *et al.,* 2007a).

Biofilm formation occurs as a result of the following events:

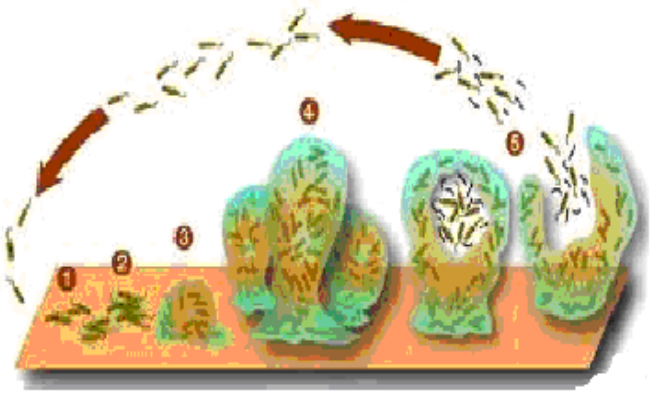


Plate1.3 Biofilm formation. Graphic by Peg Dirckx and David Davies. 2003 Center for Biofilm Engineering Montana State University.

1. Microbial surface attachment.
2. Irreversible attachment
3. Maturation stage I
4. Maturation stage II
5. Dispersion

Microbial attachment is the first step in the colonization of surfaces (1) and precedes the process of consolidation, during which the initially weak adhesive forces are strengthened by exopolymer formation (2). When irreversibly attached, the micro-organisms continue to multiply forming micro-colonies or stacks (3 and 4). Portions of these stacks can shear off and colonize other parts of the system (5) (Denyer *et al.,* 1993).

1.8.1 Factors supporting biofilm formation

Water flowing around biofilms carries nutrients, supporting growth and re-growth, and aiding colonization downstream (Le Chevalier *et al.,* 1988, Morton *et al.,* 1998, Newsome, 2001, Murga *et al.,* 2001).

Biofilms are more likely to form where there are areas of low water flow and where water is allowed to stagnate. Biofilms are, therefore, found in anthropogenic water systems in dead-end pipes, which are closed at one end through which no water passes, and dead-leg pipes, through which water only passes when there is draw-off from the fitting (Donlan, 2002).

Liu *et al.,* (2006), found, however, the lowest biofilm-associated *Legionella* counts with a stagnant flow and the highest counts with a turbulent flow. This could be explained by the fact that turbulent flow results in a higher overall mass transfer rate compared with laminar flow. Mass transfer can be described as the efficiency of suspended solids (nutrient) delivery from the bulk phase (flowing water) to the attached phase (biofilm). A higher mass transfer rate would result in greater particle deposition onto the pipe surface. It could be possible that turbulent flow increases oxygen and nutrient availability at the attachment surface, which in turn may lead to an increase in *Legionella* under turbulent conditions. The lowest concentration of *Legionella* recovered from stagnant flow pipes could be explained by the limited availability of oxygen and nutrients under these conditions (Lui *et al.,* 2006).

Temperature also is an important factor in the formation of biofilms. Villanueva *et al.,* (2010) found that although biofilms developed at temperatures as low as 11ºC, formation was faster when the temperatures were higher (Villanueva *et al.,* 2010).

A study by Else *et al.,* (2003) found that when the temperature was held at 30°C there was an increase in values of heterotrophic plate counts per coupon of the materials tested, which were stainless steel, nickel and titanium, but that when the temperature was held at 60°C and 70°C, the counts decreased for all three coupon types to undetectable levels (Else *et al.,* 2003).

Materials such as synthetic rubber (ethylene-propylene rubber), polyethylene and copper are commonly used in water distribution systems. These materials can encourage or resist biofilm formation.

Studies have demonstrated that synthetic rubber encourages biofilm formation (Schofield and Locci, 1985, Keevil *et al.,* 1993, Rogers *et al.,* 1994, BSRIA Technical Note TN 9/96). The persistence of *L. pneumophila* in water systems was also attributed to their survival within biofilms on rubber materials in taps and showers

(Colburne *et al.,* 1984b, Schofield and Locci 1985, Memish *et al.,* 1992). Rubber grommets within a cooling tower probably provided a nidus of infection which caused Legionnaires' disease in 16 patients of the Glasgow Royal Infirmary. (Timbury *et al.,* 1986). Shock absorbers, installed within water lines to absorb pipe vibration, usually consist of an inflatable balloon that is made of synthetic rubber and that is in constant contact with water (Timbury *et al.,* 1986). Memish *et al.,* (1992) found that these absorbers were a reservoir for *Legionella* in one hospital, due to *Legionella* sloughing off from biofims that had formed on the rubber surfaces of the absorbers (Memish *et al.,* 1992).

Biofilms have been found on the surfaces of synthetic rubber lined flexible hoses, which are often used in water systems to connect pipes to taps. Investigations into the occurrence of *Legionella* in hospitals have shown that some rubber lined flexible hoses were heavily infected with biofilm which included *Legionella* (Water Regulations Advisory Scheme, January 2006, EPDM (Ethylene Propylene Diene Monomer (M-class) Rubber Flexible Hoses). A 2010 alert by the UK Department of Health highlighted the removal of rubber lined flexible hoses due to the risk of colonization that they present (Estates and Facilities Alert Department of Health Ref. DH (2010) 03. 05/05/10).

Polyethylene also seems to encourage biofilm formation. Biofilm formation was found to be higher on polyethylene pipes than on copper pipes by Van der Kooij, *et al.,* (2005).

Copper seems to resist biofilm formation because copper is naturally biocidal (Schofield and Locci, 1985, Keevil *et al.,* 1993, Rogers *et al.,* 1994, BSRIA Technical Note TN 9/96).

Iron pipes coated with a protective coating of zinc, called galvanized pipes, are also used in water distribution systems. The corrosion rate of the protective zinc layer increases at temperatures in the range of 55ºC to 95ºC, exposing the underlying iron. Iron pipes facilitate the development of tubercles due to the iron hydroxide or loose porous rust slowly transforming into a crystallized form. If other ions like calcium or carbonate are present they make a variety of precipitates that mix in with the iron hydroxide producing a crusty twisted coating which can grow into convoluted shapes (tubercles), see plate 1.4 below. These tubercles can affect the flow of water and

because they also absorb organic material they can provide nutrients and a habitat for micro-organisms including *Legionella* (Haas *et al*.,1983, BSRIA Application Guide Ag 2/93, Camper, 1996, Geldreich and Le Chevalier, 1999, www.corrosion-doctors.org).

Plate 1.4. Inner surfaces of an eroded galvanized pipe. (ProEconomy Ltd, 2006)

Biofilm formation can be higher on rough surfaces because the 'valleys' present can allow microbes to reside in a protected area with reduced shear forces and the surface roughness can provide a surface with increased surface area for bacterial attachment (Donlan, 2002).

Micro-organisms have also been found to attach more rapidly to hydrophobic than hydrophilic surfaces (Donlan, 2002, Flemming and Wingender, 2001a), and corrosion of pipes and scale formation on pipes attracts nutrients which also aids bacterial attachment and biofilm formation (Research meeting, University of Plymouth, 2009).

Biofilm formation can vary depending upon the characteristics of the micro-organisms forming the biofilms, and is influenced by an interbacterial communication mechanism, called quorum sensing (Sauer *et al.,* 2002).

Bacteria can oppose or promote *Legionella* attachment. For instance, Schofield and Locci, (1985), found that *Flavo*bacteria aided the attachment of *Legionella,* and Mampel *et al.,* (2006), observed attachment of *L. pneumophila* to biofilms formed by *Empedobacter breve*, *Microbacterium sp*. and *Acinetobacter baumanii* but not to *Pseudomonas* species, *Corynebacterium glutamicum* or *Klebsiella pneumoniae* biofilms.

Biofilm formation is also aided by protozoa grazing the surfaces of biofilms which causes the loss or 'sloughing' of parts of biofilms which then colonize other parts of the system (Keevil *et al.,* 1993, Abu Kwaik *et al.,* 1997).

Jass *et al.,* (1995) used a chemostat coupled modified Robbins device to monitor the colonization of soil isolates, *P. fluorescens* and *P. putida*, on silastic rubber surfaces. The authors found that *P. fluorescens* formed confluent dense biofilms in less than 24 hours, whereas *P. putida* adhered as single cells or microcolonies after the same period (Jass *et al.,* 1995).

1.9 Detecting *Legionella* in water systems

The association of *Legionella* with protozoa and its occurrence within biofilms, in which they hide, complicates its detection.

The current standard and validated technique for detecting *Legionella* in water is based on culturing for *Legionella* (ISO 11731:1998). This technique is however lengthy and complex, and detects only planktonic, free-living, and extracellular *Legionella*.

Rapid molecular tests for the detection of *Legionella* Deoxyribonucleic Acid (DNA) have been developed (Mahbubani *et al.,* 1990). Although these tests detect planktonic, sessile, extracellular and intracellular *Legionella*, nonviable *Legionella* cells are also detected. Since it is likely that water samples contains nonviable *Legionella* cells, which were killed by disinfection measures, false-positive readings of *Legionella* samples are possible which could lead to unnecessary and expensive emergency decontamination procedures (Shih and Lin, 2006).

1.10 Models built to examine biofilm formation and *Legionella* growth on plumbing materials

Schofield and Loci (1985), built a model hot water system to examine the survival of *L. pneumophila* and the formation of biofilms on different materials, see Figure 1.1. The model consisted of a 1 litre conical flask, which acted as a reservoir and was connected via a flow meter and peristaltic pump to three glass chambers; one chamber contained twisted strips of copper; one contained twisted strips of stainless steel and; one contained glass beads interspersed with aluminium discs. The outflow silicone tubes from the top of the chambers were connected and returned, via a sample port, to the conical flask. The temperature of the reservoir was controlled by placing it in a water bath. When fluid was actively circulating the temperature was kept at 45ºC. During static phases the temperature was allowed to drop to ambient. The water was circulated. Ten weeks after the start of the experiment the apparatus was dismantled and samples were taken for examination. The materials sampled included the rubber plugs on top and bottom of each chamber, the connecting silicone tubing, the glass beads and the copper and stainless steel twisted strips.

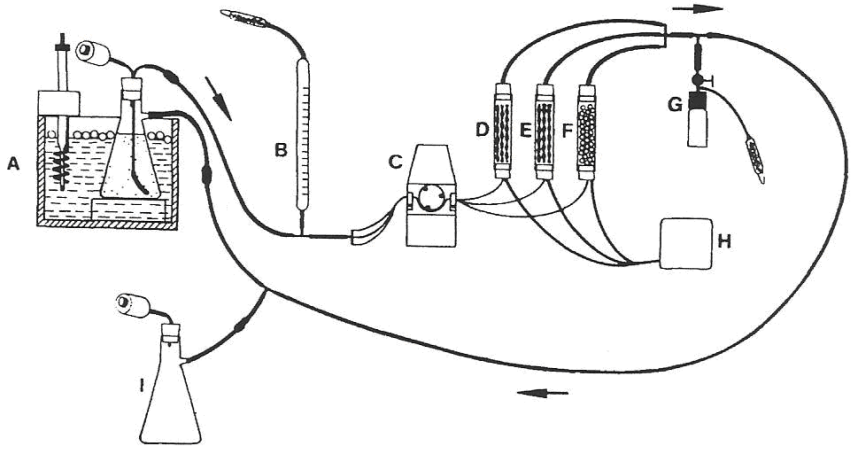


Figure 1.1 Diagram of the model hot water system. A, water bath; B, flow meter; C, peristaltic pump; D, rubber-stoppered glass tube containing copper turnings; E, rubber stoppered glass tube containing stainless steel turnings; F, rubber-stoppered glass tube containing glass beads and aluminium discs; G, sample port; H, air pump; I, return flask. All tubing was a silicone (Schofield and Loci 1985).

The authors observed that mats of cells and slime-like debris were heaviest on natural rubber and least on copper materials. Abundant growth of *L. pneumophila* on rubber

was also observed. *L. pneumophila* also appeared to be able to colonize silicone tubing and adhere to stainless steel (Schofield and Loci, 1985).

Keevil *et al.* (1993) and Rogers *et al.* (1994) used a model water distribution system, which consisted of two glass vessels linked in series to analyse biofilm formation on plumbing materials, see Figure 1.2. The first (seed) vessel simulated a storage tank, and the second (biofilm) vessel (which was constantly fed by the seed vessel) modelled the distribution system. Biofilms were generated in the second vessel by inserting $1cm^2$ coupons of sterile plumbing materials, commonly used in the construction of water systems, into the culture suspended on titanium wire. The model system was run continuously for the duration of the work. Each experiment had plumbing materials of only one type. The coupons were removed and tested after 1, 4, 7, 14, 21 and 28 days from the onset of each experiment.

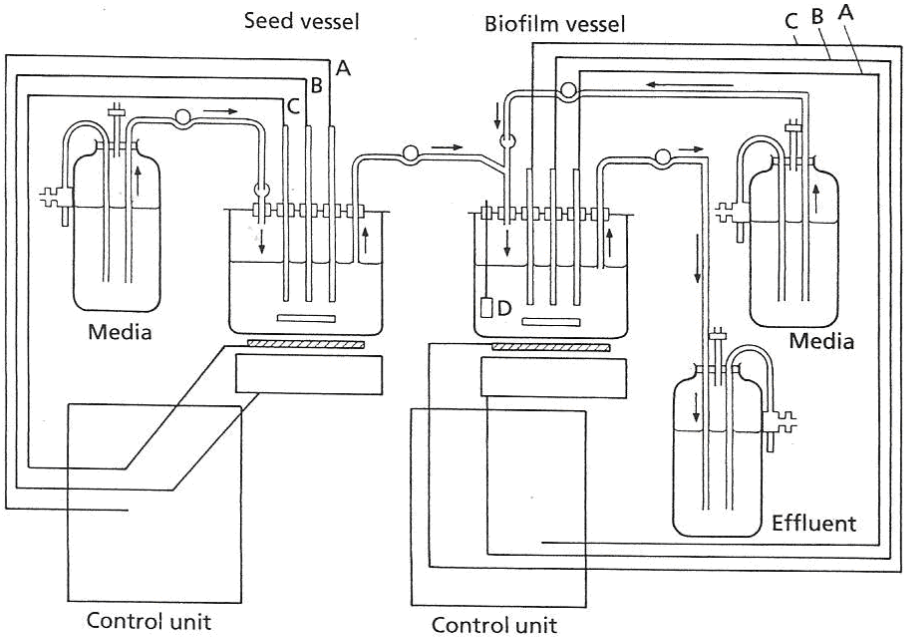


Figure 1.2 Continuous-culture biofilm model. A, pH monitoring; B, oxygen/stirrer control; C, temperature control; D, plumbing material coupon (Keevil *et al.,* 1993, Rogers *et al.,* 1994).

The pH was monitored but not controlled. The temperatures of the vessels were maintained between 20ºC and 60ºC. The temperature was corrected with an external heater pad beneath the fermenters. Dissolved oxygen tension was maintained at 20% of air saturation via feedback control of the stirrer speed.

Keevil *et al.*, (1993) found that copper supported sparse microbial growth and *Legionella* comprised a very low proportion of the population. The low colonization of copper surfaces was presumed to be due to the inhibitory effect of copper ions, either by selectively inhibiting *Legionella* or by inhibiting the organisms that support their growth. The most prolific biofilms were those that developed on the surface of elastomeric materials. The biofilm covered the entire elastomer surfaces after only 24 hours and contained more than $8.9x10^6$cfu/cm^2 on latex and $2.7x10^6$cfu/cm^2 on ethylene propylene rubber. The highest populations of *L. pneumophila* were found on latex as well (Keevil *et al.*, 1993).

Rogers *et al.*, (1994) found that the lowest concentration of flora was on the stainless steel surface and that the latex and ethylene-propylene rubber were most heavily colonised. Of the plastic materials used, polyethylene appeared to be most heavily colonised (Rogers *et al.*, 1994).

Three full size hot and cold water services rigs were constructed by the Building Services Research and Information Association (BSRIA) in 1996 to evaluate the efficacy of temperature and copper and silver ionization techniques to control *Legionella*, see Figure 1.3. Each rig replicated the water system of a small home for the elderly. Two of the rigs were fitted with ionization water treatment from silver/copper alloy electrodes and were operating at reduced hot water temperatures; one was filled with hard water and one with soft water. The third rig relied solely upon temperature as disinfection and was filled only with hard water. The cold water system in each rig consisted of a glass reinforced plastic cistern, which served a cold water copper pipework circuit of 30m in length, which included two cold taps and one shower. The hot water system consisted of a vertical hot water storage calorifier of copper construction, which served a hot water copper pipework circuit of 40m length, which included two hot taps and one shower. Copper and glass reinforced plastic coupons were inserted at various locations in each rig. These coupons were regularly removed for biofilm and *Legionella* analysis.

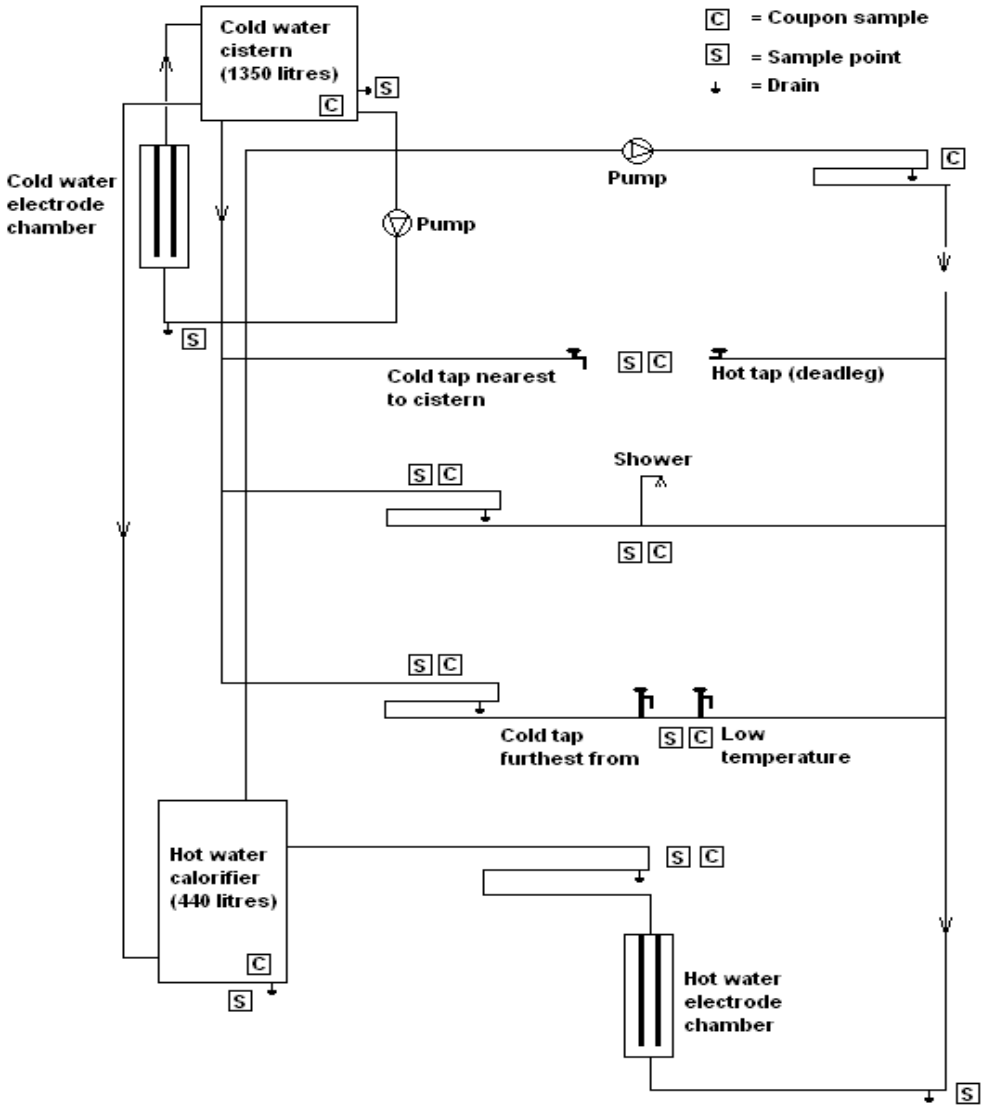


Figure 1.3 Schematic of the BSRIA test facility (BSRIA Technical Note TN6/96).

Legionella and heterotrophic bacteria were recovered from the biofilms that had formed on the copper and glass reinforced plastic coupons before copper and silver ionisation was applied but numbers were only significant on the glass reinforced plastic coupons and not on the copper pipework because this appeared to be naturally biocidal (BSRIA Technical Note TN 9/96).

The effects of stainless steel, polyethylene and copper pipe materials on the promotion of biofilm formation and growth of *Legionella* were investigated using a model system that simulated domestic consumption of hot tap water at a water temperature of around 37ºC by Van der Kooij *et al.* (2005), see Figure 1.4. The system included 3 separate identical electric water heaters fed with cold tap water. The water was not

stored and the heated water was re-circulated. Each heater fed either two duplicate stainless steel, or copper or polyethylene pipes. These pipes were 5.9m long and included four pipe segments, each with a length of 15cm, for biofilm analysis. The pipe segments were frequently removed for biofilm and *Legionella* analysis (Van der Kooij *et al.,* 2005).

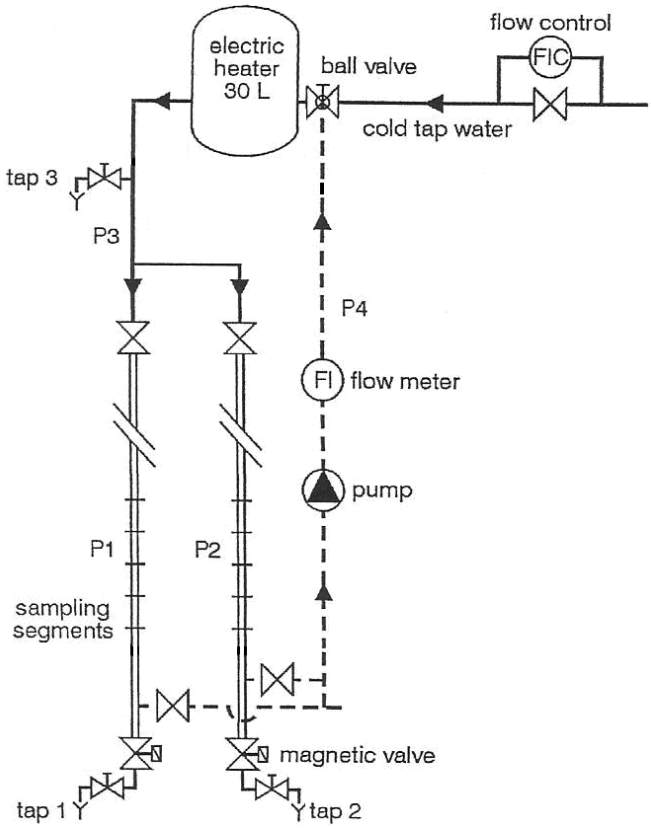


Figure 1.4 Diagram of the model warm water system. P1 and P2, pipes of the test material. P3, PVC-C pipe for the supply of warm water. P4, PVC-C pipe for recirculation. Tap 1 and Tap 2, sampling for collecting water from the pipes, Tap 3, heater outlet and sampling point for collecting water from the heater (van der Kooij *et al.,* 2005).

The results suggested that biofilm formation on the polyethylene surfaces was higher than that on the copper and stainless steel surfaces and that *Legionella* concentrations in water from the polyethylene pipes were more than 10 times higher than those from the copper pipes. After about 2 years of operating the system the *Legionella* concentrations from the copper pipes and surfaces were similar to those observed with the stainless steel pipes (Van der Kooij *et al.,* 2005).

1.11 Controlling *Legionella* in hot and cold water distribution systems

The risk from exposure to *Legionella* can be controlled by not allowing *Legionella* to proliferate in water systems. However, *Legionella* contaminate anthropogenic water supplies from the environment, and when these systems are contaminated, preventing proliferation and gaining control of *Legionella* is difficult because *Legionella* survive extreme ranges of conditions and grow and are protected in biofilms and in protozoan cells.

Evidence suggested that, once, colonized, anthropogenic water systems, particularly those in large complex buildings and hospitals, retain a relatively constant population of *Legionella* for many years (Rangel-Frausto *et al.,* 1999, Garcia *et al.,* 2003, Perola *et al.,* 2005).

National legislation in most countries, however, enforces preventing or controlling the risk of legionellosis. The Health and Safety at Work Act (HSWA) and the Control of Substances Hazardous to Health Regulation (COSHH) make this obligatory in the UK.

To comply with the UK legal duties, employers and those with responsibilities for the control of *Legionella* in water systems of premises should identify and assess sources of risk, prepare a scheme for preventing or controlling the risk, implement, manage and monitor precautions, keep records of the precautions, and appoint a person to be managerially responsible.

1.12 UK guidance for controlling *Legionella*

Practical advice on how to prevent or control *Legionella* in water systems is given in the Health and Safety Executive's Approved Code of Practice (L8) (ACoP, (L8), 2000). Specific guidance for National Health Trusts is given in the Department of Health's Technical Memorandum 04-01 (HTM 04-01, 2006).

Both documents recommend that hot and cold water distribution systems should be designed to aid safe operation by preventing or controlling conditions which permit the growth of *Legionella* and to allow easy cleaning and disinfection. Water supply quality should always be of equivalent quality as mains water and the materials used should avoid biofilm formation and *Legionella* growth. Water should flow consistently and stagnation should be avoided.

The documents also recommend that prevention or control of the risk from exposure to *Legionella* should include the use of water treatment techniques. The techniques recommended for continuous control are:

1.12.1 Temperature control

Temperature control involves storing hot water at 60ºC and distributing it so that it reaches a temperature of above 50ºC within one minute at outlets. Cold water temperatures should be below 20ºC after running outlets for up to two minutes.

Whilst Dennis *et al.,* (1984) reported that *L. pneumophila* serogroup 1 had a decimal reduction time at 50ºC *in-vitro*, there is a lack of *in-vivo* controlled evaluation tests on the efficacy of controlling *Legionella* at these temperatures.

Controlling *Legionella* by temperature may also not always be possible or practical. The ACoP (L8), therefore, advises that alternative techniques instead of temperature control can be used as long as proliferation of *Legionella* is prevented. The HTM 04-01 document also recommends the use of alternative techniques but only together with and not instead of temperature control.

The alternative techniques for continuous control described in the ACoP (L8) and HTM 04-01 documents are:

1.12.2 Chlorine Dioxide

According to the ACoP (L8) levels of 0.5mg/l of chlorine dioxide can, if properly managed, be effective against *Legionella* in hot water systems.

Chlorine dioxide is an oxidizing biocide with an extremely high oxidation potential, which probably accounts for its potent bactericidal powers through inactivation of critical cell enzyme systems or disruption of cell protein synthesis (Metcalf and Eddy, 1991).

It is a gas in solution that is typically generated on site at the facility. Methods for producing chlorine dioxide include controlled mixing of chemical precursors or electrochemical generation. Although only a limited number of controlled evaluations have been published, chlorine dioxide has been used for water treatment in Europe since the 1940s, and numerous systems have been installed in the US for *Legionella* disinfection (Lin *et al.,* 2011).

Compared to chlorine, chlorine dioxide has superior penetration into biofilms, it does not produce carcinogenic by-products, and it is less corrosive, (Lin *et al.,* 2011).

Studies have demonstrated that a chlorine dioxide residual at outlets of 0.3mg/l to 0.5mg/l controlled *Legionella* (Hamilton *et al.,* 1996, Pavey and Roper, 1998, Hill *et al.,* 2000, Hood *et al.,* 2000). It is, however, difficult to maintain such levels, especially, in hot water systems. Sidari *et al.,* (2004) reported that chlorine dioxide residuals decreased with increasing distance from the application point and with raised temperature (Sidari *et al.,* 2004). Higher doses may, therefore, be necessary (Walker *et al.,* 1995, Makin, 1998, Harris and Rendell, 1999) although the Drinking Water Inspectorate only allows a maximum of 0.5mg/l in drinking water (www.dwi.defra.gov.uk). A prolonged time may also be necessary to demonstrate significant reductions in the *Legionella* positivity rate (Lin *et al.,* 2011).

1.12.3 Copper and silver ionization

Copper and silver ionization involves the continuous release of copper and silver ions in water. The ions can be generated either by passing a low electrical current between two pure copper and two pure silver electrodes or by passing a current between copper and silver alloys, usually consisting of 70% copper and 30% silver.

The biocidal efficacy of copper and silver is well documented. The copper ion is thought to be responsible for the cascading effect of copper toxicity in bacterial cells. Suggested mechanisms involve an initial cell membrane compromise, followed by rapid accumulation of copper ions in the bacterial cells, and subsequent protein damage resulting in cell death and DNA degradation (Grass *et al.,* 2011). It was suggested that the silver ion primarily affected the function of membrane-bound enzymes, such as those in the respiratory chain, through binding to thiol groups (Bragg and Rainnie, 1974, McDonnell and Russell, 1999, Uchida *et al.,* 2003). Yamanaka *et al.,* (2005) found, however, that the silver ion also readily infiltrated in the interior of *Escherichia coli*, rather than residing in the cell membrane area, and that one of the major bactericidal actions of the silver ion was caused by its interaction with the ribosome and subsequent suppression in the expression of enzymes and proteins essential to ATP production (Yamanaka *et al.,* 2005).

Studies carried out *in-vitro* demonstrated that copper and silver ionization inactivated *L. pneumophila* with 0.4mg/l copper and 0.04mg/l silver (Landeen *et al.,* 1989, Lin *et al.,* 1996, BSRIA TN6/96). *In-vitro* studies also demonstrated that biofilm formation was controlled with copper and silver ionization when levels of 0.4mg/l copper and 0.04mg/l silver were maintained (BSRIA TN6/96, Walker *et al*, 1997, Shih and Lin, 2010).

Evaluation tests of copper and silver ionization installed treating hot and cold water distribution systems of hospitals mainly in the US showed that *L. pneumophila* was controlled (Liu *et al.,* 1994, Miuetzner *et al*, 1997, Liu *et al*, 1998, Stout *et al.,* 1998, Biurrun *et al.,* 1999, Kusnetsov *et al*, 2001, Stout and Yu, 2003, Chen *et al.,* 2008). A residual effect of copper and silver throughout the treated systems was also observed. (Lui *et al.,* 1994, Lui *et al.,* 1998).

The ACoP (L8) and HTM 04-01 documents recommend concentrations for *Legionella* control of 0.4mg/l copper and 0.04mg/l silver in hot water systems, and 0.4mg/l copper and 0.02mg/l silver in soft water systems. Both documents advise, however, that it can be difficult to maintain silver ion concentrations in hard water systems due to build–up of scale on the electrodes and that the ionization process is pH sensitive, which may need extra water treatments. The documents also suggest that it is difficult to maintain silver ion concentrations above a pH of 7.6.

Although Lin *et al.,* (2002), showed that there was no impact by hardness ions, calcium and magnesium, on the biocidal efficacy of copper and silver ions, calcium and magnesium ions can convert in water with a high pH into insoluble particles, which can precipitate and cause rapid scale formation on the copper-silver electrodes, potentially obstructing copper and silver ions release and reducing the presence of copper and silver ions in the water (Lin *et al.,* 2002).

Lin *et al.,* (2002) demonstrated that a high pH had little effect on the *Legionella* inactivation action of the silver ions and that at a pH as high as 9 only the copper ions, at 0.4mg/l, failed to kill *L. pneumophila,* achieving only a 10-fold reduction in 72 hours. There is, however, a lack of studies on the influence of a pH above 7.6 on the efficacy of copper and silver ionization in controlling *Legionella* in hot and cold water distribution systems (Lin *et al.,* 2002).

Copper and silver ions are positively charged ions (cations) and are attracted to negatively charged ions (anions) in water, which can result in the formation of insoluble chemical compounds, potentially resulting in less biocidal efficacy. A chloride ion is an anion that enters water distribution systems via treatment processes in which chlorine or chloride is used. It also enters water systems from run-off containing road de-icing salts, the use of inorganic fertilizers, landfill leachates, septic tank effluents, animal feeds, industrial effluents, irrigation drainage, and seawater intrusion in coastal areas (www.who.int).

Lin *et al.,* (2002) also demonstrated that as the chloride concentration in solution increases, silver complexation reduces the available silver cations by 56% at 15mg/l

chloride to 26% at 50mg/l chloride. The authors suggested that it was possible that chloride concentrations in water may decrease the availability of silver cations and reduce its biocidal potential (Lin *et al.,* 2002). Studies evaluating the affect of chloride on the efficacy of copper and silver ionization in controlling *Legionella* in hot and cold water distribution systems are, however, lacking.

One French hospital reported failure of copper and silver ionization (Blanc *et al.,* 2005). According to Lin *et al.,* (2005) this could have been due to a phosphate compound that was added to the water system to control corrosion, which may have interfered with the efficacy of ionization (Lin *et al.,* 2005). Although a reduction of the biocidal effect of copper and silver has been reported when copper and silver were complexed by phosphates *in-vitro* (Wuhrmann and Zobrist, 1958, Zevenhuizen *et al.,* 1979, Landeen *et al.,* 1989), there is a lack of studies on the influence of phosphates on the efficacy of copper and silver ionization in controlling *Legionella* in hot and cold water distribution systems.

In two German hospitals copper and silver ionization systems were unable to control *Legionella.* Possible resistance of *Legionella* against the metal ions was suggested as the reason for the lack of control (Rohr *et al.,* 1999, Mathys *et al.,* 1999). According to Lin *et al.,* (2011), the lack of control in the hospitals was more likely due to the concentration of the silver ions being below the recommended levels at 0.01mg/l (Lin *et al.,* 2011).

Long term treatment with copper and silver ions could, however, theoretically result in the development of resistance to these ions, although Chopra, (2007), suggested that copper and silver resistance is metabolically expensive for bacteria (Chopra, 2007), which could make resistance less likely. Although studies have demonstrated development of resistance in *Salmonella, Pseudomonas, Enterococcus, Klebsiella,* and *E. coli* (McHugh *et al*., 1975, Bridges *et al.*, 1979, Haefeli *et al*., 1984, Kaur and Vadehra 1986, Solioz and Odermatt 1995, Silver, 1996, Gaillard and Webb, 1997, Gupta *et al*., 1999, Gupta, *et al.*, 2001), resistance has not yet been demonstrated in *Legionella.* Studies on *Legionella* resistance affecting the efficacy of copper and silver ionization in controlling *Legionella* in hot and cold water distribution systems are, however, lacking.

Kuchta *et al.*, (1995) showed that copper and silver ions inhibit the amoeba *H. vermiformis*. Rohr *et al.*, (2000) reported that the amoebas *T. pyriformis* and *H. vermiformis* were inactivated by 0.1mg/l of silver and 1mg/l of copper. Cassells *et al.,* (1995), reported a T_{99} value of 3.9 minutes (exposure time required to achieve a 99% reduction) of the amoeba *Naegleria fowleri* by 0.8mg/l copper and 0.08mg/l silver with 1mg/l of free chlorine (Cassells *et al.,* 1995).

At present, each country in Europe specifies individual maximum permitted copper and silver levels for water intended for human consumption. These levels apply until maximum concentration values for copper and silver are given in the European Council Biocidal Products Directive (98/8/EC).

The UK Water Supply (Water Quality) Regulations 2000 and the European Council Directive 98/83/EC, which currently govern the maximum permitted levels of a range of substances in water intended for human consumption in the UK, revealed a surprising situation in the case of silver as the regulations and the directive do not specify a maximum concentration value for silver. Theoretically, this state of affair allows any level of silver to be used. It could also mean, however, that previous permitted levels should be applied, which would be, if silver is used in a water treatment process, 0.08mg/l (The UK Water Supply (Water Quality) Regulations 1989). Levels for copper are much more clearly defined, with a maximum permitted level of 2mg/l being specified in the UK Water Supply (Water Quality) Regulations 2000.

The US Environmental Protection Agency (EPA) has set maximum concentration levels for drinking water of 1.3mg/l for copper and 0.1mg/l for silver (Lin *et al.,* 2011).

The World Health Organisation (WHO) gives a guideline value for copper in drinking water of 2mg/l. No specific guideline value for silver is given because there are no adequate data with which to derive a health-based guideline value in drinking water. The guidelines advise, however, that silver, up to 0.1mg/l, in drinking water gives a total dose over 70 years of half the human no observable adverse effect level

(NOAEL) of 10g, and, therefore, could be tolerated without risk to health (WHO Guidelines for Drinking Water Quality, 2011).

Being biocidal, copper and silver fall within the scope of the European Council Biocidal Products Directive (98/8/EC) and evidence has to be presented to the Council that they do not pose significant risk to humans, animals or the environment.

Task Forces were created to share the costs for the preparation and evaluation of technical dossiers specifying copper and silver toxicity and assessing the risk of exposure to copper and silver. Dossiers were submitted to the relevant authorities for evaluation. These authorities are currently assessing the dossiers so that an acceptable copper and silver value can be determined, which will be then be published in the European Council Biocidal Products Directive (98/8/EC).

However, due to its extensive use in the photographic industry, considerable research has already been carried out upon the effects of silver in the environment. Although laboratory investigations have indicated that it is potentially very toxic with acute toxicity values of 0.023mg/l to 0.357mg/l for saltwater invertebrates, it seems to have little actual impact upon organisms in an ecosystem. This could be due to its readiness to form complexes with a wide variety of substances, so reducing its availability for absorption, which reduces toxicity and the biocidal effectiveness of silver in an ecosystem. Bioaccumulation data also suggest that it is not concentrated in species to any great extent. Copper shows a higher level of bioaccumulation, although again it does not seem to be regarded as presenting any significant risk to the environment. There is also no indication of substantive biomagnification occurring in the case of either metal.

When assessing the level of toxicity of copper and silver to humans, instances of fatalities caused by acute copper poisoning have been recorded, although doses required were considerable. Instances of acute silver poisoning, however, are unknown. Cases of chronic copper poisoning are rare, whilst for silver the only known long term effect of ingesting quantities of the element is argyria, a condition in which skin and hair are heavily discoloured by silver in the tissues. Silver in particular seems to be readily excreted from the body, only a small percentage of silver is absorbed, retention rates in humans and laboratory animals range between 0% and 10%. The

evidence available indicates that neither copper nor silver is carcinogenic or mutagenic, despite extensive exposure via water pipes in the former and therapeutic and industrial use of the latter. With regard to teratogenicity, the few studies done for silver suggest that it is not toxic in this respect. More studies have been done on copper but results are conflicting (Bedford, 2003).

1.12.4 Ozone (O_3) and Ultraviolet Light (UV)

According to the ACoP (L8) and HTM 04-01 documents Ozone (O_3) and UV light are not intended to be dispersive and are designed to have their effect only at or very close to the point of application.

Ozone (O_3) is a powerful oxidizing agent, far more powerful then oxygen (O_2). It is generally believed that bacterial kill through ozonation occurs directly because of cell wall disintegration (cell lysis). Ozone is generated either from air or pure oxygen when a high voltage is applied across the gap of narrowly spaced electrodes. It is, however, chemically unstable and decomposes rapidly to oxygen after generation and must be generated on-site. (Metcalf and Eddy, 1991).

The effect of ozonation of supply water for one wing of an unoccupied hospital building which had positive cultures for *L. pneumophila* from multiple potable water fixtures was studied by Edelstein *et al.,* (1982). Although mean ozone residual concentrations of 0.79 mg/l eradicated *L. pneumophila* from the fixtures, the authors suggested that whether or not ozone could be considered to be an effective biocide to use for the eradication and continuous suppression of *L. pneumophila* in a potable water system was not answered because *L. pneumophila* was also eradicated from by non-ozonated water in the control wing fixtures (Edelstein et al., 1982).

In-vitro studies developed on a 38 litres plumbing system using ozone, among other biocides, was evaluated by Muraca *et al.,* (1986). The studies showed effectively control of *L. pneumophila* by a residual concentration of 1 to 2 mg/l of ozone and the data suggested that ozone could potentially remove *L. pneumophila* in a large water distribution system. However, the authors also suggested that because of the rapid decomposition of the ozone residual in water, its main utility may be limited as a supplemental disinfectant (Muraca *et al.,* 1986).

Several authors have also noted that corrosion could also be a potential problem with the application of ozone (Bird, 1987, Keenahan, 1990, Ford, 1991), and Newsome, (2001) suggested that the major problem with ozone was poor penetration to all parts of the water system (Newsome, 2001).

UV light of wavelength 254nm can destroy bacteria. *Legionella pneumophila* has been reported to be sensitive to UV dosages of 2500-7000µW-s/cm^2 at 254nm (Antopol and Ellner, 1979).

Muraca *et al.,* (1986) showed that UV irradiation at 30000µW-s/cm^2 at 254 nm produced a 5 log decrease in the concentration of *L. pneumophila* within 20 minutes. (Muraca *et al.,* 1986). Makin and Hart, (1992) found that during an 8-month period of 254 nm UV lamps being lit, no *Legionella* or amoebae were found in the water tested at one shower (Makin and Hart, 1992).

Franzin *et al.,* 2002 demonstrated that UV irradiation was useful to protect a water system but only in a small area. UV irradiation was found to be effective only at the point immediately after the UV light lamps but *Legionella* persisted especially at the furthest point from the UV light. Because of the lack of residual activity, the authors recommended combining UV irradiation with other methods of disinfection (Franzin *et al.,* 2002).

1.12.5 Intermittent disinfection of hot and cold water distribution systems

The ACoP (L8) and HTM 04-01 documents recommend that hot and cold water distribution systems are cleaned and disinfected thermally or chemically when inspections consider it to be necessary, if the system or part of it has been substantially altered or entered for maintenance purposes in a manner which may lead to contamination, and during or following an outbreak or suspected outbreak of legionellosis.

Thermal disinfection is recommended to be carried out by ensuring that the temperature at a calorifier is high enough so that the temperatures at the outlets do not fall below 60°C. Each outlet should also be run sequentially for at least five minutes at above 60ºC. The risk of scalding should be considered and particular care should be taken to ensure that water services are not used, other than by authorised personnel, until water temperatures have dropped to their normal operating levels.

As well as disinfecting water systems thermally, the ACoP (L8) and HTM 04-01 documents recommend that disinfection may also be carried out using oxidizing

chemicals such as chlorine dioxide, chlorine, and bromine. It is recommended that all parts of the system are disinfected and not just those which are readily accessible.

When chlorine is used the free residual level in the cold water storage tank should be between 20 and 50mg/l, which should be allowed to flow to all parts of the system by successively opening the outlets in the system until there is a smell of chlorine. The outlets should then be closed for an appropriate period, which depends on the chlorine concentration – from at least one hour at 50mg/l to at least two hours at 20mg/l. The chlorine concentration needs to be monitored throughout the disinfection process and after disinfection the system should be thoroughly flushed.

The ACoP (L8) and HTM 04-01 documents point out that because chemical disinfection is hazardous it may only be carried out by trained personnel and should be closely supervised. All building occupants should also be warned.

The ACoP (L8) also advises that biodispersants may be needed to remove organic fouling from pipework and that chemical descaling may also be necessary.

The HTM 04-01 document recommends that after disinfection, when water is run to waste into a natural watercourse, or a drain leading to it, it should be de-chlorinated using either sulphite or bisulphite or meta-bisulphite to avoid contamination.

1.12.6 Point-of-use filtration

The HTM 04-01 document briefly discusses using point-of-use filters in the operational considerations part of the document.

The risk of exposure to *Legionella* in water systems can be controlled by attaching 0.2µm filters to outlets (Salvatorelli *et al.,* 2005).

Plate 1.5 Outlet with point-of-use water filter attached (Sheffer *et al.,* 2005)

Results of a study conducted on a water system of a hospital building in the US, colonized with *L. pneumophila,* suggested that point of use filters can prevent exposure of high risk patients to waterborne bacteria (Sheffer *et al.,* 2005).

The HTM 04-01 document highlights that it is feasible for bacteria to colonize the filter material. It is, therefore, recommended to replace the filters at appropriate intervals in accordance with the manufacturer's recommendations, typically at least once a month.

2. AIMS AND OBJECTIVES

Hospital-acquired Legionnaires' disease is linked to the presence of *Legionella* in hospital water systems.

Results of studies suggest that cases of hospital-acquired Legionnaires' disease are reduced by the implementation of copper and silver ionization systems.

Measures to control *Legionella* in hospital water systems in the UK are recommended in the HTM 04-01 documents. Little data is however available on the efficacy of these measures.

Although the HTM 04-01 document recommend using copper and silver ionization as an alternative measure, it is recommended to only be used together with and not instead of temperature control. Temperature control is, therefore, seen by UK National Health Trusts as the main control method.

The overall aim of this project is, therefore, to investigate the efficacy of controlling *Legionella* by applying copper and silver ionization in UK hospital water systems and to examine efficacy by applying the HTM 04-01 recommended temperatures, taking into consideration the formation of biofilms on plumbing materials that are commonly used.

The objectives of this project are:

(a) To study the efficacy of copper and silver ionization systems in controlling *Legionella* in water systems of 10 hospitals in the UK.

(b) To study the efficacy of maintaining hot water temperatures above 50ºC after running hot outlets for 1 minute in controlling *Legionella* in water systems in the UK.

(c) To study the efficacy of maintaining cold water temperatures below 20ºC after running cold outlets for 2 minutes in controlling *Legionella* in water systems in the UK.

(d) To design and build small scale rigs to simulate circulating water flow systems in copper and polyethylene piping to compare:

    i. The growth of *Legionella*.

ii. The efficacy of controlling *Legionella* with elevated temperatures.

iii. The efficacy of controlling *Legionella* with copper and silver ionization.

(e) To evaluate the impact of chloride and phosphate on the efficacy of copper and silver ionization in controlling *Legionella* in 10 hospital water systems in the UK.

(f) To assess the growth of biofilms on plumbing materials commonly found in hospital water systems in the UK using a Robbins device.

3. MATERIALS AND METHODS

3.1 Evaluation studies in UK hospitals

Studies on the efficacy of copper and silver ionization in controlling *Legionella* in water distribution systems of hospitals in the UK were carried out. These studies were conducted by contractual arrangement with these hospitals for control of *Legionella.*

Results of *Legionella* analyses that may have been carried out outside the contractual arrangement with these hospitals before the copper and silver ionization systems were activated were not available.

One set of samples was taken before activation of the systems from each hospital, which were analysed for *Legionella.* Temperatures at the outlets were recorded whenever samples were taken. The outlets selected were outlets that were identified as being at risk of *Legionella* contamination and outlets that were known to be contaminated. At least double the number of outlets was tested before activation of the copper and silver ionization systems than after activation.

The probability of contamination at outlets at which *Legionella* were found before the copper and silver ionization systems were activated would be higher than at outlets at which no *Legionella* were found. Also due to financial limitations in the number of samples to be analysed, the *Legionella* contamination at outlets at which *Legionella* were found before activation was, therefore, primarily monitored after activation.

The water system near to outlets at which *Legionella* persisted after activation was also investigated by taking samples for *Legionella* analysis from outlets close to the *Legionella* contaminated outlets.

Table 3.1 below shows the approximate number of beds that accommodated these hospitals, the date the copper and silver ionization systems were activated, the number

of outlets that were sampled before the systems were activated, and the number of outlets that were sampled on a monthly basis after these systems were activated.

Study hospital	Approximate number of beds	Date system commissioned	Number of outlets sampled before commissioning	Number of outlets sampled after commissioning	Frequency of sampling
1	800	11/09/2007	46	21	Monthly (21 outlets)
2	350	10/01/2008	30	15	Monthly (15 outlets)
3	70	04/02/2008	20	10	Monthly (10 outlets)
4	100	08/04/2008	12	6	Monthly (6 outlets)
5	550	25/07/2008	51	25	Monthly (20 outlets)
6	550	06/10/2008	60	25	Monthly (13 outlets)
7	150	25/11/2008	26	20	Monthly (13 outlets)
8	200	27/04/2009	45	22	Monthly (21 outlets)
9	100	03/08/2009	30	15	Monthly (15 outlets)*
10	850	21/12/2009	60	30	Monthly (30 outlets)

*- From September 2010 onwards samples were taken once every two months from 15 outlets and once a month from 6 outlets.

Table 3.1 – Study hospitals - Size, number of sample outlets and sampling frequencies.

The copper and silver ionization systems installed in these hospitals were designed and manufactured by ProEconomy Limited, Bedfordshire, UK.

3.1.1 The Orca copper and silver ionization system

With the Orca copper and silver ionization system, see Plate 3.1, water flows through the turbine of a flow sensor, which sends a signal to a control unit. The control unit then passes a low direct current between two 99.9% copper and two 99.9% silver electrodes, housed in electrode chambers, called 'Orca pods'. This causes the release of copper and silver ions into the flowing water. To ensure that the necessary levels of copper and silver are released by the system, the system has been designed to deliver automatic adjustment taking into account variable flow rates and water qualities. Copper and silver levels, electric current settings, and flow rates can be interrogated remotely. The system is commonly installed on the mains water pipe.

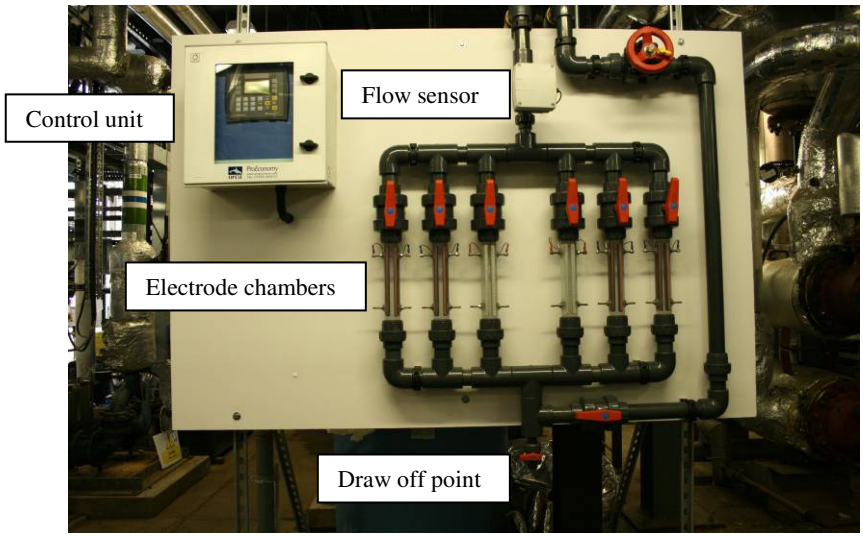


Plate 3.1 The Orca copper and silver ionization system (ProEconomy Ltd)

Potential formation of scale on the electrodes, which can obstruct the release of copper and silver ions in hard water, is prevented with the Orca system by using pure copper and pure silver electrodes instead of copper/silver alloys. This provides controlled release of the copper and silver ions by being able to separately increase or reduce the electrical current output, which is not possible when copper/silver alloys are used. Also, the polarity of the electrodes is automatically switched from anode to cathode and visa versa, which results in cations, such as calcium and magnesium ions, that may have become stuck on the on the surface of the cathode, desorbing from its surface when the polarity switches to anode.

3.1.2 Sampling and system maintenance procedures

Samples were taken before the copper and silver ionization systems were activated and once a month after activation of the systems from pre-selected outlets of the water distributions systems of the hospitals studied. These samples were analyzed by a UKAS accredited laboratory for *Legionella,* copper and silver.

The outlets from which the samples were taken were either identified, through risk assessments conducted by the hospitals, as being at risk of *Legionella* contamination or were known to be contaminated with *Legionella*, therefore, potentially presenting a risk of legionellosis in patients. Outlets identified through risk assessment as being at risk of *Legionella* contamination were mostly outlets that were not or infrequently used, cold outlets at which temperatures were not maintained below 20ºC, and hot outlets at which temperatures were not maintained above 50ºC.

When samples were taken, the type of outlet that was sampled from was recorded, showing if it was a single hot or cold, a mixer, a shower, or an assisted bath outlet. When rubber lined flexible hoses and mixing valves were attached to the outlets, this was also recorded.

The sample for *Legionella* analysis was taken after running the outlets for 1 to 2 minutes in a sterilized one litre plastic bottle which also contained 1ml of sodium thiosulfate to de-chlorinate the sample. After the sample for *Legionella* analysis was taken, the sample for copper and silver analysis was collected in a 125ml plastic bottle containing 1ml of nitric acid to ensure that the metal content stayed in solution, free from particulate matter. After the sample for copper and silver analysis was taken, the temperature was recorded using a non-contact TN1 infrared thermometer, positioned as close to the running water as possible.

The creation of aerosols was avoided when known *Legionella* contaminated outlets were sampled by attaching a clean hose between the outlet and its drain, and, where this was not possible, by running the outlet slowly, avoiding splashing. Samples from showers that were known to be contaminated with *Legionella* were taken either by dismantling the showerhead and attaching a clean hose between the outlet and its drain, or, when this was not possible, by cutting a corner of a food grade plastic bag,

inserting the shower head into the plastic bag, securing the bag closed around the shower head with an elastic band, and then running the water slowly into the sample bottle, avoiding splashing.

The *Legionella*, copper and silver samples were placed in a carton box, away from UV light. All samples were then transported to a UKAS accredited laboratory for analysis.

When *Legionella* were found, the hospitals were advised to flush the contaminated outlet, avoiding the creation of aerosols, to encourage the copper and silver levels and to avoid water stagnation. Re-samples were also taken for copper, silver and *Legionella* analysis immediately after opening the contaminated outlet and also after running it for ten minutes. This was in-line with the recommendations given in the ACoP (L8) and the HTM 04-01 documents, and the British standard 7592:1992.

The copper and silver ionization systems were checked monthly. Where necessary, the electrodes were cleaned and re-gapped, worn electrodes were replaced with new ones, and the control unit settings were adjusted.

Samples to determine the pH, chloride, and phosphorus levels in the water supply of the hospitals were taken. These samples were taken where the water entered the hospitals, and were also analyzed by a UKAS accredited laboratory.

The pH samples were taken in a clean and empty plastic 150ml bottle. The samples for chloride analysis were taken in a clean and empty plastic 1 litre bottle and the samples for phosphorus analysis were taken in a plastic 125ml bottle containing 1ml of nitric acid to ensure that the phosphorus content stayed in solution, free from particulate matter.

3.1.3 Study hospital 1

Study hospital 1 was a large site consisting of many buildings, built in the 1960s and modernized over the years. The hospital accommodated approximately 800 beds.

The hospital's water distribution system was complex and extensive. The temperature regime, as recommended in the ACoP(L8) and HTM 04-01 documents, was applied as the sole *Legionella* control measure but it proved to be difficult to maintain hot water temperatures above 50ºC and cold water temperatures below 20ºC in some parts of the hospital due to cross heating and poor insulation.

Approximately 1000 mixing valves had to be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, below 45ºC. Water below 45ºC and the debris that built up in the mixing valves was, however, considered ideal for *Legionella* proliferation. Rubber lined flexible hoses and rubber fittings were also attached to several outlets and water stagnation was possible due to poor use of outlets and ward closures, which was also considered ideal for *Legionella* proliferation.

The water was supplied to the site via a mains pipe and was passed to two large concrete tanks from which water was provided to the whole site. The storage capacity of these tanks was approximately 600000 litres. The water was not softened.

One copper and silver ionization system with nine copper electrode chambers and nine silver electrode chambers was installed treating the incoming mains supply water, see Plate 3.2.

The system was installed on the incoming mains pipe before the two storage tanks. The systems were commissioned on the 11th September 2007.

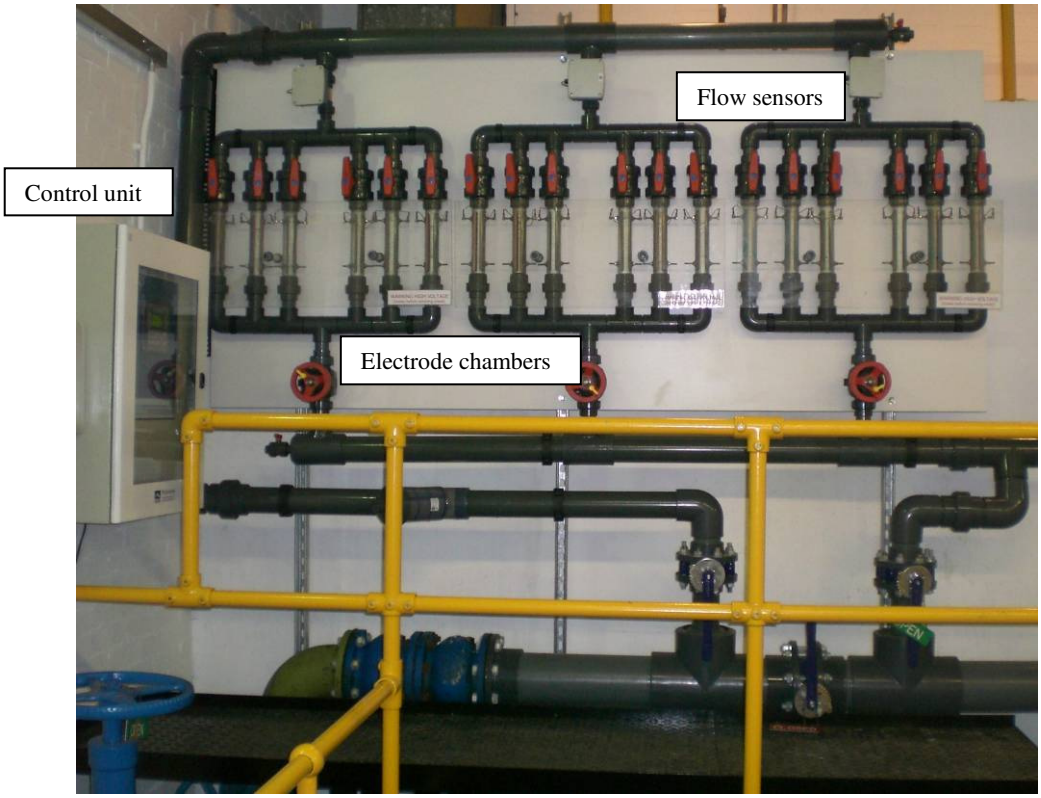


Plate 3.2. Photograph of the copper and silver ionization system installed in study hospital 1

The copper and silver ionization system installed was a digital system. The system was activated when the storage tanks were filling up with water. The system, therefore, released copper and silver ions into the storage tanks. The electrical currents adjusted automatically to the different flow rates and could also be manually adjusted to either increase or reduce the silver and copper ions output. The polarity of the electrodes reversed automatically, from anode to cathode, every fifty seconds to prevent scale build-up on the surfaces of the electrodes.

On the 05th September 2007, before the system was activated, samples were taken from 46 outlets.

On the 05th October 2007, after the system was commissioned, samples were taken from 21 outlets at which *Legionella* were found in the samples taken before the system was commissioned.

Samples were taken monthly from the 05th October 2007 onwards from 21 outlets.

3.1.4 Study hospital 2

Study hospital 2 was a smaller site with newer buildings than study hospital 1. The hospital accommodated approximately 350 beds.

As well as with study hospital 1, the temperature regime was applied as the sole *Legionella* control measure, which also proved to be difficult to maintain. Mixing valves had to be fitted and rubber lined flexible hoses and rubber fittings were attached to several outlets. Water stagnation was also possible due to poor use of outlets and ward closures. This was considered ideal for *Legionella* proliferation.

The water was supplied to the site via a mains pipe and was passed to two small break tanks, which prevented contamination of the mains water supply by incorporating an air gap which stopped water from flowing backwards into the mains. From these break tanks the water was passed to four underground concrete tanks, which supplied approximately 26 roof tanks scattered around the site. The water was again not softened.

One copper and silver ionization system, with eight copper electrode chambers and four silver electrode chambers, was installed to treat the incoming mains supply. The system was installed on the incoming mains pipe before the 'break' tanks, see Figure 3.1. The system was also digital, similar to the system installed in study hospitals 1, and consisted of two control units controlling two flow sensors. The system was commissioned on the 10th January 2008.

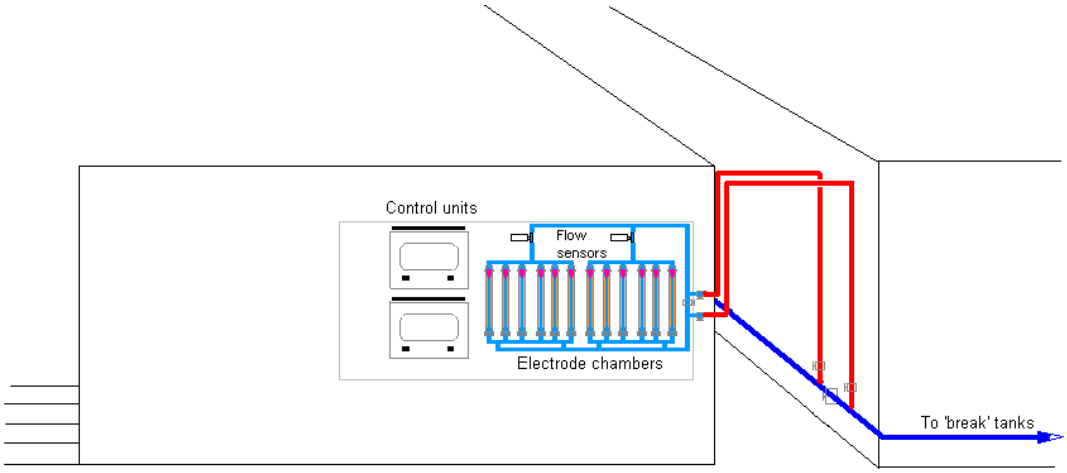


Figure 3.1 Schematic drawing of the copper and silver ionization installation in study hospital 2

Before the system was activated samples were taken from 30 outlets.

On the 04th February 2008, 3 weeks after the system was commissioned, samples were taken from 15 outlets. Six of these outlets were outlets at which *Legionella* were found in the samples taken before the system was commissioned.

Samples were taken monthly from the 04th February 2008 onwards from 15 outlets.

3.1.5 Study hospital 3

Study hospital 3 was a new hospital commissioned in 2007. The hospital accommodated 70 beds over two floors. The temperature regime was the sole method used to control *Legionella* in the water system.

The water system was less complex compared to study hospitals 1 and 2 but it was again difficult to maintain temperatures, particularly, in the cold water system and mixing valves had to be fitted to reduce hot water temperatures to non-scalding temperatures. Rubber lined flexible hoses and rubber fittings were again attached to several outlets. This was considered ideal for *Legionella* proliferation.

As with study hospital 1, the water was supplied to the site via a mains pipe and was passed to two tanks from which water was provided to the whole site. The capacity of these tanks was 20000 litres each. The water was again not softened.

One copper and silver ionization system, with two copper electrode chambers and two silver electrode chambers, was installed to treat the incoming mains supply. The system was installed on the incoming mains pipe before the two tanks, see Figure 3.2. The system was digital, similar to the systems installed in study hospitals 1 and 2. The system was commissioned on the 04th February 2008.

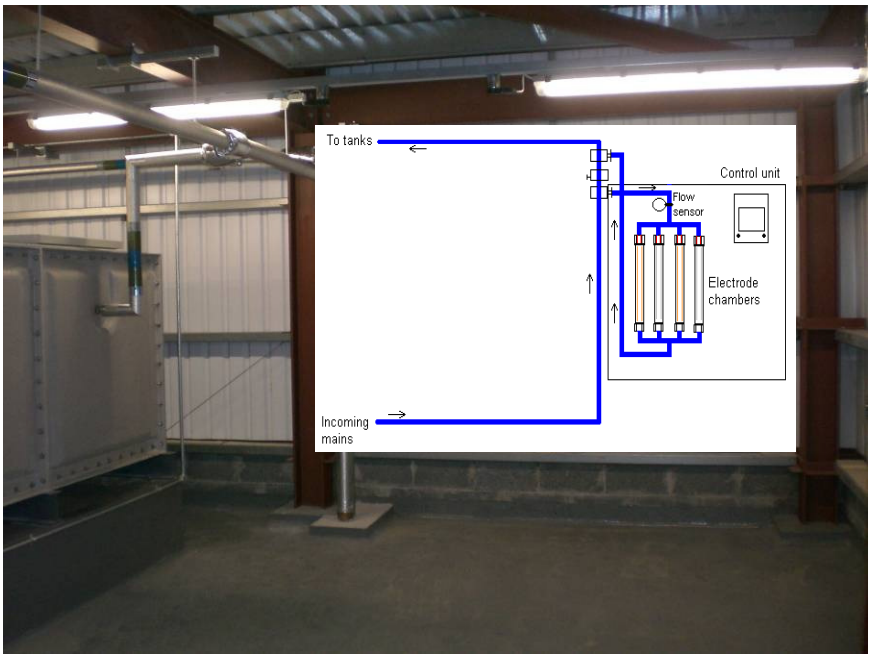


Figure 3.2 Schematic drawing of the copper and silver ionization installation in study hospital 3.

On the 04th February 2008, before the system was activated, samples were taken from 20 outlets.

On the 06th March 2008, after the system was commissioned, samples were taken from 10 outlets. Three of these outlets were outlets at which *Legionella* were found in the samples taken before the system was commissioned.

Samples were taken monthly from the 06th March 2008 onwards from 10 outlets. The outlet points were rotated from June 2010.

3.1.6 Study hospital 4

Study hospital 4 was built in the 1980s and accommodated approximately 100 beds. The temperature regime was again the sole method used to control *Legionella* and the advocated temperatures were being maintained. Mixing valves had to be, however, fitted to reduce hot water temperatures to non-scalding temperatures and rubber lined flexible hoses and rubber fittings were attached to several outlets, which were considered ideal for *Legionella* proliferation.

The water was supplied to the site via a mains pipe and was passed to two tanks from which water was provided to the whole site. The capacity of each tank was approximately 20000 litres. The hot water was de-scaled by electronic de-scaling devices.

One copper and silver ionization system, with three copper electrode chambers and one silver electrode chamber, was installed to treat the incoming mains supply. The system was installed on the incoming mains pipe before the two tanks, see Figure 3.3. The system was digital, similar to the systems installed in study hospitals 1, 2, and 3. The system was commissioned on the 08th April 2008.

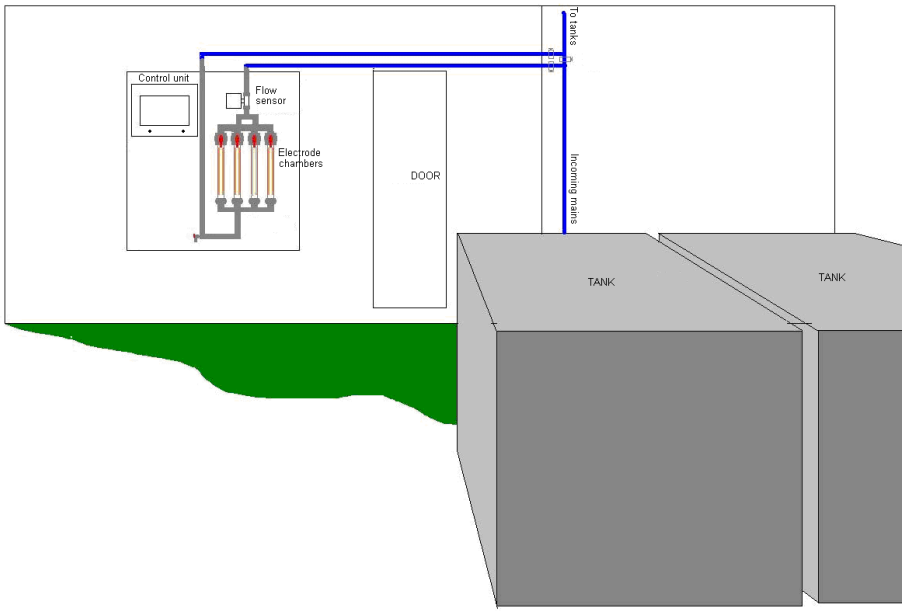


Figure 3.3 Schematic drawing of the copper and silver ionization installation in study hospital 4.

On the 08th April 2008, before the system was activated, samples were taken from 12 outlets.

On the 24th April and the 07th May 2008 re-samples were taken from the outlets at which *Legionella* were found in the samples taken on the 08th April.

Samples were taken monthly from the 07th May 2008 onwards from 6 outlets.

3.1.7 Study hospital 5

Study hospital 5 was a three storey building, built in the 1970s, which accommodated approximately 550 beds.

The temperature regime was applied as the sole *Legionella* control measure but it proved to be difficult to maintain cold water temperatures below 20ºC in some parts of the hospital due to cross heating and poor insulation. Approximately 600 mixing valves had to also be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, rubber lined flexible hoses and rubber fittings were also attached to several outlets, and water stagnation was possible due to poor use of outlets and ward closures. This was considered ideal for *Legionella* proliferation.

The water was supplied to the site by a mains pipe, which was divided in two, one side delivering water via two softening systems to a softened water storage tank, and the other side delivering water to a raw water storage tank.

One copper and silver ionization system with four copper electrode chambers and two silver electrode chambers was installed to treat the softened water and one copper and silver ionization system with eight copper electrode chambers and four silver electrode chambers was installed to treat the raw water. These systems were installed on the incoming mains pipe before the storage tanks, see Figure 3.4. Because water softening with salt reduces the concentration of positively charged ions (cations) in the water, such as calcium and magnesium, it also reduces the concentrations of copper and silver, which is why the system that treated the softened water was installed after the softening systems.

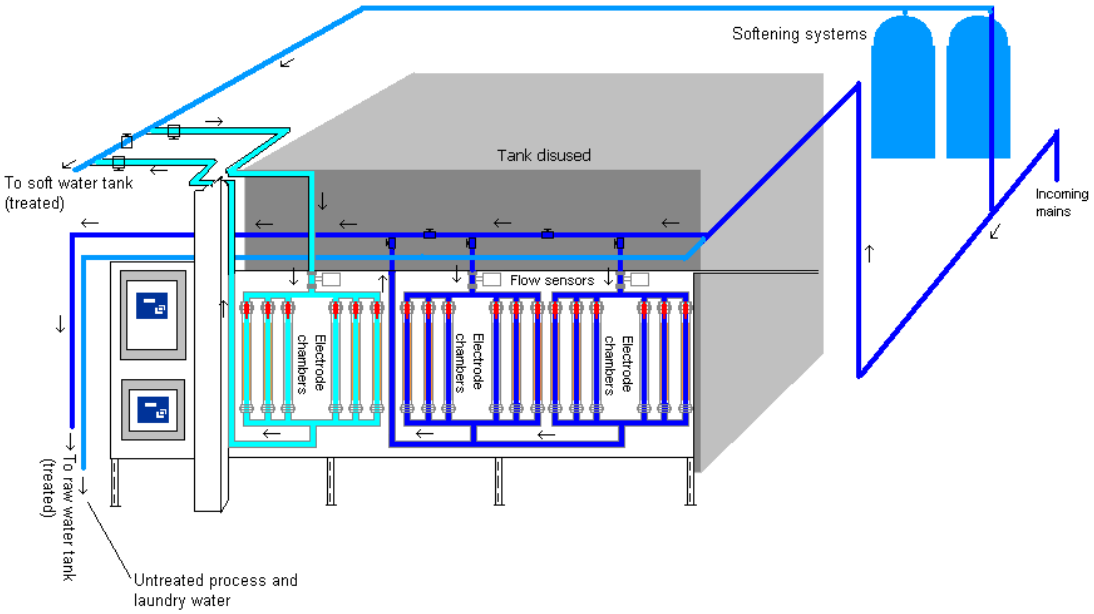


Figure 3.4 Schematic drawing of the copper and silver ionization installation in study hospital 5

The copper and silver ionization systems installed were digital systems, similar to the systems installed in the other study hospitals. The systems were commissioned on the 25th July 2008.

Before the systems were activated samples were taken from 51 outlets. Samples were taken from pure cold and pure hot outlets but in order to obtain a wider view of the potential contamination of the cold and hot water system, samples were also taken by filling the sample bottles half with hot water and half with cold water from 17 outlets. Less than one month after the systems were commissioned, samples were taken, on the 18th August 2008, from 25 outlets at which *Legionella* were found in the samples taken before the systems were commissioned.

From the 18th August 2008 onwards, samples were taken once every month from 20 outlets.

3.1.8 Study hospital 6

Study hospital 6 consisted of a combination of old and new buildings, dating from the 1950's to 2005, spread over a large site. The hospital accommodated approximately 550 beds.

The temperature regime was applied as the sole *Legionella* control measure but it proved to be difficult to maintain cold water temperatures below 20ºC in some parts of the hospital due to cross heating and poor insulation. Approximately 600 mixing valves had to also be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, rubber lined flexible hoses and rubber fittings were also attached to several outlets, and water stagnation was possible due to poor use of outlets and ward closures. This was considered ideal for *Legionella* proliferation.

The water was supplied to the site via two mains pipes and also from a borehole. The borehole water was used for the hot water supply. The water from the two mains was used for drinking and the cold water supply. The water was passed to numerous tanks around the site, all with varied capacities. The borehole water was softened. The water from the two mains was not softened.

One copper and silver ionization system, with four copper electrode chambers and four silver electrode chambers, was installed to treat the borehole water. As with study hospital 5, because water softening with salt reduces the concentrations of copper and silver, this system was installed after the softening systems and before the storage tanks, see Figure 3.5.

One copper and silver ionization system, with four copper electrode chambers and two silver electrode chambers, treated one of the two mains supplies, and one copper and silver ionization system, also with four copper electrode chambers and two silver electrode chambers, treated the other mains supply. These systems were installed before the storage tanks, see Figures 3.6 and 3.7.

The systems were digital, similar to the systems installed in the other study hospitals. The systems were commissioned on the 06th October 2008.

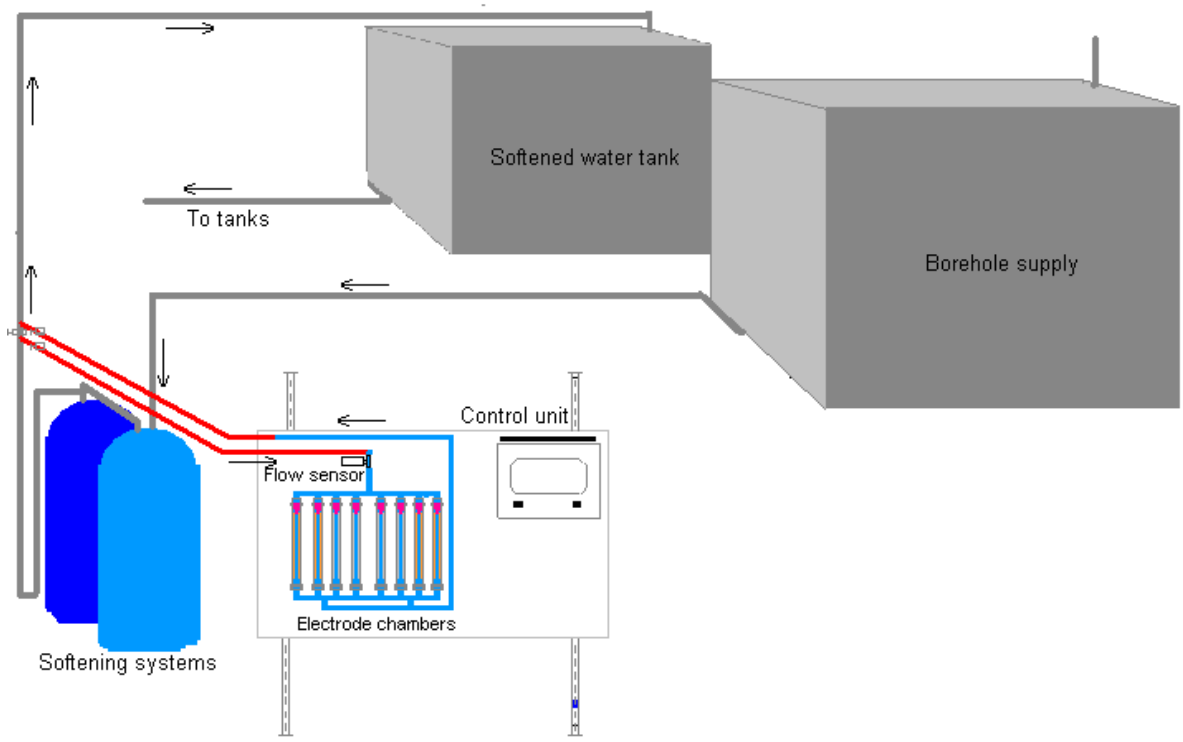


Figure 3.5 Schematic drawing of the copper and silver ionization installation in study hospital 6 – Borehole

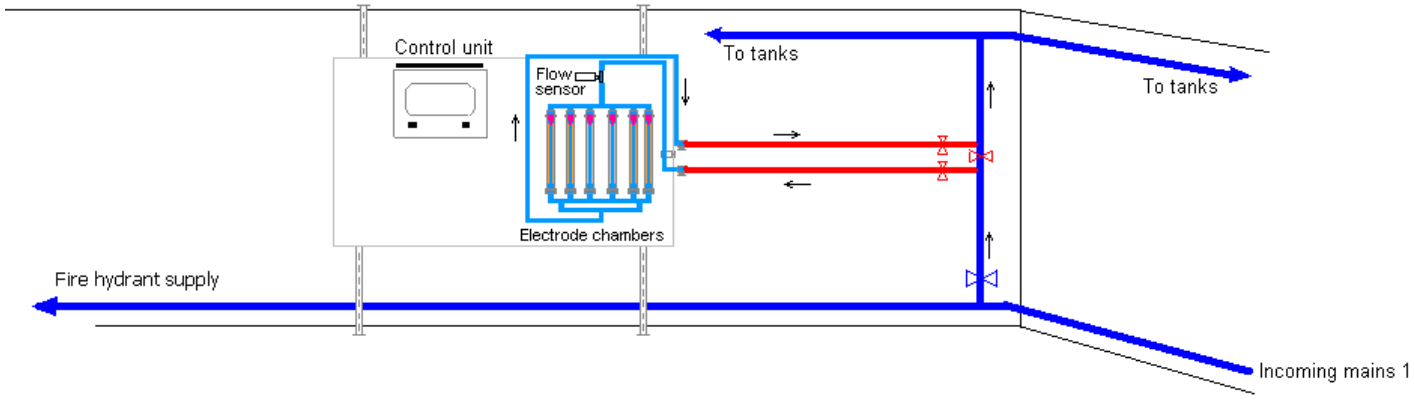


Figure 3.6 Schematic drawing of the copper and silver ionization installation in study hospital 6 – Incoming mains 1.

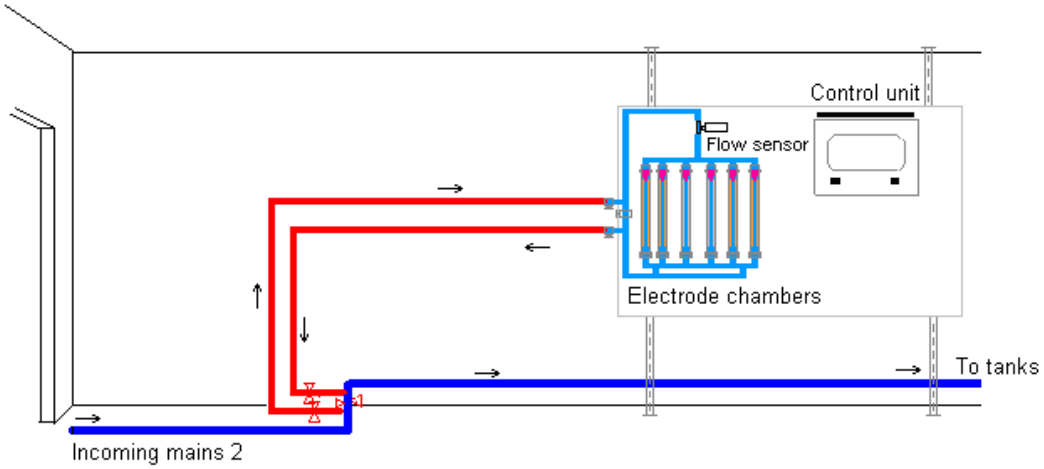


Figure 3.7 Schematic drawing of the copper and silver ionization installation in study hospital 6 – Incoming mains 2

On the 02nd October 2008, before the systems were activated, samples were taken from 60 outlets.

On the 27th November 2008, after the systems were activated, samples were taken from 25 outlets. Eleven of these outlets were outlets at which *Legionella* were found in the samples taken before the systems were commissioned.

Samples were taken monthly from the 27th November onwards from 25 outlets.

3.1.9 Study hospital 7

Study hospital 7 was a combination of old and new buildings, dating back to the 1930s, that spread over a large site. These buildings accommodated approximately 150 beds in total.

The temperature regime was applied as the sole *Legionella* control measure but it proved to be difficult to maintain cold water temperatures below 20ºC in some parts of the hospital due to cross heating and poor insulation. Mixing valves had to also be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, and rubber lined flexible hoses and rubber fittings were also attached to several outlets. This was considered ideal for *Legionella* proliferation.

The water was supplied to the site via two mains pipes and was passed to many tanks with various capacities. The water was not softened.

One copper and silver ionization system, with two copper electrode chambers and two silver electrode chambers, was installed to treat one of the incoming mains supplies, and one system, with one copper electrode chamber and one silver electrode chamber, was installed to treat the other incoming mains supply. Both systems were installed on the incoming mains pipes before the storage tanks, see Figures 3.8 and 3.9.

The systems were digital, similar to the systems installed in the other study hospitals. The systems were commissioned on the 25th November 2008.

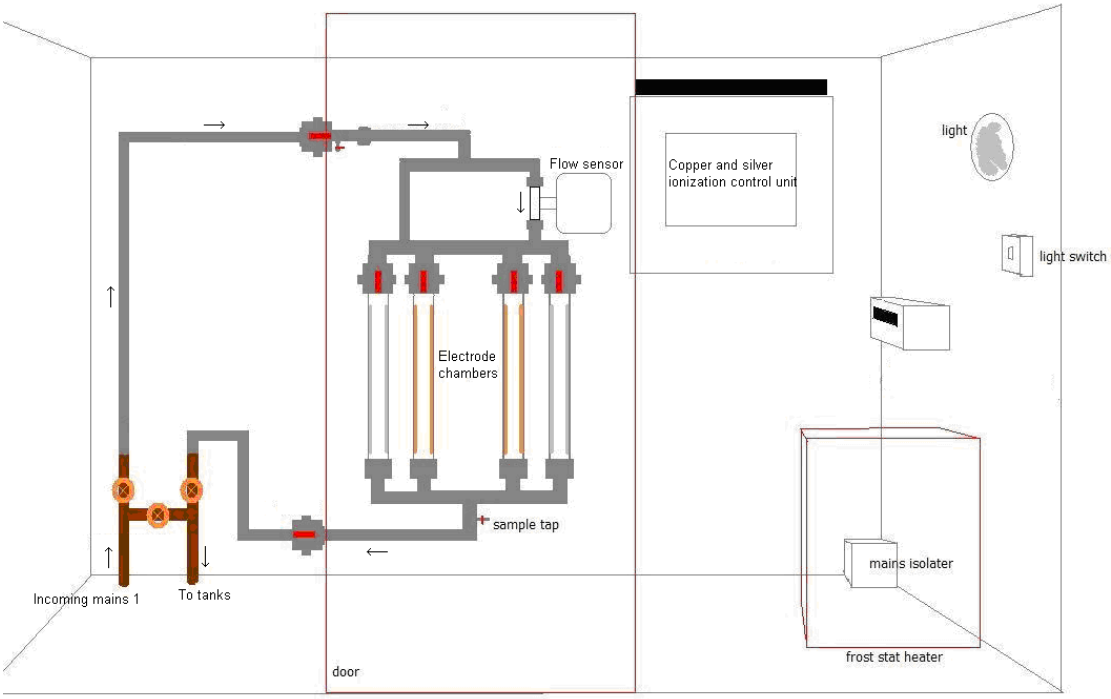


Figure 3.8 Schematic drawing of the copper and silver ionization installation in study hospital 7 – incoming mains 1.

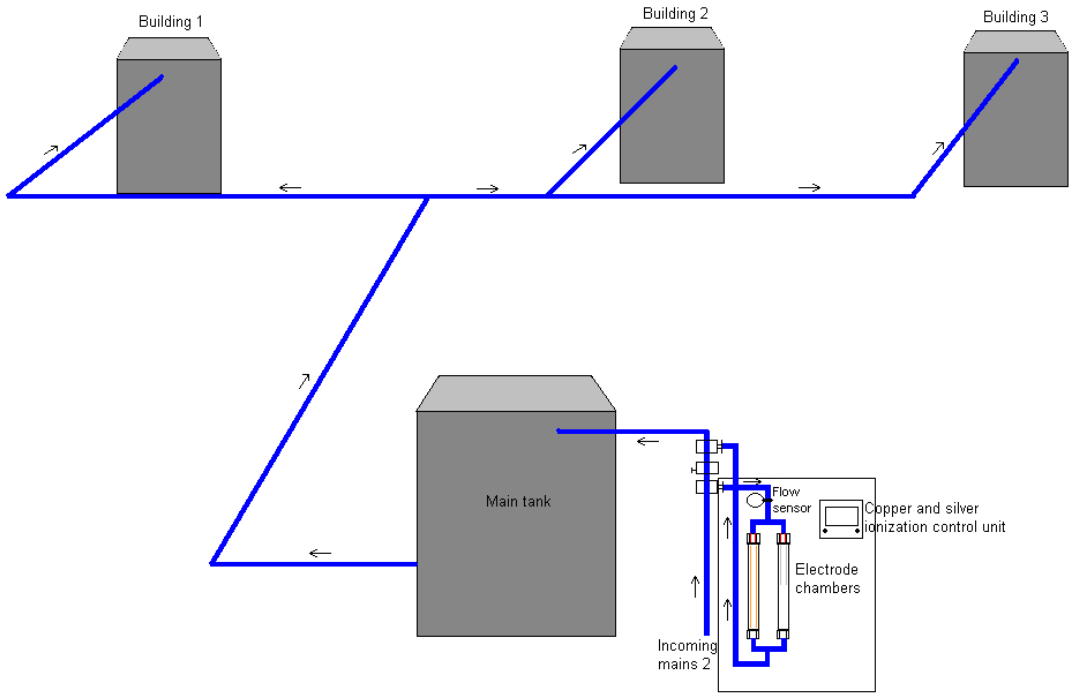


Figure 3.9 Schematic drawing of the copper and silver ionization installation in study hospital 7 – incoming mains 2

Before the systems were activated samples were taken from 26 outlets.

Six weeks after the systems were commissioned samples were taken, on the 05th January 2009, from 20 outlets at which *Legionella* were found in the samples taken before the systems were commissioned. Samples were again taken on the 04th February 2009 from 11 outlets at which *Legionella* were found in the samples taken on the 05th January 2009.

Samples were taken monthly from the 04th February 2009 onwards from 13 outlets.

3.1.10 Study hospital 8

Study hospital 8 consisted of buildings going back to the Victorian times. The hospital was, however, re-piped in 2000. The hospital accommodated approximately 200 beds.

The temperature regime was applied as the sole *Legionella* control measure but it proved to be difficult to maintain the advocated temperatures in some parts of the hospital. More than 600 mixing valves had to also be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, and rubber lined flexible hoses and rubber fittings were also attached to several outlets. This was considered ideal for *Legionella* proliferation.

The water was supplied to the site via a mains pipe and was passed to two tanks both with a storage capacity of 44000 litres, which supplied water to smaller tanks throughout the buildings. The water was not softened.

One copper and silver ionization system, with eight copper electrode chambers and four silver electrode chambers, was installed to treat the incoming mains supply. The system was installed on the incoming mains pipe before the two large tanks, see Figure 3.10. The system was digital, similar to the systems installed in the other study hospitals. The system was commissioned on the 27th April 2009.

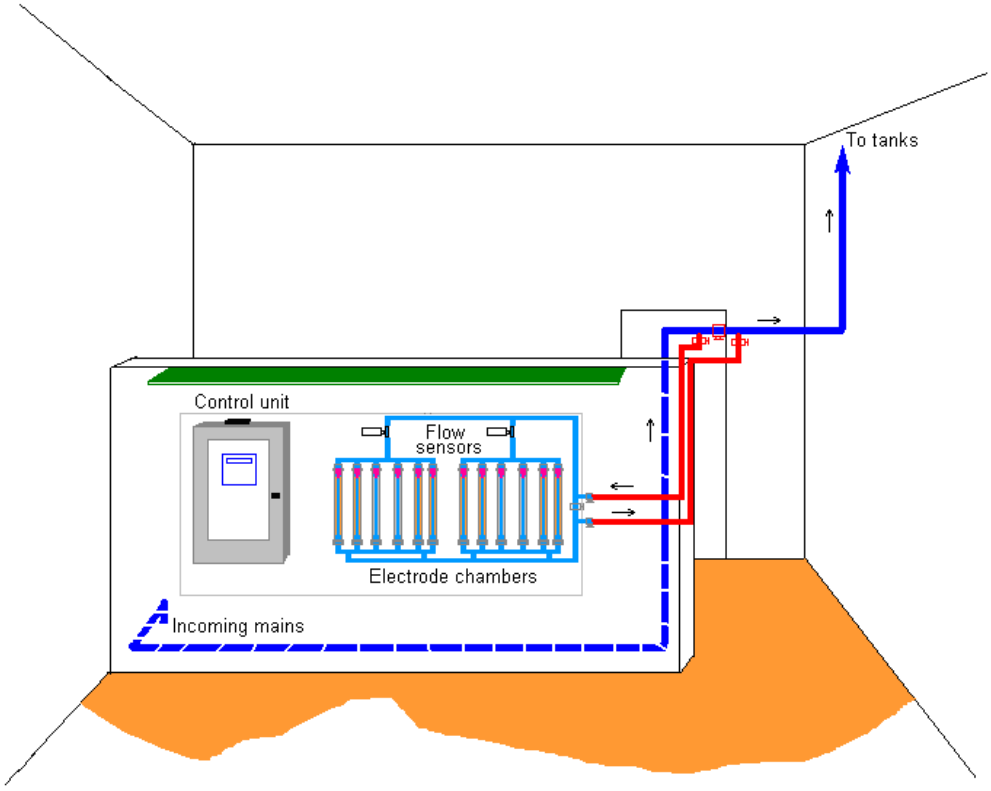


Figure 3.10 Schematic drawing of the copper and silver ionization installation in study hospital 8.

On the 27th April 2009, before the system was activated, samples were taken from 45 outlets.

One month later, on the 27th May 2009, samples were taken from 22 outlets. Eight of these outlets were outlets at which *Legionella* were found in the samples taken before the system was commissioned.

Samples were taken monthly from the 27th May onwards from 21 outlets.

3.1.11 Study hospital 9

Study hospital 9 was an orthopaedic hospital and consisted of a combination of new and old buildings dating from the 1940s to 2003. The hospital accommodated approximately 100 beds.

The temperature regime was applied as the sole *Legionella* control measure but it proved to be difficult to maintain cold water temperatures below 20ºC in some parts of the hospital due to cross heating and poor insulation. More than 100 mixing valves had to also be fitted to hot outlets to reduce the hot water temperatures to non-scalding temperatures, and rubber lined flexible hoses and rubber fittings were also attached to several outlets. This was considered ideal for *Legionella* proliferation.

The water was passed around the site directly from the incoming mains. No storage tanks were on site. However, from 2010 onwards extensive modifications to the water distribution system included the installation of two storage tanks. The water was not softened.

One copper and silver ionization system, with four copper electrode chambers and two silver electrode chambers, was installed in an enclosure outside the hospital. The system treated initially the mains water only. After the two tanks were installed the system also treated the water before it entered the two tanks. The system was installed on the incoming mains pipe, see Figure 3.11. The system was digital, similar to the systems installed in the other study hospitals, and was commissioned on the 03rd August 2009.

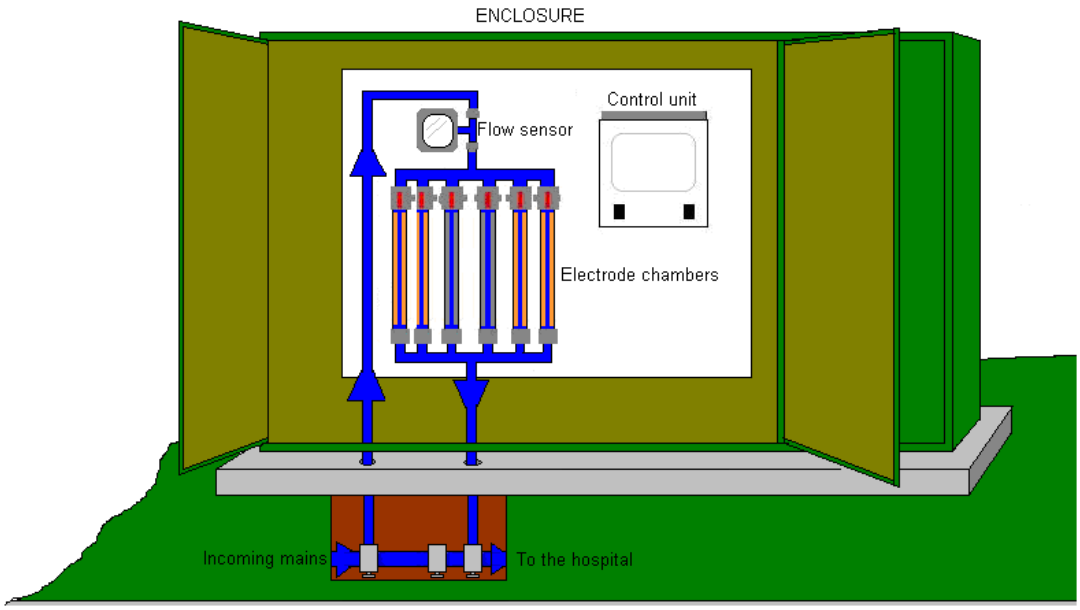


Figure 3.11 Schematic drawing of the copper and silver ionization installation in study hospital 9.

On the 29th July 2009, before the system was activated, samples were taken from 30 outlets.

On the 02nd September 2009, after the system was commissioned, samples were taken from 15 outlets, which included 13 outlets at which *Legionella* were found in the samples taken before the system was commissioned.

Samples were taken monthly from the 02nd September 2009 onwards from 15 outlets. From September 2010 onwards samples were taken once every two months from 15 outlets and once a month from 6 outlets.

Extensive construction work, including major modifications to the water system, see Plate 3.3 below, were started in May 2010. This work was not completed at the time of writing.

Plate 3.3 Study hospital 9 – photograph of construction work started in May 2010.

3.1.12 Study Hospital 10

Study hospital 10 is a nine-storey building, originally constructed in the 1960s and added to since then. The hospital accommodated approximately 850 beds.

The temperature regime was applied as a *Legionella* control measure but it proved to be difficult to maintain the advocated temperatures. The water system was, therefore, also treated with chlorine dioxide. *Legionella* was, however, not controlled by both measures and 3 patients contracted Legionnaires' disease from *Legionella* that was present in the water system.

The water was supplied to the site via one mains pipe and was passed to two large tanks. The hot water was softened. The softened water was stored in one of the tanks, holding 320m^3, and the raw water was stored in the other tank, holding 800m^3.

One copper and silver ionization system with four copper electrode chambers and four silver electrode chambers was installed to treat the softened water and water softening with salt reduces the concentrations of copper and silver, this system was installed after the softening systems and before the storage tank, see Figure 3.12.

One copper and silver ionization system with eight copper electrode chambers and four silver electrode chambers was installed to treat the raw water. This system was installed on the incoming mains pipe before the storage tank, see Figure 3.13. Both systems were digital, similar to the systems installed in the other study hospitals. The systems were commissioned on the 21st December 2009.

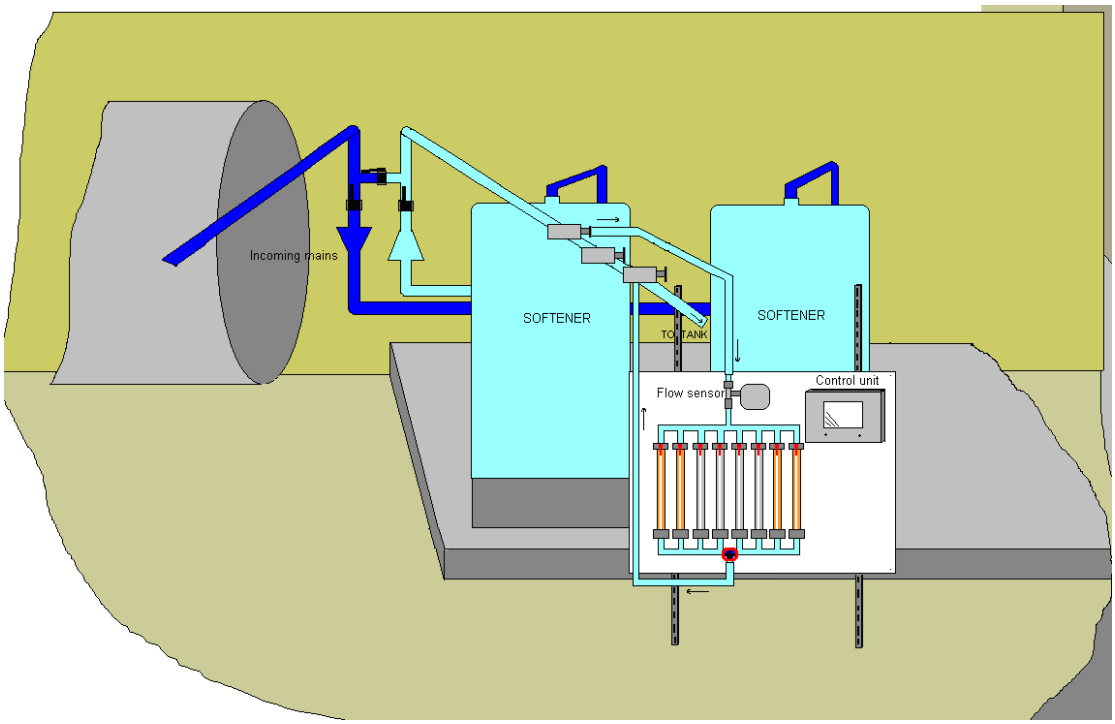


Figure 3.12 Schematic drawing of the copper and silver ionization installation in study hospital 10 – Soft water.

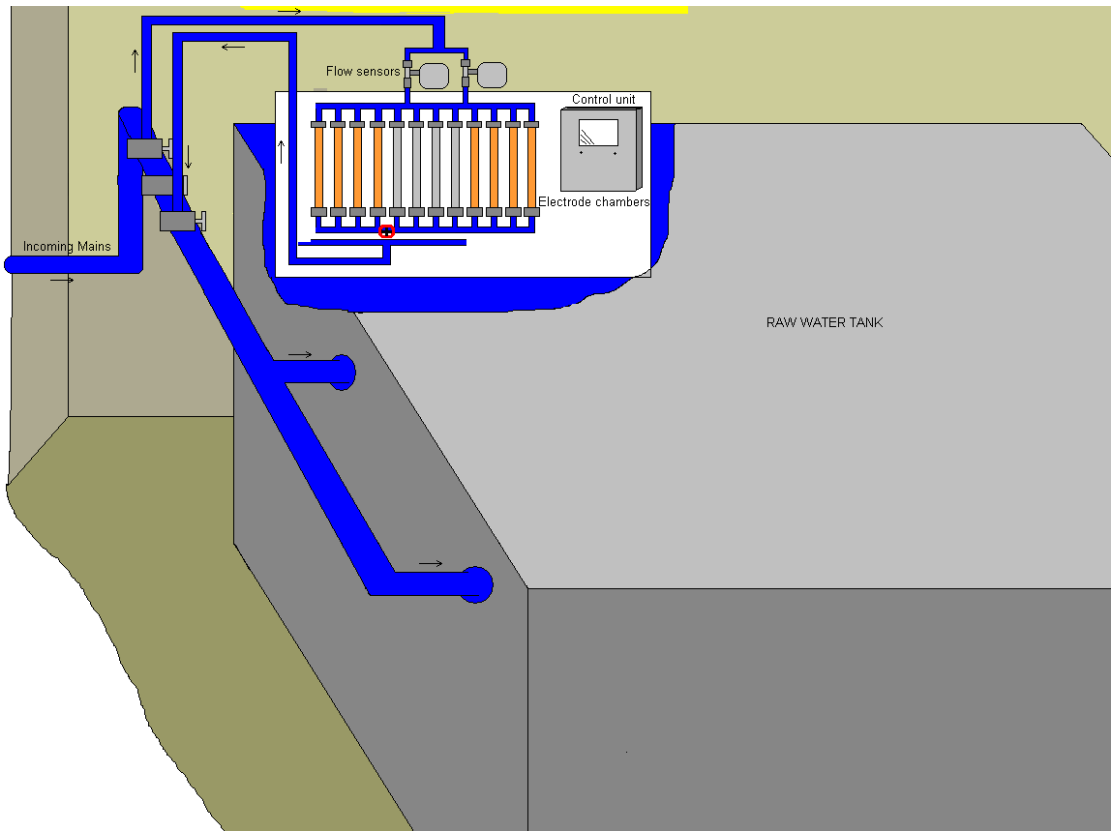


Figure 3.13 Schematic drawing of the copper and silver ionization installation in study hospital 10 – Raw water.

On the 15th December 2009, before the systems were activated, samples were taken from 60 outlets.

One month later, on the 13th January 2010, samples were taken from 30 outlets, which included 15 outlets at which *Legionella* were found in the samples taken before the systems were commissioned.

Samples were taken monthly from the 13th January 2010 onwards from 30 outlets. From April 2011 onwards samples were taken monthly immediately after opening 30 outlets and also after running them for 2 minutes.

3.2 The copper and polyethylene rigs

Pneumonia is the leading cause of death in care residents and the risk of contracting Legionnaires' disease is, particularly, higher in the elderly. *Legionella* also tends to proliferate more in blended water systems than in pure hot and pure cold water systems.

Managing risk factors, such as a water temperature between 30ºC and 45ºC and the presence of biofilms, can limit the growth of *Legionella* in these systems but these factors can be difficult to manage, especially, in systems in which water is blended to avoid scalding and in systems in which materials are used that can promote biofilm formation.

Copper and polyethylene are plumbing materials that are commonly used in UK water systems and experiments with model systems have indicated that polyethylene can promote growth more than copper (Keevil *et al.,* 1993, van der Kooij *et al.,* 2005). Although the model systems constructed for these experiments endeavoured to replicate small hot water systems, they did not simulate a copper and a polyethylene piped hot water system of a typical small hospital in the UK, in which water is stored, blended and circulated. Biofilm formation and *Legionella* growth were also only examined in these model systems and *Legionella* control measures were not tested.

For this project, copper and polyethylene piped rigs were, therefore, constructed to not only examine the differences in biofilm and *Legionella* growth in copper and polyethylene piped water systems that simulate a typical blended water system of a small UK hospital but also to examine the effect of hot water temperatures of 50ºC and the effect of copper and silver ionization at temperatures below 45ºC in controlling *Legionella* in the rigs.

Initially two small scale rigs, simulating circulating hot water flow systems in copper and polyethylene piping, were designed and built. Four more identical rigs were built later on.

Each of these rigs consisted of 15mm diameter ‘push fitted’ pipe. For one rig copper material was used, and for the other rig polyethylene pipe (PE-X) BS 7291 material was used.

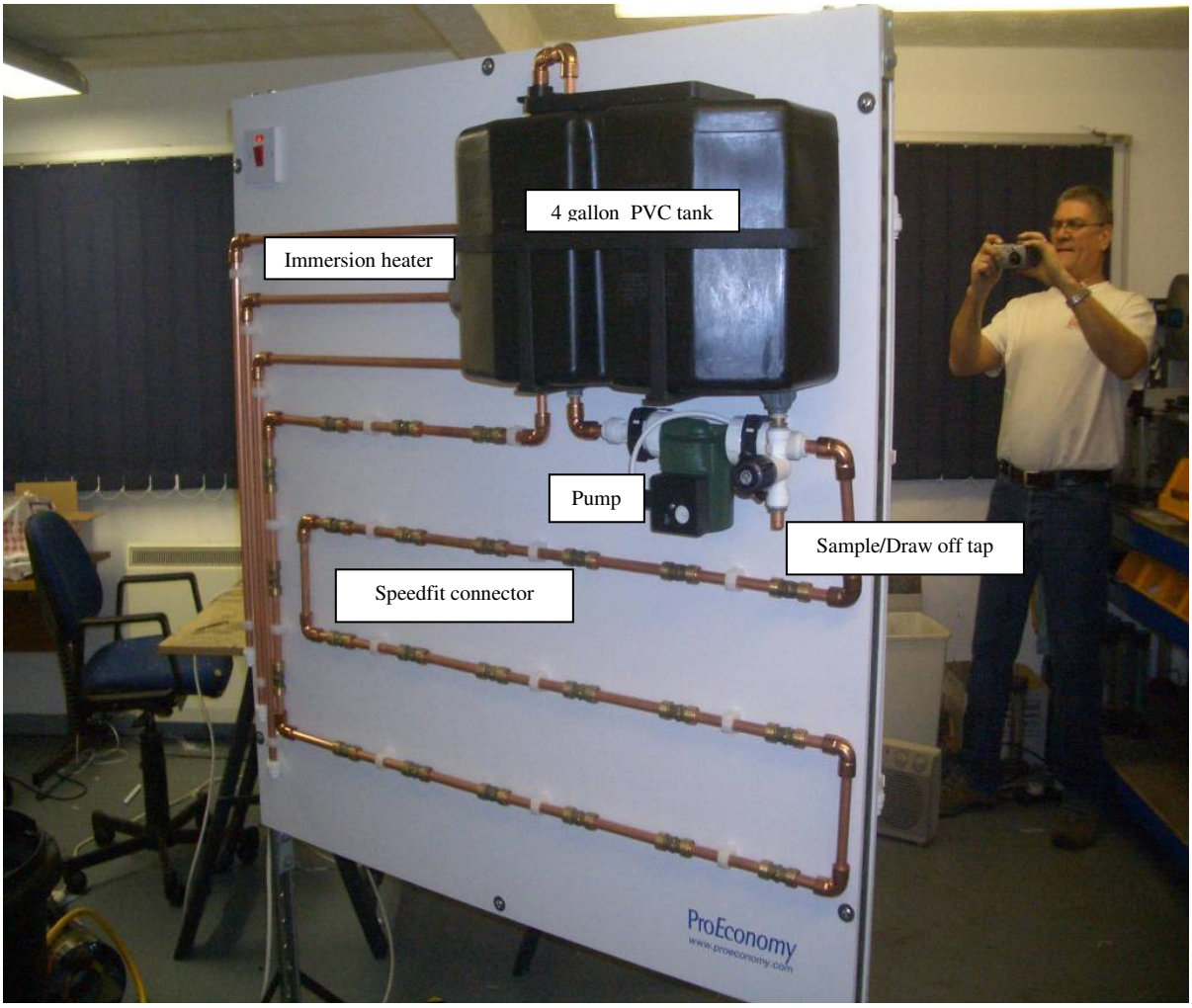


Plate 3.4 Rig with copper push fitted piping.

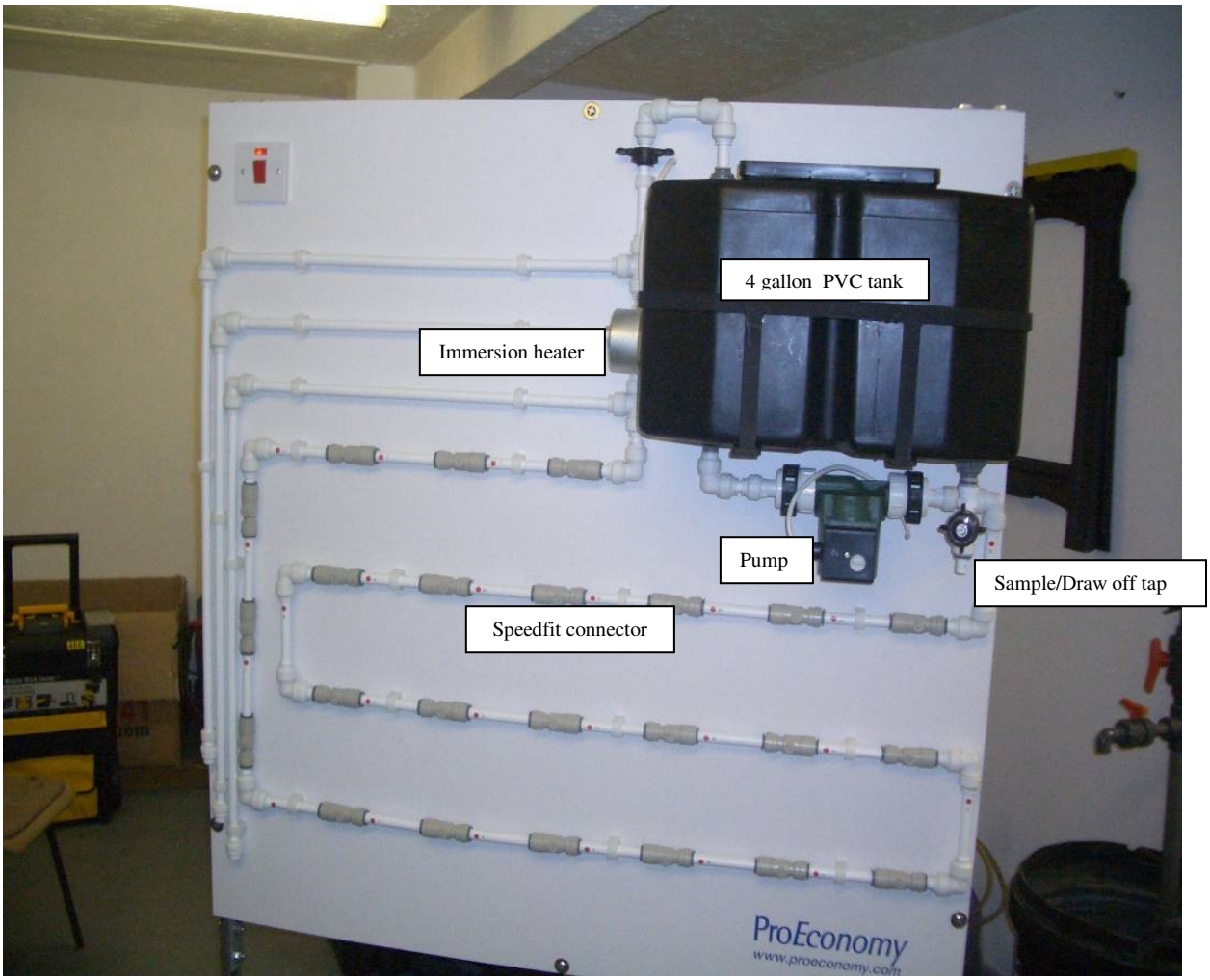


Plate 3.5 Rig with polyethylene push fitted piping.

In order to be able to collect samples to determine biofilm formation, the presence of total viable counts and *Legionella* in biofilm, 24 removable sections were introduced in each rig.

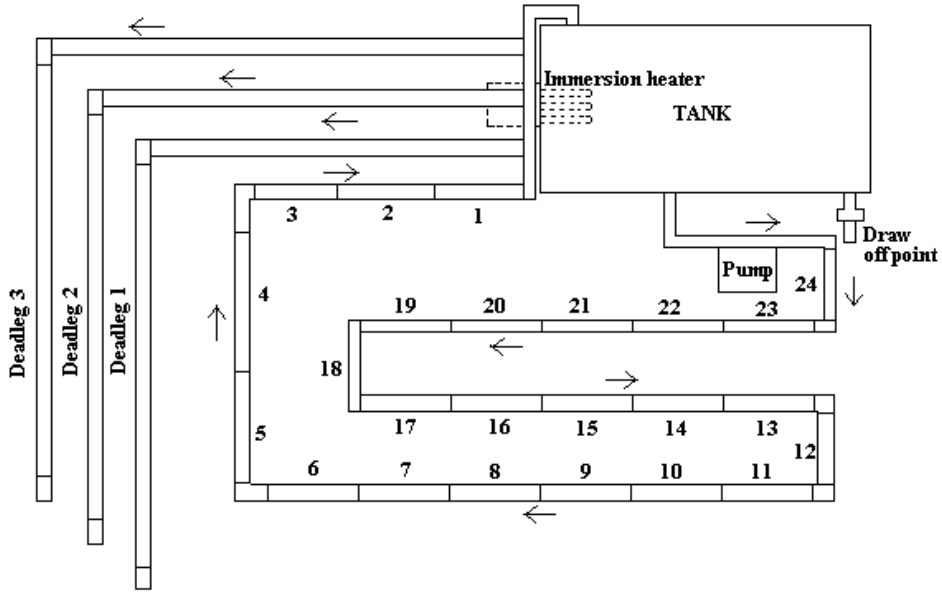


Figure 3.14 Rig plus 24 sections

These sections were connected by 15mm diameter ‘Speedfit’ pipe connectors on each end, which were commonly used in water systems to connect pipes. A ‘Speedfit’ connector contained a collet which had stainless steel teeth that gripped the pipe. It also contained two rubber ‘O’ rings, which provided a leak-proof seal, and a valve to enable isolation.

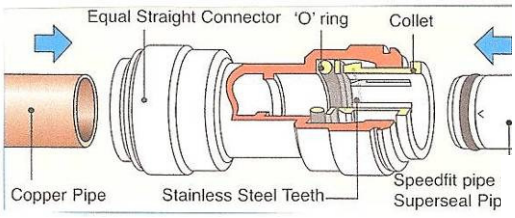


Figure 3..15 Speedfit connector (www.speedfit.co.uk)

The pipe sections were each 15mm diameter, 15.24cm long, and were made of either copper or polyethylene. The sections could be easily removed using a special tool and when removed were replaced with new sections.

The height of each rig was 1.22m, the width was 1.22m. All rigs were mounted, back to back, on a movable board. The total length of the pipes of each rig was 538cm each.

The PVC tank of each rig held approximately 15 litres of water. The water was circulated through each rig using a brass circulating pump. The water was heated

using an 11 inch long stainless steel immersion heater (immersed in each tank). The flow-rate of the water going through each rig was approximately 2 litres per minute. The flow could be reduced, if necessary, by changing the settings of the valve nearest to the tank input.

3.2.1 Initial experimental procedures – Rigs A

The experiment with the first built rigs (Rigs A) was designed to develop and monitor a microbial population for comparison between the copper piped rig and the polyethylene piped rig.

Both rigs were run at a temperature of ~ 42ºC.

Mains water was introduced into the rigs and, to simulate hot water use whilst not loosing the introduced micro-organisms, three litres of water were manually drawn from the rigs every day. The rigs were also manually re-filled with three litres of mains water every day. This represented storage of ~5 days. In reality, it is, however, advised not to store water for longer than 24 hours.

To introduce bacteria into the rigs and to encourage biofilm formation, both rigs were deliberately filled with one litre of water with pre-established counts of viable bacteria (Total Viable Counts or TVCs) as shown in Table 3.2 below.

TVC @ 37º 2 days CFU/ml	**TVC @ 22º 3 days CFU/ml**	***Legionella* Species CFU/l**	***L. pneumophila* Serogroup 1 CFU/l**	**Day introduced to Copper rig**	**Day introduced to Polyethylene rig**
770	330	Not found	Not found	None	102
3470	2260	Not found	Not found	102	None
330	440	Not found	Not found	None	123
605	550	Not found	Not found	123	None
5450	7430	Not found	Not found	None	179
3960	9410	Not found	Not found	179	None
14	2	Not found	**1800**	260	260
3	16	**900**	Not found	285	285

Table 3.2 Initial experiment - Total viable bacteria and *Legionella* bacteria added to rigs A

Analysis for viable bacteria present in the rigs was carried out following the standard culture method (BS EN ISO 6222:1999). Table 3.3 below shows the pattern of water sampling for this analysis.

Day	Day
0	417
7	424
14	431
19	438
27	445
33	453
70	459
80	466
90	473
97	478
110	480
119	487
128	494
137	501
155	508
162	515
193	522
211	529
239	536
246	543
268	550
284	557
302	564
324	569
333	570
344	571
355	572
372	575
383	576
387	577
395	578
404	579
409	582
410	

Table 3.3 Initial experiment Rigs – Frequency of TVC analysis.

Sixty seven samples were taken from each rig. The last samples were taken on day 582.

On day 260 the rigs were seeded with one litres of water that contained 1800CFU/l of *L. pneumophila* serogroup 1, and on day 285 the rigs were seeded with one litre of water that contained 900CFU/l of *Legionella* non-pneumophila as shown in Table 3.2. One litre samples were, therefore, taken from both rigs for *Legionella* analysis from day 324 onwards as shown in Table 3.4 below.

Day	Day
324	473
333	480
344	487
355	494
372	501
383	508
387	515
395	522
404	529
409	536
410	543
417	550
424	557
431	564
438	571
445	578
453	585
459	
466	

Table 3.4 Initial experiment Rigs - Frequency of *Legionella* analysis.

Thirty seven samples were taken from each rig. The last samples were taken on day 585.

The pattern of biofilm development was followed by periodic removal of sections. Pipe sections of each rig were isolated by closing the valves of the 'Speedfit' connectors. The pipe sections were then removed by using a special tool to separate the connectors from the pipe. The pipe sections were then aseptically released in a sterilized, plastic bottle containing 500ml of distilled water.

In order to investigate the best way to remove and to quantify any biofilm from the inner surfaces of the pipe sections a number of different techniques were attempted as shown in Table 3.5 below.

Section	Time in rigs Days	Procedure
1	19	Shaken/biomass/TVC
2	27	Shaken/biomass/TVC
3	70	Shaken/biomass/TVC
4	80	Shaken/biomass/TVC
5	89	Shaken/biomass/TVC
6	128	Shaken/biomass/TVC
7	128	Sonicated/biomass/TVC
8	211	Control section
9	211	Sonicated/biomass/TVC
10	211	Shaken/biomass/TVC
11	211	Brushed/biomass/TVC
12	284	Brushed/biomass/TVC
13 and 14	387	Brushed/biomass/TVC
15	409	Brushed/biomass/TVC
16	478	Brushed/biomass/TVC

Table 3.5 Initial experiment Rigs - Pattern and procedures of biofilm harvesting and time of sections in rigs A.

The first method involved placing removed sections together with 500ml distilled water in a sonification bath for 1 hour after which the suspended solids were collected and weighed. This was done by recording the weight of a filter, placing it on a filtration manifold, filtering the sample through the filter, placing the filter in an oven at 40ºC, drying it, then weighing the filter again, and deducting the initial weight result from that of the dried filter. An analytical balance was used for the weighing. The second method involved shaking removed sections in 500ml distilled water on a rotary shaker for one hour after which the suspended solids obtained were dried and weighed. In the third method, removed sections were brushed through 5 times with a sterilized 50cm long bottle brush holding the section half way into the bottle filled with 500ml of distilled water, after which the sample was also dried and weighed. The third method was introduced as it was felt that strongly adherent biofilms could withstand the first 2 treatments and not be fully removed by them.

The highest weight was found in the brushed samples. All sections were from then on, therefore, brushed through to remove biofilm.

Initially, viable biological material within the brushed samples was also analyzed by the standard culture method (BS EN ISO 6222:1999), shown also in Tables 3.6 and 3.7. The result, expressed in colony forming units per ml, was then multiplied by 500, because the sample was 500ml, which was then divided by 7000mm^2, approximately the inner surface area of the pipe section (surface area = circumference (πd = 3.14 x 15mm = 47.1mm) x length (15.24cm or 152.4mm) $\approx$ 7000mm^2), which gave the approximate colony forming units per mm^2. A result of >1000/mm^2 was considered a substantial biolayer.

The presence of *Legionella* in biofilms on the inner surfaces of pipe sections of the rigs was monitored by analyzing brushed through pipe section samples by the culture method (ISO 11731:1998). Table 3.6 below shows the pattern of the sampling for *Legionella* analysis of biofilm in the sections.

Section	**Time in rigs Days**
1	19
13 and 14	387
15	409
16	478
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16	547, 539, 496, 486, 477, 436, 436, 353, 353, 353, 353, 220, 177, 177, 155, 86

Table 3.6 Initial experiment Rigs - Pattern of sampling for *Legionella* analysis of biofilm of rigs A.

Legionella sampling of the biofilm in the pipe sections commenced after a population of *Legionella* organisms was detected in the rigs' water. Sections representing residence times of between 86 and 547 days were all analyzed at one time point, on day 564, to investigate whether a pattern of biolayer development could be shown.

3.2.2 Additional experimental procedures – Rigs B and C

Two more copper and two more polyethylene rigs (Rigs B and C), identical to rigs A, were built to compare the efficacy of controlling *Legionella* by elevated temperatures with copper piping and polyethylene piping, and to compare the efficacy of controlling *Legionella* by copper and silver ionization with copper piping and polyethylene piping, see Table 3.7 below.

Rigs	**Treatment**	**Temp. ºC**
A	Copper and silver ionization	< 45
B	None	< 45
C	Temperature	~ 50

Table 3.7 Treatment of rigs A, B and C.

To obtain a similar microbial load as in rigs A, water from rigs A was introduced into rigs B and C. The copper rigs B and C were each filled with one third of the water from the copper rig A and the polyethylene rigs B and C were each filled with also one third of the water from the polyethylene rig A.

To obtain similar biofilm layers and to encourage biofilm formation in rigs B and C, sections of rigs A were placed in rigs B and C.

Figures 3.16, 3.17, and 3.18 below show the sections removed from and left in the polyethylene rig A and the sections replaced in the polyethylene rigs B and C.

Sections 18, 17, 16, 15, 14, 13, 12, and 11 of the polyethylene rig B were replaced with sections 18, 17, 16, 15, 14, 13, 12, and 11 of the polyethylene rig A. Sections 11, 10, 9, 8, 7, 6, 5, and 4 of the polyethylene rig C were replaced with sections 10, 9, 8, 7, 6, 5, 4, and 3 of the polyethylene rig A.

Figures 3.19, 3.20, and 3.21 below show the sections removed from and left in the copper rig A and the sections replaced in the copper rigs B and C.

Sections 23, 22, 21, 20, 19, 18, 17, and 16 of the copper rig B were replaced with sections 23, 22, 21, 20, 19, 18, 17, and 16 of the copper rig A. Sections 23, 22, 21, 20,

19, 18, 17, and 16 of the copper rig C were replaced with sections 15, 14, 13, 12, 11, 10, 9, and 8 of the copper rig A.

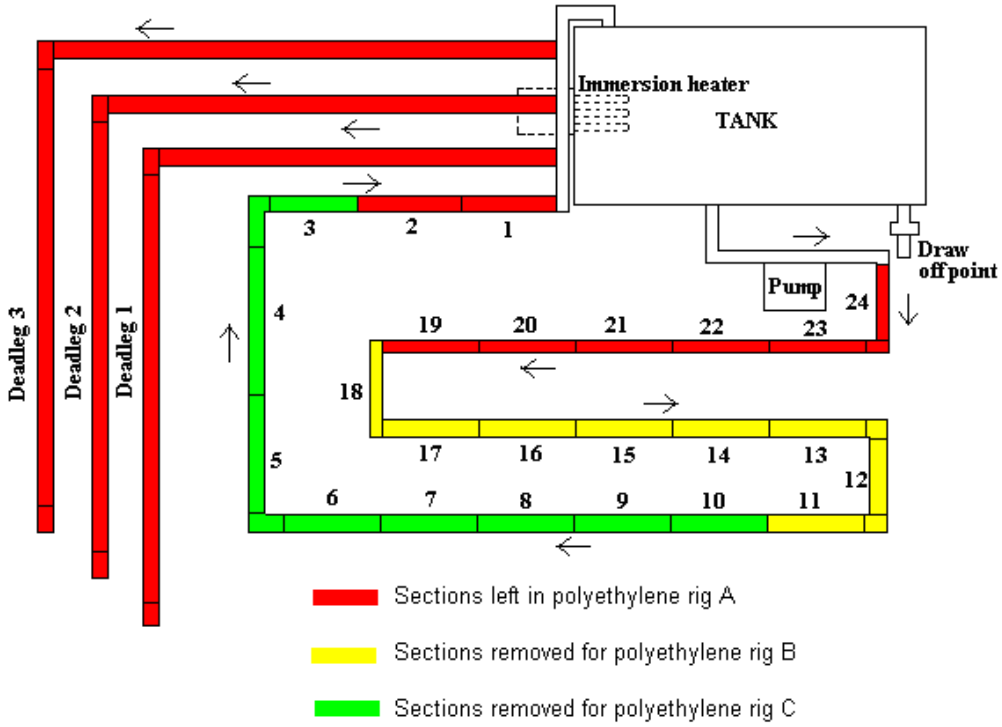


Figure 3.16 Polyethylene rig A sections left and removed.

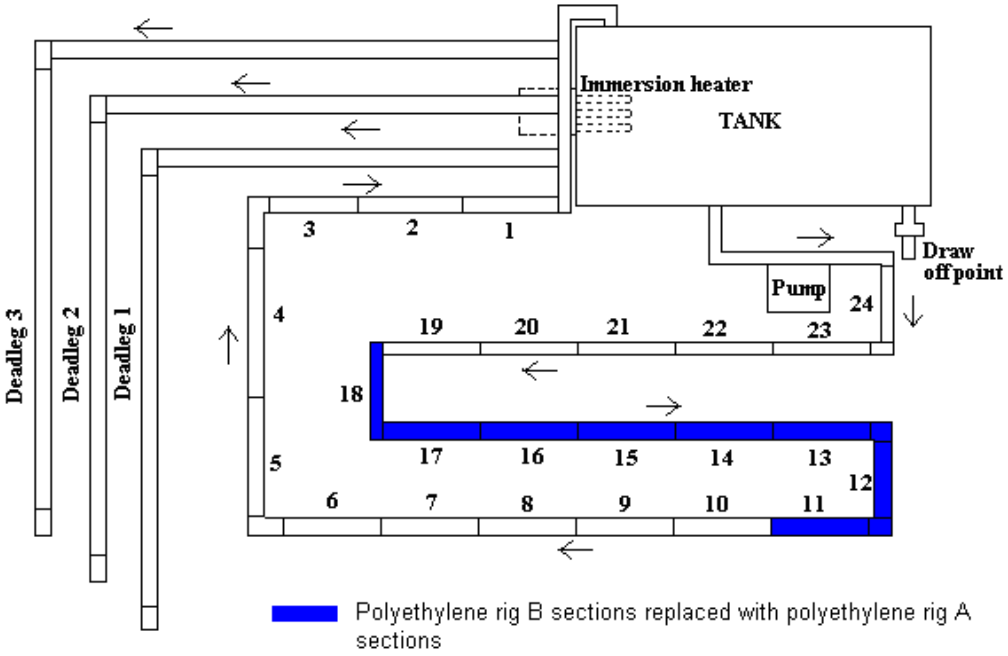


Figure 3.17 Polyethylene rig B sections replaced with polyethylene rig A sections.

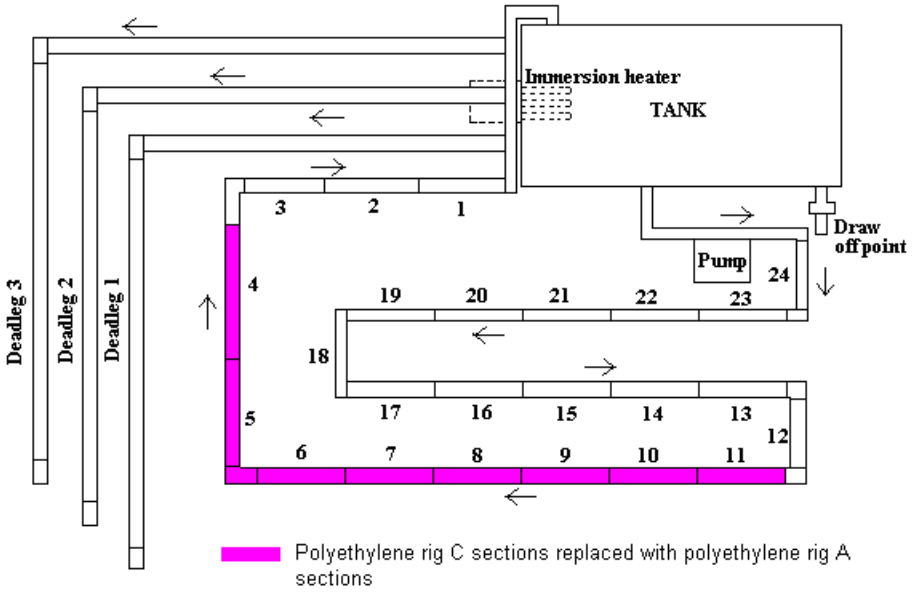


Figure 3.18 Polyethylene rig C sections replaced with polyethylene rig A sections.

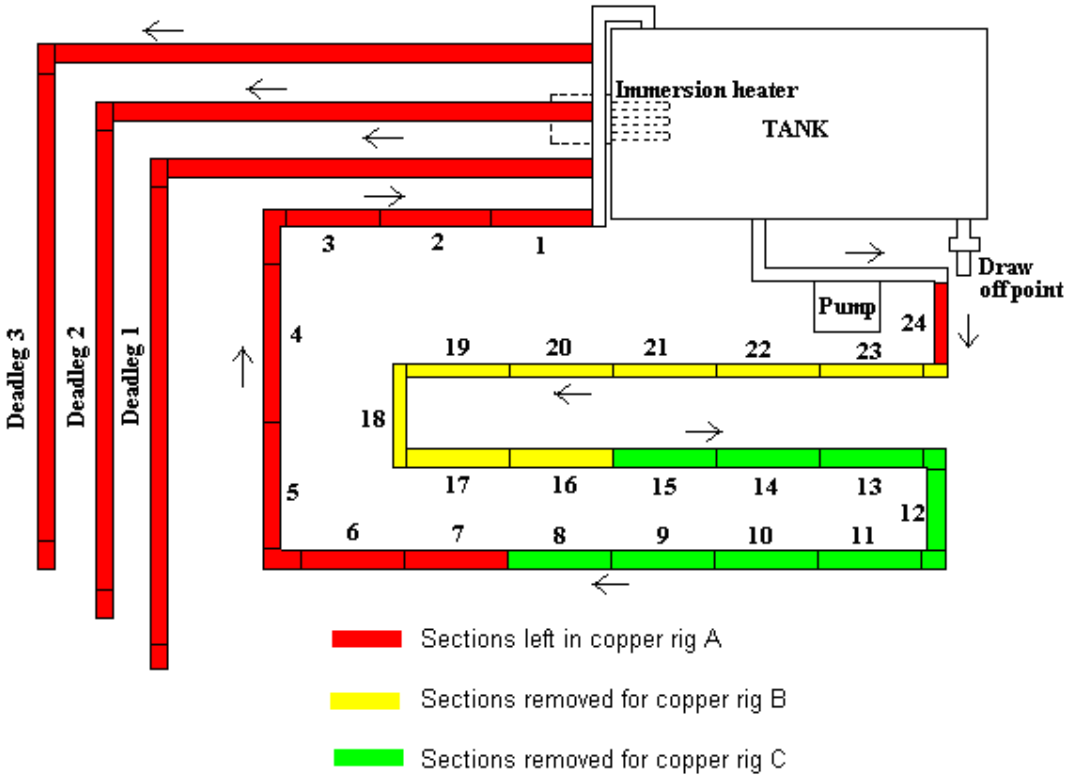


Figure 3.19 Copper rig A sections left and removed.

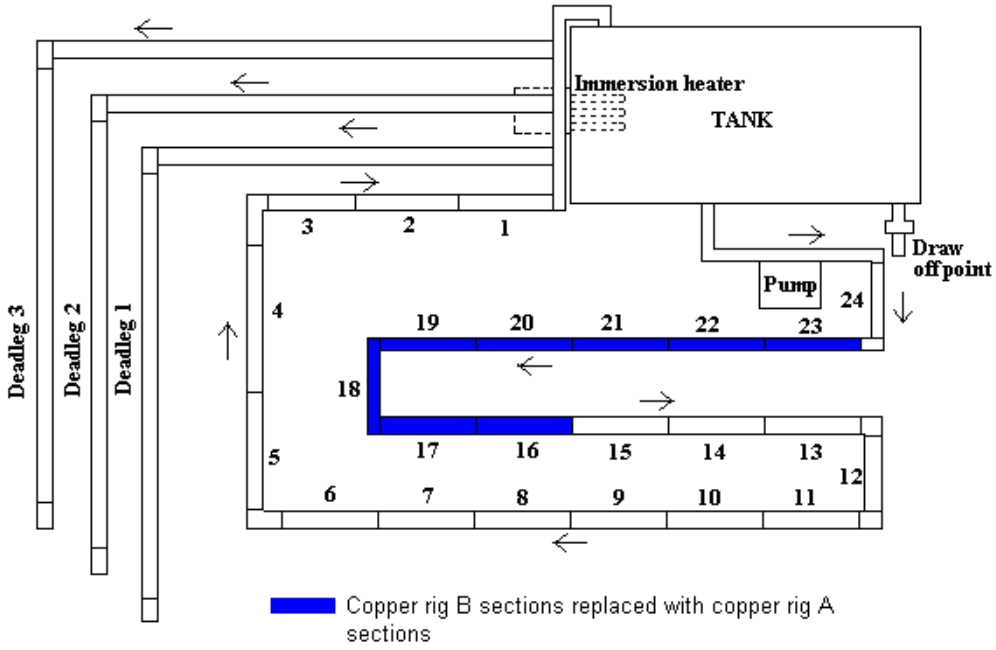


Figure 3.20 Copper rig B sections replaced with copper rig A sections.

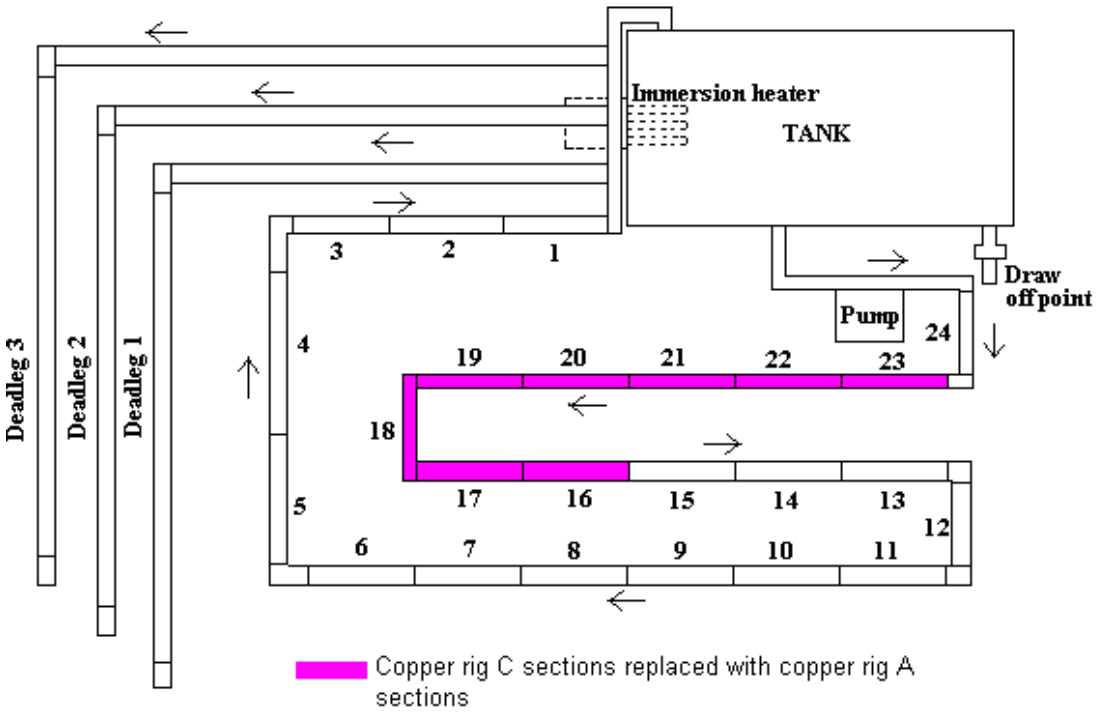


Figure 3.21 Copper rig C sections replaced with copper rig A sections

Three litres of water were manually drawn from all six rigs every day to simulate hot water use. Rigs B and C were automatically re-filled with three litres of mains water by a ball valve mechanism inserted in each tank and rigs A were re-filled manually every day.

Once *Legionella* populations were found in all rigs, treatment was started. Three litres of water from rigs A were manually replenished every day with water containing copper and silver ions. The temperatures in rigs A were kept below 45ºC.

The Orca copper and silver ionization system produced the copper and silver ions that were introduced into rigs A. This system was not attached directly to the rigs, as it was unsuitable for the small size of the experimental rigs. The system produced approximately 0.1mg/l of silver and 3.6mg/l of copper and three litres of this water were introduced into the rigs daily in a separate operation.

No treatment was added to the B rigs. The temperatures in these rigs were maintained below 45ºC, simulating a typical hot water circulating system in which mixing valves are used to blend water to a temperature below 45ºC to avoid scalding.

The temperatures in rigs C were maintained above 50ºC, simulating a pure hot water circulating system to which the temperature control regime as recommended in the ACoP (L8) and the HTM 04-01 documents is applied.

The temperatures in the six rigs tanks were recorded in ºC using an infrared non-contact thermometer. Recordings were done daily when the water was being drawn from the rigs.

125ml samples for analysis for copper and silver by Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) and Inductively Coupled Plasma Mass Spectrometry (ICPMS) were collected from the draw off points of all six rigs, also daily.

One litre of sample for *Legionella* analysis was collected once a week from the draw off points of all six rigs. The analysis for *Legionella* was carried out by the culture method (ISO 11731:1998).

The presence of *Legionella* in biofilms was monitored once a month by analyzing samples of pipe sections, brushed through in 500ml distilled water, by the culture method (ISO 11731:1998).

3.3 Analytical methods

3.3.1 Water analysis

Water analysis was carried out by a UKAS accredited laboratory.

(a) Total Viable Count (TVC) (BS EN ISO 6222:1999)

The standard culture method for analysis for total viable bacteria was applied. 1ml of the sample was plated, within 12 hours, onto prepared Petri dishes. The sample was incubated at 37ºC for 48 hours and at 22ºC for 72 hours, after which the number of colony forming units that had formed in the medium were calculated using a standard plate counter. The results were expressed in colony forming units per 1ml (CFU/ml). A colony forming unit represents a number of bacterial cells that has the ability to form a colony on the media.

(b) *Legionella* (ISO 11731:1998)

The standard culture method for analysis for *Legionella* bacteria was applied. The sample was filtered through a 0.2µm membrane filter. The filter was placed in a stomaching bag containing 10ml of 'Ringer's solution' and rubbed between thumb and finger to 'wash' the bacteria from the filter into the solution.

10ml of the solution produced was then split into 3 portions, one was tested without further treatment, one was heat treated at 50ºC (± 1ºC) for 30 minutes (± 2 minutes), and one was treated with acid (pH 2.2) left for 5 minutes (± 30 seconds). *Legionella* are resistant to these treatments whereas other bacteria may be killed, therefore, reducing the total amount of bacteria in the test solutions without (ideally) losing any *Legionella*.

0.1ml of each of the 3 solutions was then spread onto agar containing the selective agents: Glycine, vancomycin, polymyxin and cyclohexamide (GVPC) and incubated at 36ºC for 10 days. The plates were inspected 4 times during the 10 day incubation, on days 3, 5, 7 and 10. If colonies grew on any of the 3 plates during the 10 day period with the correct morphology, these were scored as 'presumptive' *Legionella*

positive. The number of 'presumptive' colony forming units present on the medium was then calculated. The 'presumptive' colonies from these plates were then sub-cultured onto two further plates. One with and one without supplements to determine the presence of *Legionella* species, which grow only on the plate with supplements: Buffered charcoal yeast extract agar with cysteine (BCYE+), and fail to grow on the plate without supplements, BCYE- (without cysteine).

To identify *L. pneumophila* serogroup 1 and serogroup 2-14 coloured latex beads coated in antibodies were used. These antibodies agglutinate if those species are present. The results were expressed in colony forming units per litre (CFU/l).

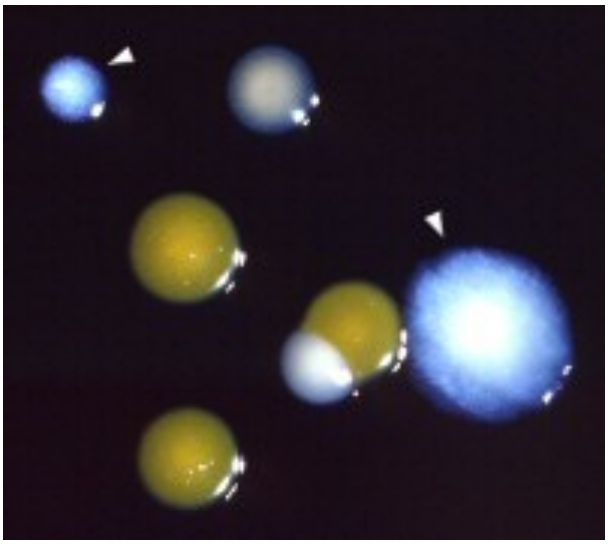


Plate 3.6 Two *Legionella pneumophila* colonies on BCYE+

(c) Phosphorus, copper and silver (Inductively Coupled Plasma Optical Emission Spectroscopy/Mass Spectrometry - ICPOES/ICPMS)

The sample was released into inductively coupled plasma, which was created from highly ionized argon gas. The sample was released into it as an aerosol suspended in argon gas formed by a nebulizer. When the sample aerosol stream passed through the plasma, desolvation and atomization occurred, this resulted in the constituent elements achieving an excited state.

In the case of optical emission spectroscopy (ICPOES), after excitation, the phosphorus and metal ions in the sample emitted light of characteristic wavelengths,

which were converted to an electrical current which was proportional to the concentration of the specific ions in the sample.

In the case of mass spectrometry (ICPMS), after excitation, the specific mass to charge ratio of the ions was measured and an electrical current was generated, which was proportional to the concentration of the specific ions in the sample. ICPOES was used to obtain phosphorus and copper concentrations, whereby, ICPMS was used to obtain the lower silver concentrations.

(d) Chloride (AQUA-800 Analyzer)

Analysis for chloride was carried out by the AQUA-800 Analyzer. The sample was placed on a sample wheel. Aliquots of the sample and reagents were dispensed into reaction vials and the reaction and colour development were allowed to proceed. After an appropriate delay the reaction mixture passed through a read head, which measured the intensity of the colour formed. By comparison to the readings obtained from previously analyzed standards of known strength the concentration of each analyte was determined.

(e) pH

Analysis for pH was carried out by measuring the electromotive force (EMF) of a cell comprising of an indicator electrode (which was responsive to hydrogen ions, such as a glass electrode) and a reference electrode, both immersed in a test solution. The EMF of this cell was measured with a pH meter, which was a high-impedance electrometer calibrated in terms of pH.

3.3.2 Statistical analysis

The results of all analyses and the temperature recordings were entered and interpreted using Microsoft Excel software.

The variability of the values was measured by taking the root mean square deviation of the values from their mean.

$$S=\sqrt{\frac{\sum(X-M)^2}{n-1}}$$

Where:

Σ = Sum of

X = Individual score

M = Mean of all scores

N = Sample size (Number of scores)

3.4 Experiments using the Robbins device

The Tyler Research Corporation (Edmonton, Alberta, US) manufactures a range of devices for the analysis of biofilms on surfaces, which includes the modified Robbins device.

Synthetic rubber, as well as copper and polyethylene, is also commonly used in UK water systems and it was suggested that synthetic rubber encouraged biofilm growth (Schofield and Locci, 1985, Keevil et al., 1993, Rogers et al., 1994, BSRIA Technical Note TN 9/96).

The Robbins device was, therefore, used to compare biofilm formation on rubber, copper and polyethylene discs. The device used consisted of 12 sampling ports in a linear array, see Plate 3.7 below. These ports accepted press-fit plugs that held 1 sample disc each with a surface area of 50mm^2. These discs were made of rubber, copper, and polyethylene. The design of the press-fit plugs was such that the surfaces of the rubber, copper and polyethylene sample discs became part of the channel wall. The sample discs were, therefore, exposed to the water that was circulated through the channel of the device.

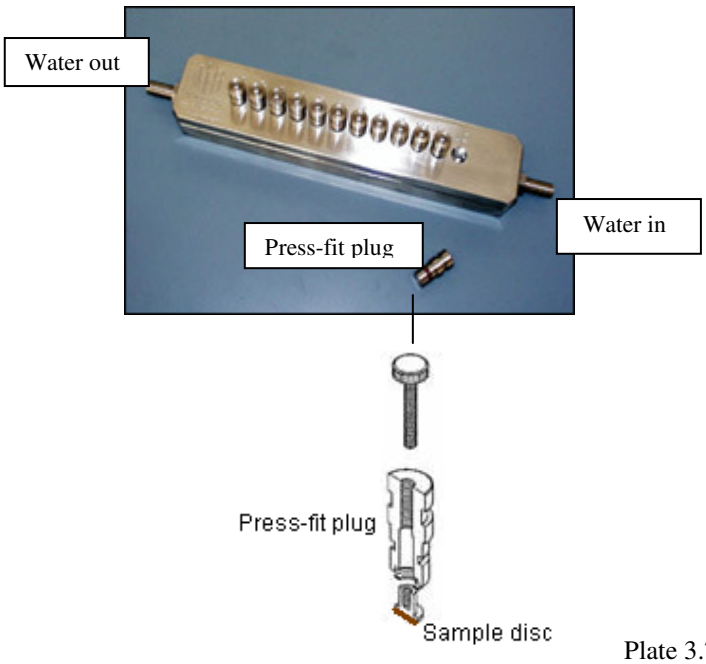


Plate 3.7 The Robbins device including drawing of press-fit plug (www.tylerresearch.com).

Before the experiments, the Robbins device was disinfected by pumping 20ml of methanol through the device, using a peristaltic pump (Watson Marlow Limited, Falmouth, Cornwall) set at 12 reps per minute, followed by 50ml of distilled water.

Six copper discs were first placed on the surface of six press-fit plugs. These plugs were fitted in the first six ports of the Robbins device.

The water that was circulated through the channel of the device as an inoculum source was inoculated by leaving unbrushed sections 20 and 21 from the polyethylene rig A in 500ml distilled water for 1 week. 500ml of this water was circulated through the device for 24 hours, using the peristaltic pump again set at 12 reps per minute. The temperature of the water was ~ 20ºC.

The sample discs were aseptically removed from the plugs after 24 hours. The discs were released in 10ml distilled water, and shaken for 30 seconds. 1ml of this sample was serial diluted from neat to 10^{-1}, 10^{-2}, and 10^{-3}. 0.1ml of the 10^{-2} and 10^{-3} dilutions were then spread onto trypto soya agar plates. The 10^{-1} dilutions were too overgrown and were therefore not used. Two plates were not spread and were used as controls. The plates were then incubated at 20ºC (± 2ºC), and checked after twenty-four hours.

In total three individual tests, as described above, were conducted with the copper discs, three individual tests with the polyethylene discs, and three individual test were done with the rubber discs (Tests 1, 2, and 3).

It was felt that strongly adherent biofilms would not be totally removed by shaking the discs in 10ml distilled water for 30 seconds. The surfaces of 4 copper, 4 polyethylene and 4 rubber discs that were exposed to the inoculated water for 48 hours, were, therefore, brushed using interdental brushes (Tests 4).

4. RESULTS

4.1 Study hospital 1

The results of samples taken on the 05th September 2007, before the copper and silver ionization system was activated, are shown in Table 4.1 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
71 142 HT	25	0.0003	0.116	**2100 s1**
71 142 CT	24	0.0003	0.142	**200 s1**
0.2.0016 HT	43	0.0003	0.049	**100 s1**
0.2.0016 CT	20	0.0003	0.029	**100 s1**
58-00-87 HT	40	0.0003	0.031	**1700 s1**
58-00-87 CT	21	0.0003	0.014	<LOD
58-03-112 HT	39	0.0003	0.043	**100 s1**
58-03-112 CT	22	0.0003	0.027	**100 s1**
67-0-008 HT	57	0.0003	0.041	<LOD
67-0-008 CT	28	0.0003	0.068	<LOD
68-01-045 HT	43	0.0003	0.043	<LOD
68-01-045 CT	21	0.0003	0.023	<LOD
68-01-05 HT	42	0.0003	0.037	**9800 s1**
68-01-05 CT	21	0.0003	0.019	<LOD
69-010-70 HT	38	0.0003	1.050	**7500 s1**
38-0-029 HT	34	0.0003	0.057	**1600 s1**
38-0-029 CT	27	0.0003	0.026	**1200 s1**
14-01-023 HT	47	0.0003	0.059	**200 s2-14**
14-01-023 CT	20	0.0003	0.021	<LOD
35-0-028 HT	28	0.0003	0.043	**4200 s1 + 100 np**
35-0-028 CT	20	0.0003	0.019	**200 s1**
62-02-067 shower	26	0.0003	0.068	<LOD
62-02-067 CT	19	0.0003	0.014	**200 s1**
39-01-86 HT	51	0.0003	0.081	<LOD
39-91-86 CT	20	0.0003	0.183	<LOD
39-00-65 HT	57	0.0003	0.053	<LOD
39-00-65 CT	22	0.0003	0.02	**3500 s1**
25-00-31 shower	26	0.0003	0.273	<LOD
25-00-31 HT	39	0.0003	0.067	**200 s1 + 1200 np**
25-00-31 CT	19	0.0003	0.04	<LOD
22-086 shower	40	0.0003	0.037	<LOD
22-086 HT	37	0.0003	0.029	<LOD
22-086 CT	20	0.0003	0.013	<LOD
21-1-137 shower	40	0.0003	0.118	<LOD
21-1-137 HT	42	0.0003	0.038	<LOD
21-1-137 CT	19	0.0003	0.013	<LOD
23-01-78 shower	43	0.0003	0.132	**1100 np**
23-01-78 HT	40	0.0003	0.083	**1100 np**
23-01-78 CT	19	0.0003	0.03	<LOD
29-00-07 HT	56	0.0003	0.062	<LOD
29-00-07 CT	34	0.0003	0.035	**8200 s1**
41-0-072 shower	40	0.0003	0.064	**500 s1**
41-0-052 HT	43	0.0003	0.073	<LOD
41-0-052 CT	19	0.0003	0.067	<LOD
681-08-22 HT	43	0.0003	0.065	<LOD
71-01-30 HT	39	NA	NA	<LOD

CT = Cold Tap, HT = Hot Tap, np = *Legionella* non-*pneumophila,* s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/L).

Table 4.1 Study hospital 1 - Results from samples taken on the 05th September 2007, before commissioning of copper and silver ionization system.

Legionella were found at 21 out of the 46 outlets tested, around 46% of the outlets tested were, therefore, contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 9800CFU/l. The average *Legionella* count of the 46 outlets sampled was 983CFU/l (± 331CFU/l). Hot and cold outlets were contaminated.

The highest *Legionella* counts were found in samples taken from hot outlets at which low hot water temperatures, below 50ºC, were recorded and in cold outlets at which elevated temperatures, above 20ºC, were recorded. Although not visible, thermostatically controlled mixing valves (TMVs) may have been blending the water at the hot outlets at which low hot water temperatures were recorded.

The copper found in the samples was due to copper leaching from copper pipes. The average was 0.08mg/l (± 0.023mg/l), which included the highest level of 1.050mg/l. Excluding the highest copper level the average copper found was 0.058mg/l (± 0.007mg/l).

The copper and silver ionization system was activated on the 11th September 2007.

Table 4.2 below shows the results of samples taken on the 05th October 2007 from the 21 outlets that were contaminated before the system was activated.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
71 142 HT	24	0.011	0.217	<LOD
71 142 CT	23	0.006	0.245	**2000 s1**
02.0016 HT	41	0.027	0.132	<LOD
02.0016 CT	18	0.041	0.186	<LOD
58-00-87 HT	40	0.031	0.131	**700 s1**
58-03-112 HT	39	0.026	0.104	<LOD
58-03-112 CT	18	0.047	0.181	<LOD
68-01-05 HT	41	0.021	0.116	<LOD
69-010-70 HT	55	0.014	0.111	<LOD
38-0-029 HT	33	0.038	0.173	**400 s1**
38-0-029 CT	25	0.048	0.17	<LOD
14-01-023 HT	50	0.009	0.065	<LOD
35-0-028 HT	27	0.04	0.183	**100 s1**
35-0-028 CT	17	0.048	0.179	<LOD
62-02-067 CT	19	0.059	0.176	<LOD
39-00-65 CT	21	0.004	0.157	<LOD
25-00-31 HT	39	0.027	0.147	<LOD
23-01-78 shower	41	0.029	0.579	<LOD
23-01-78 HT	39	0.03	0.173	**200 np**
29-00-07 CT	30	0.021	0.126	<LOD
41-0-072 shower	37	0.013	0.193	<LOD

CT = Cold Tap, HT = Hot Tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

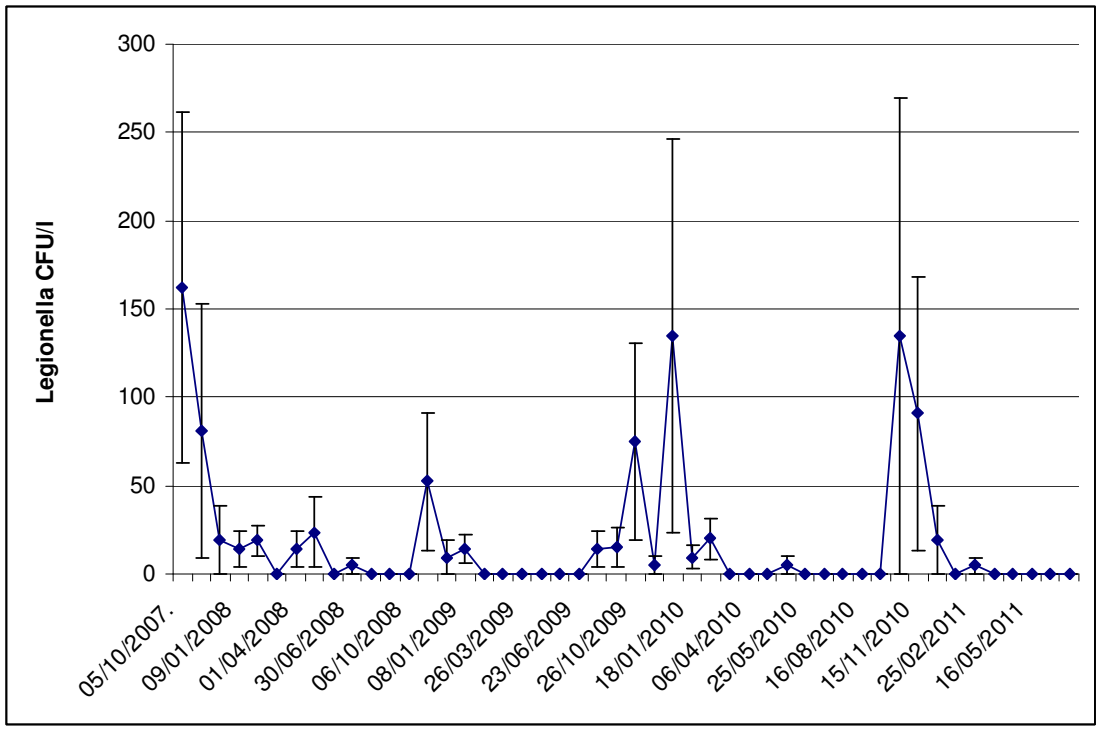
Table 4.2 Study hospital 1 - Results from samples taken on the 05th October 2007, one month after commissioning of copper and silver ionization system.

No *Legionella* were found in samples taken from 16 outlets that were previously contaminated. The *Legionella* colony forming unit counts also reduced. The average *Legionella* count was 161CFU/l (± 99CFU/l). The highest *Legionella* count was 2000CFU/l, found in a sample taken from a cold outlet. The temperature recorded at this outlet was 23ºC.

The cold temperatures remained above 20ºC at 4 out of the 8 cold water outlets tested and the hot water temperatures were again predominantly below 50ºC. A TMV was blending the water at the 38-0-029 hot outlet at which a *L. pneumophila* serogroup 1 count of 400CFU/l was found. TMVs were, however, not visible at the other hot outlets tested at which also *L. pneumophila* serogroup 1 organisms were found. The average hot water temperature was 39ºC (± 2ºC).

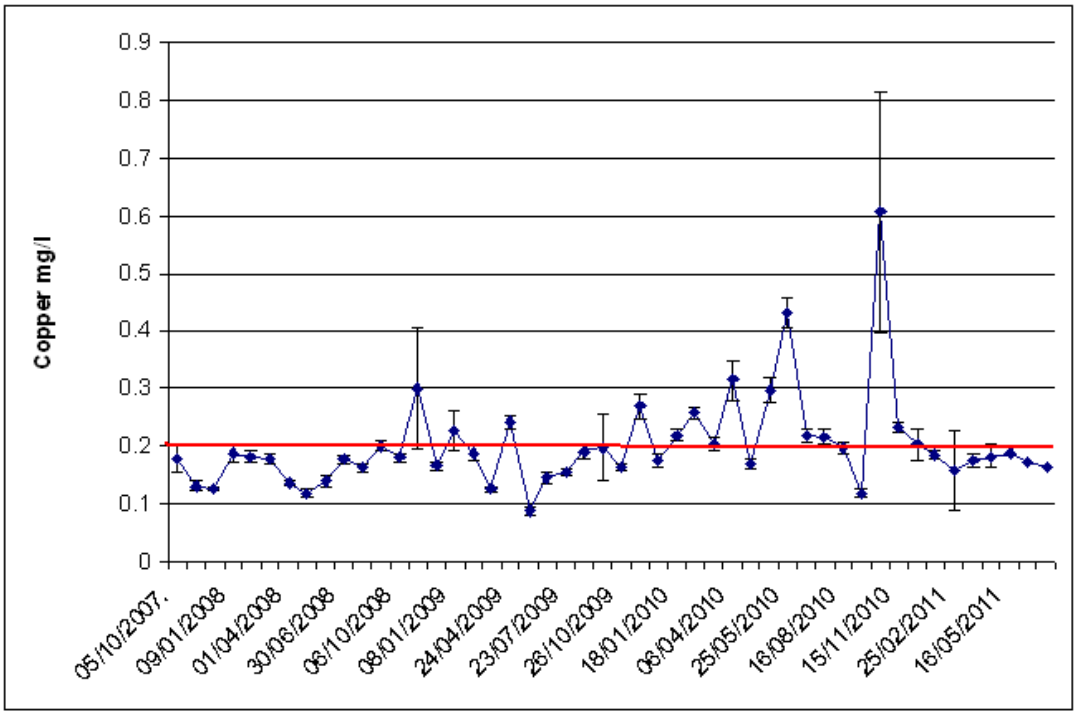
The silver found in the 21 outlets tested ranged from 0.004mg/l to 0.059mg/l. The average silver level was 0.028mg/l (± 0.003mg/l). The copper ranged from 0.104mg/l to 0.579mg/l. The average copper level was 0.178mg/l (± 0.022mg/l).

From the 05th October 2007 onwards, the *Legionella* contamination continued to decline in the blended and cold water system, see Graph 4.1 below. This graph shows the average *Legionella* counts in samples taken monthly from 21 outlets from the 05th October 2007 up to the 11th July 2011.



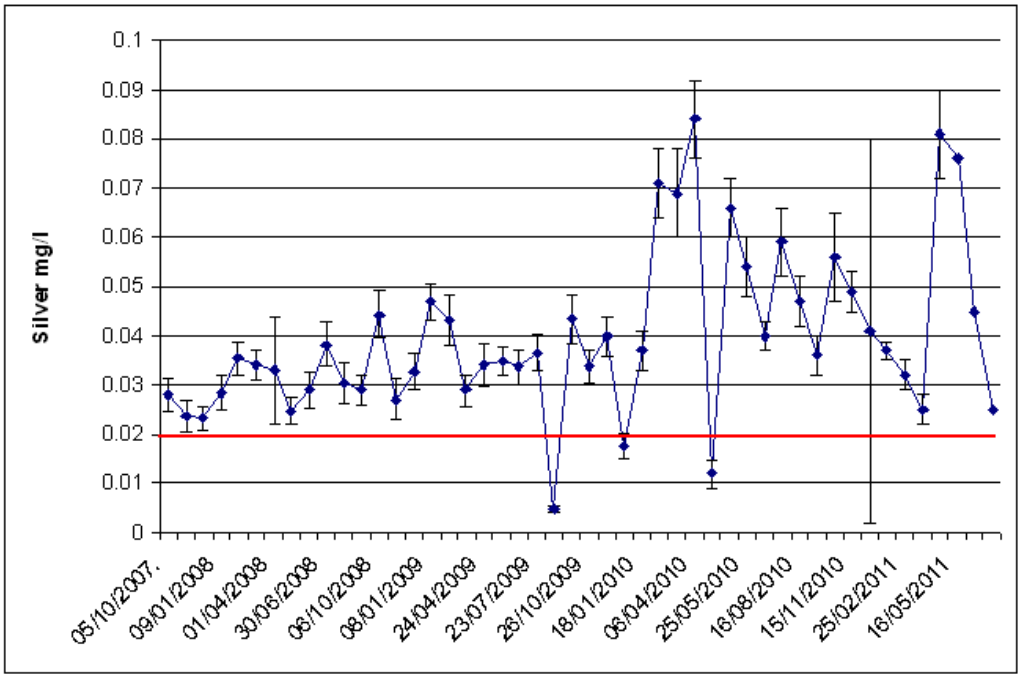
Graph 4.1 Study hospital 1 – Average *Legionella* results from the 05th October 2007 to the 11th July 2011.

Graphs 4.2 and 4.3 below show the average copper and silver levels found in samples taken monthly from the 21 outlets tested from the 05th October 2007 to the 11th July 2011.



Graph 4.2 Study hospital 1 – Average copper levels from the 05th October 2007 to the 11th July 2011 (the red line is the target level for copper of 0.2mg/l).

The average copper level from the 05th October 2007 to the 11th July 2011 was 0.201mg/l (± 0.012mg/l).



Graph 4.3 Study hospital 1 – Average silver levels from the 05th October 2007 to the 11th July 2011 (the red line is the target level for silver of 0.02mg/l).

The average silver level from the 05th October 2007 to the 11th July 2011 was 0.04mg/l (± 0.002mg/l).

Legionella persisted, albeit at reduced counts, in samples taken from outlets at which either less than the copper target level or less than the silver target level was found, and at outlets at which less than both the copper and the silver target levels were found. No *Legionella* were found in samples taken from cold water outlets at which water temperatures above 20ºC were recorded when the copper and silver target levels were being maintained. No *Legionella* were also found in samples taken from hot water outlets at which water temperatures below 50ºC were recorded when the copper and silver target levels were being maintained.

The average cold water temperature recorded monthly at the outlets samples were taken from, from the 05th October 2007 to the 11th July 2011, was 17ºC (± 0.3ºC). The average hot water temperature recorded was 41ºC (± 0.4ºC).

The chloride level found in a sample taken on the 05th April 2011 from the incoming mains was 15.1mg/l, the phosphorus was 455µg/l, and the pH was 8.5.

4.2 Study hospital 2

The results of samples taken on the 10th January 2008, before the copper and silver ionization system was activated, are shown in Table 4.3 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
43.32.1 MT hot	28	0.0003	0.088	<LOD
49-023-1 HT	57	0.0003	0.070	<LOD
49-023-1 CT	13	0.0003	0.018	**100 np**
56-030-1 MT hot	14	0.0003	0.156	**2200 np**
19-069-01 MT hot	45	0.0003	0.039	**100 np**
17-029-01 HT	55	0.0003	0.064	<LOD
17-029-01 HT	15	0.0003	0.063	<LOD
23-024-01 MT hot	18	0.0003	0.036	**100 s1**
18-054-01 HT	41	0.0003	0.059	<LOD
18-054-01 CT	13	0.0003	0.026	<LOD
16-027-01 HT	57	0.0003	0.053	<LOD
16-027-01 CT	12	0.0003	0.013	<LOD
13-027-01 HT	53	0.0003	0.044	<LOD
13-027-01 CT	11	0.0003	0.026	<LOD
14-040.2 HT	54	0.0003	0.039	<LOD
14-040.2 CT	12	0.0003	0.018	<LOD
12-049-01 MT hot	39	0.0003	0.035	<LOD
10-0-014 MT hot	36	0.0003	0.071	<LOD
10-131-1 MT hot	44	0.0003	0.047	<LOD
12-107-1 HT	56	0.0003	0.043	<LOD
12-107-1 CT	14	0.0003	0.014	<LOD
14-137.1 MT hot	38	0.0003	0.030	<LOD
13-128-1 HT	55	0.0003	0.042	<LOD
13-128-01 CT	12	0.0003	0.015	<LOD
16-128-1 MT hot	32	0.0003	0.057	**5900 np**
17-103-1 MT hot	41	0.0003	0.042	**100 np**
19-156 1 HT	59	0.0003	0.034	<LOD
19-156-1 CT	12	0.0003	0.009	**200 np**
20-136-1 HT	57	0.0003	0.387	<LOD
20-136-1 CT	11	0.0003	0.047	<LOD

MT = Mixer Tap, CT = Cold Tap, HT = Hot Tap, np = *Legionella* non-*pneumophila,* s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.3 Study hospital 2 - Results from samples taken on the 10th January 2008, before commissioning of copper and silver ionization system.

Legionella were found at 7 out of the 30 outlets tested, around 23% of the outlets tested were, therefore, contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 5900CFU/l. The average *Legionella* count of the 30 outlets sampled was 290CFU/l (± 207CFU/l). Cold and blended outlets were contaminated.

The average temperature recorded at the cold water outlets was 12ºC (± 0.3ºC). The temperatures recorded at the two contaminated cold water outlets were 12ºC and 13ºC. The average temperature at the hot water outlets was 43ºC (± 3ºC).

The temperatures recorded at the 5 contaminated hot outlets were all below 50ºC mainly because the hot water was blended. The highest *Legionella* count, of 5900CFU/l, was found in a sample taken from a blended outlet (a shower). The temperature recorded here was 32ºC.

The average copper level found due to copper leaching from copper pipes was 0.056mg/l (± 0.013mg/l).

The copper and silver ionization system was activated on the 10th January 2008.

Table 4.4 below shows the results of samples taken on the 04th February 2008 from the 7 outlets that were contaminated before the system was activated and from 8 outlets that were identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
49-023-1 CT	12	0.052	0.341	<LOD
56-030-1 MT hot	42	0.009	0.179	**200 np**
19-069-01 MT hot	45	0.031	0.224	<LOD
23-024-01 MT hot	18	0.05	0.364	<LOD
16-128-1 MT hot	34	0.028	0.225	<LOD
17-103-1 MT hot	42	0.029	0.193	<LOD
19-156-1 CT	45	0.031	0.224	<LOD
10.0-014 MT hot	33	0.033	0.231	<LOD
14.0-0391 HT	51	0.033	0.209	<LOD
14.0-0391 CT	12	0.052	0.347	<LOD
13.128-1 HT	54	0.019	0.109	<LOD
13.128-1 CT	11	0.054	0.346	<LOD
20.136-1 CT	11	0.055	0.345	<LOD
20-136-1 HT	56	0.02	0.167	<LOD
43.32-1 CT	11	0.049	0.338	<LOD

MT = Mixer Tap, CT = Cold Tap, HT = Hot Tap, np = *Legionella* non-*pneumophila*, <LOD = Below Limit of Detection (100CFU/l).

Table 4.4 Study hospital 2 - Results from samples taken on the 04th February 2008, one month after commissioning of copper and silver ionization system.

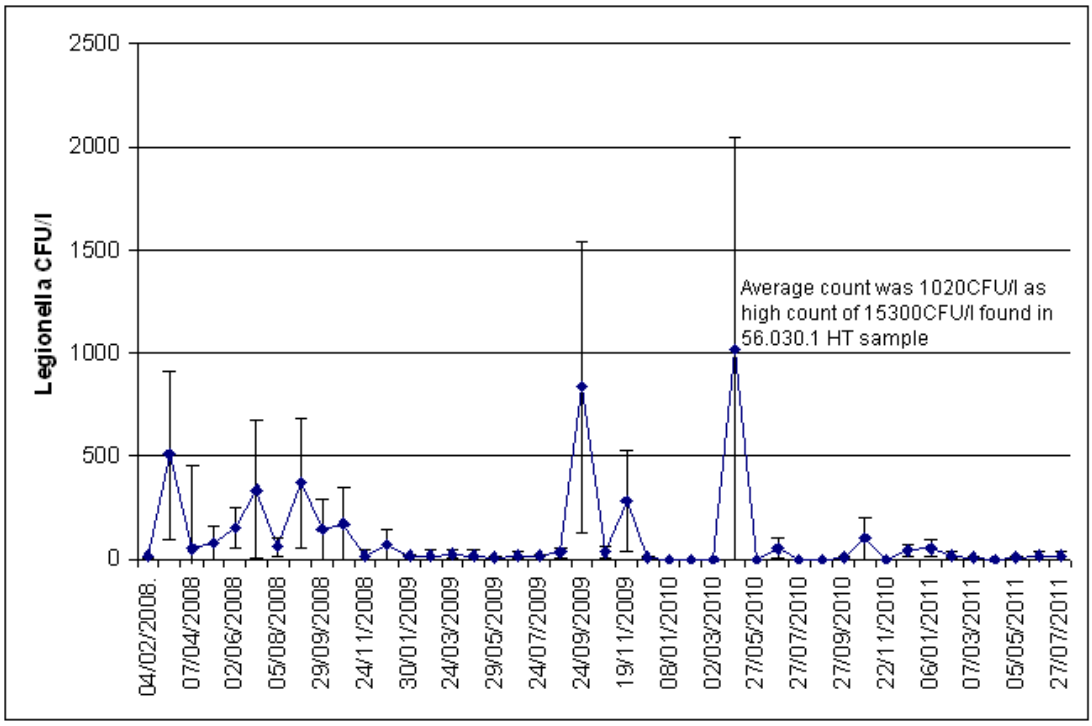
No *Legionella* were found in samples taken from 6 of the 7 outlets that were previously contaminated. *Legionella* persisted at one outlet but the *Legionella* colony forming unit count had dropped from 2200CFU/l to 200CFU/l. The temperature recorded at the contaminated outlet was 42ºC because the hot water was blended with the cold water.

The silver found in the 15 outlets tested ranged from 0.009mg/l to 0.057mg/l. The average silver level was 0.038mg/l (± 0.004mg/l). The silver found in the sample taken from the outlet in which *Legionella* were found was 0.009mg/l, which was below the target level of 0.02mg/l.

The copper found in the 15 outlets tested ranged from 0.109mg/l to 0.364mg/l. The average copper level was 0.263mg/l (± 0.022mg/l). The copper found in the sample taken from the outlet in which *Legionella* were found was 0.179mg/l, which was below the target level of 0.2mg/l.

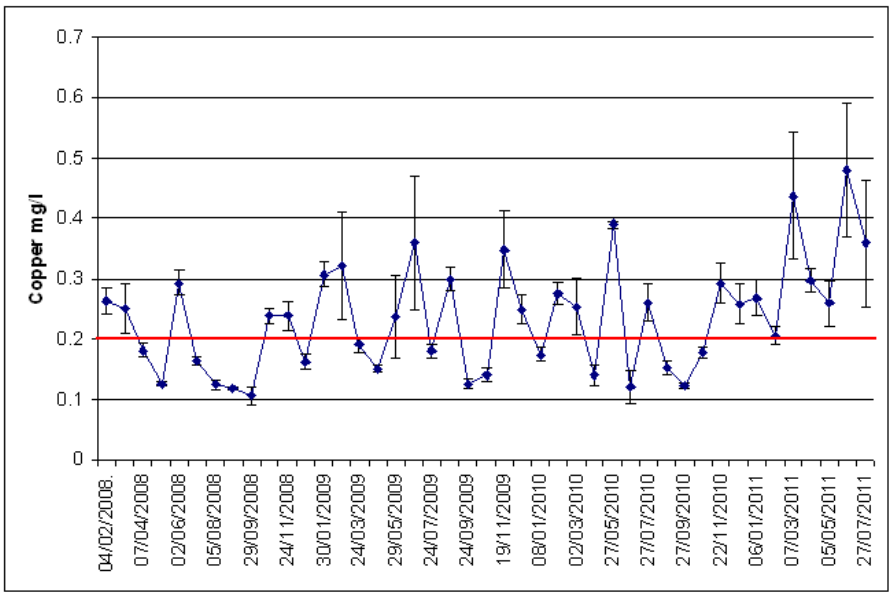
From the 04th February 2008 onwards, the *Legionella* contamination around the site continued to decline except for in samples taken from one blended outlet, the 56.030.1

hot tap. Graph 4.4 below shows the average *Legionella* counts in samples taken monthly from 15 outlets from the 04th February 2008 up to the 27th July 2011.

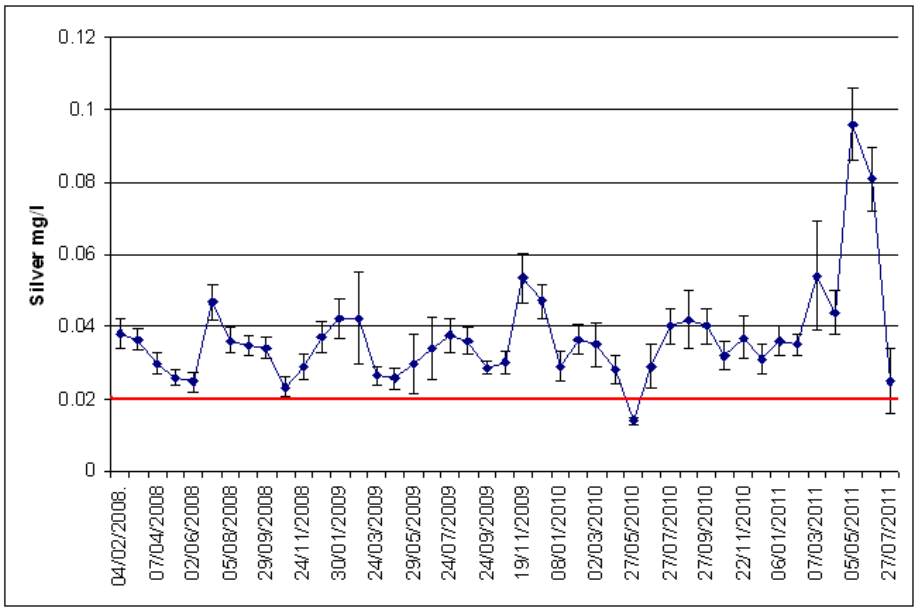


Graph 4.4 Study hospital 2 – Average *Legionella* results from the 04th February 2008 to the 27th July 2011.

Graphs 4.5 and 4.6 below show the average copper and silver levels found in samples taken monthly from the 15 outlets from the 04th February 2008 to the 27th July 2011.



Graph 4.5 Study hospital 2 – Average copper levels from the 04th February 2008 to the 27th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.6 Study hospital 2. Average silver levels from the 04th February 2008 to the 27th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average copper level from the 04th February 2008 to the 27th July 2011 was 0.235mg/l (±0.014mg/l).The average silver level was 0.037mg/l (± 0.002mg/l).

Table 4.5 below shows that the copper and silver target levels at the blended outlet at which *Legionella* persisted were not consistently maintained. Forty nine samples were taken from this outlet from the 04th February 2008 to the 27th July 2011. *Legionella* were found in 29 of these. The average silver level found in the contaminated samples was below the target level of more than 0.02mg/l at 0.013mg/l (± 0.002mg/l), the average copper level found was just above the target of 0.2mg/l at 0.213mg/l (± 0.012mg/l).

No *Legionella* were found in 20 of the 49 samples taken from the outlet. The average silver level in these samples was above the target level of more than 0.02mg/l at 0.021mg/l (± 0.005mg/l). More copper was also found than in the contaminated samples at an average level of 0.228mg/l (± 0.016mg/l).

These results suggested that both the copper and silver target levels needed to be consistently maintained at the outlet to control the *Legionella*, and that, especially, more silver was needed.

Date	Copper mg/l	Silver mg/l	Legionella CFU/l
04/02/2008	**0.179**	**0.009**	**200 np**
04/03/2008	**0.175**	**0.018**	**1500 np**
07/04/2008	**0.164**	**0.01**	**600 np**
06/05/2008	**0.129**	**0.006**	**1200 np**
02/06/2008	**0.255**	**0.008**	**1000 np**
01/07/2008	**0.156**	**0.008**	**5000 np**
05/08/2008	**0.129**	0.033	**700 np**
02/09/2008	**0.139**	**0.006**	**5600 np**
02/09/2008	**0.121**	0.033	**800 np**
29/09/2008	0.291	0.03	**2200 np**
27/10/2008	0.294	**0.003**	**2600 np**
24/11/2008	0.293	**0.003**	**300 np**
06/01/2009	**0.195**	**0.009**	**1100 np**
30/01/2009	0.331	**0.008**	**200 np**
02/03/2009	0.26	**0.002**	<LOD
24/03/2009	0.203	**0.008**	**200 np**
28/04/2009	**0.188**	**0.015**	**300 np**
29/05/2009	0.264	**0.002**	<LOD
24/06/2009	0.356	**0.002**	**200 np**
24/07/2009	0.231	**0.004**	**200 np**
17/08/2009	0.281	**0.002**	**200 np**
24/09/2009	**0.143**	0.037	<LOD
23/10/2009	**0.158**	**0.009**	**400 np**
19/11/2009	**0.145**	**0.013**	**4200 np**
19/11/2009	0.225	0.036	**300 np**
11/12/2009	0.21	**0.014**	**100 np**
08/01/2010	0.217	**0.014**	<LOD
08/01/2010	0.215	**0.015**	<LOD
02/02/2010	0.294	0.028	<LOD
02/03/2010	0.326	**0.017**	<LOD
01/04/2010	**0.142**	**0.017**	**15300 np**
27/05/2010	0.405	**0.018**	<LOD
25/06/2010	0.252	**0.005**	**100 np**
26/07/2010	**0.179**	**0.003**	<LOD
26/07/2010	0.216	0.034	<LOD
25/08/2010	**0.119**	0.022	<LOD
27/09/2010	**0.121**	**0.002**	<LOD
26/10/2010	0.209	**0.002**	<LOD
21/11/2010	**0.186**	**0.019**	<LOD
09/12/2010	0.294	**0.018**	**300 np**
06/01/2011	0.216	0.02	**100 np**
10/02/2011	**0.199**	0.024	**200 np**
07/03/2011	0.216	**0.001**	**100 np**
07/03/2011	0.265	**0.011**	<LOD
04/04/2011	0.279	0.033	<LOD
05/05/2011	0.238	0.086	<LOD
01/06/2011	0.28	0.062	<LOD
27/06/2011	0.216	**0.006**	<LOD
27/07/2011	**0.131**	**0.009**	<LOD

np = *Legionella* non-*pneumophila*, <LOD = Below Limit of Detection (100CFU/l).

Table 4.5 Copper, silver and *Legionella* results from the 04th February 2008 to the 27th July 2011 of samples taken from the 56.030.1 hot tap (highlighted in bold are copper and silver levels that were below the target levels).

The average copper found in the samples taken from the 56.030.1 hot tap from the 04th February 2008 to the 27th July 2011 was 0.219mg/l (± 0.01mg/l). The average silver level found was 0.016mg/l (± 0.002mg/l).

The average cold water temperature recorded at the cold outlets sampled from the 10th January to the 27th July 2011 was 15ºC (± 0.2ºC). The average hot water temperature recorded at the hot and blended outlets samples was 44ºC (± 0.5ºC).

The chloride level found in a sample taken from the incoming mains on the 06th October 2009 was 17mg/l, the phosphorus was 390µg/l, and the pH was 8.45.

4.3 Study hospital 3

The results of samples taken on the 04th February 2008, before the copper and silver ionization system was activated, are shown in Table 4.6 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella Cfu/l
APN 58 MT	35	0.004	0.034	<LOD
APN 49 CT	16	<0.003	0.043	**100 np**
APN 49 MT	39	<0.003	0.056	**1100 np**
NE 12 CT	17	<0.003	0.035	<LOD
NE 46 MT	34	<0.003	0.047	<LOD
PE 30 CT	16	<0.003	0.069	<LOD
DS 82 Shower Hot	34	<0.003	0.042	<LOD
DS 99 HT	57	<0.003	0.063	<LOD
DS 55 CT	20	<0.003	0.029	<LOD
DS 50 MT	39	<0.003	0.044	<LOD
ANC 18 CT	15	<0.003	0.037	<LOD
ANC 18 MT	41	<0.003	0.054	<LOD
ANC 28 MT	Not Taken	Not Taken	Not Taken	**1200 s1**
ANC 28 CT	14	<0.003	0.109	<LOD
Toilet Near 9 CT	12	0.014	0.033	<LOD
GW 1 MT	38	<0.003	0.057	<LOD
GW 25 Shower Hot	41	<0.003	0.059	<LOD
GW 50 MT	38	<0.003	0.058	**500 np**
GW 85 MT	38	0.021	0.051	<LOD
SO 37 Shower Cold	13	0.015	0.034	<LOD
SO 37 Shower Hot	42	0.006	0.073	<LOD

MT = Mixer Tap, CT = Cold Tap, HT = Hot Tap, np = *Legionella* non-*pneumophila,* s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.6 Study hospital 3 - Results from samples taken on the 04th February 2008, before commissioning of copper and silver ionization system.

Legionella were found at one cold outlet, at 100 cfu/l, and at three blended outlets, at 1200 cfu/l, 1100 cfu/l, and 500 cfu/l. Twenty one outlets were tested, around 20% were, therefore, contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 1200CFU/l. The average *Legionella* count of the 21 outlets sampled was 138CFU/l (± 77CFU/l).

The average temperature recorded at the cold water outlets was 16ºC (± 1ºC). The temperature recorded at the contaminated cold water outlet was 16ºC. The average temperature at the hot water outlets was 38ºC (± 2.5ºC). The temperatures recorded at

the 3 contaminated hot outlets were all below 50ºC mainly because the hot water was blended.

The average copper level found due to copper leaching from copper pipes was 0.051mg/l (± 0.004mg/l).

The copper and silver ionization system was activated on the 04th February 2008.

Table 4.7 below shows the results of samples taken on the 06th March 2008. Samples were taken from the 4 outlets that were contaminated before the system was activated and from 6 outlets that were identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
APN 49 CT	10	0.092	0.199	<LOD
APN 49 MT	40	0.045	0.162	<LOD
ANC 28 MT	40	0.049	0.188	<LOD
GW 50 MT	41	0.041	0.158	<LOD
DS 82 Shower Hot	41	0.039	0.155	<LOD
DS 50 MT	42	0.043	0.156	<LOD
DS 50 CT	10	0.083	0.183	<LOD
S0 37 Shower Cold	11	0.115	0.287	<LOD
SO 37 Shower Hot	42	0.044	0.150	<LOD
GW 25 Shower Hot	41	0.046	0.157	<LOD

MT = Mixer Tap, CT = Cold Tap, <LOD = Below Limit of Detection (100CFU/l).

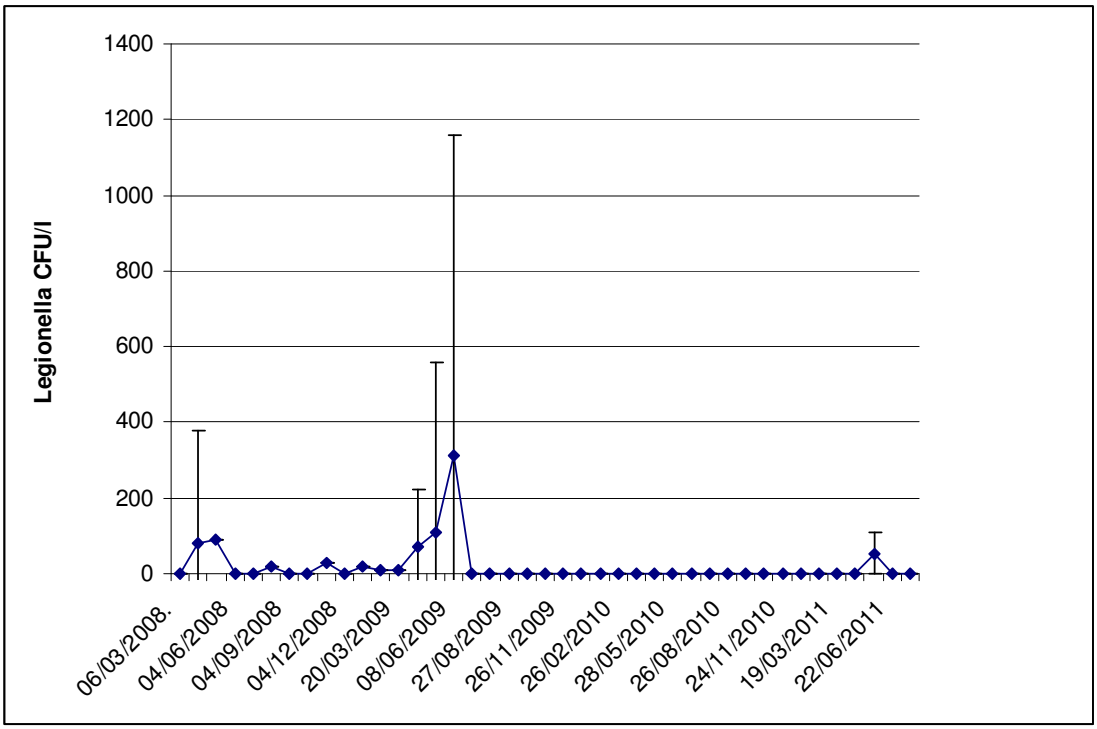
Table 4.7 Study hospital 3 - Results from samples taken on the 06th March 2008, one month after commissioning of copper and silver ionization system..

No *Legionella* were found in these samples with silver levels of between 0.039mg/l and 0.115mg/l, the average silver level was 0.060mg/l (± 0.008mg/l), and copper levels of between 0.150mg/l and 0.287mg/l, the average copper level was 0.180mg/l (± 0.013mg/l).

The hot water temperatures recorded were all below 50ºC because it was blended water. The average hot water temperature recorded was 41ºC (± 0.3ºC). The average cold water temperature recorded was 10ºC (± 0.3ºC).

Legionella persisted at 1 blended outlet, APN 49 mixer tap (set on hot), until June 2009, after which no *Legionella* were detected in samples taken to May 2011, see

Graph 4.7 below. This graph shows the average *Legionella* count of all 10 outlets sampled monthly from the 06th March 2008 to the 19th July 2011.

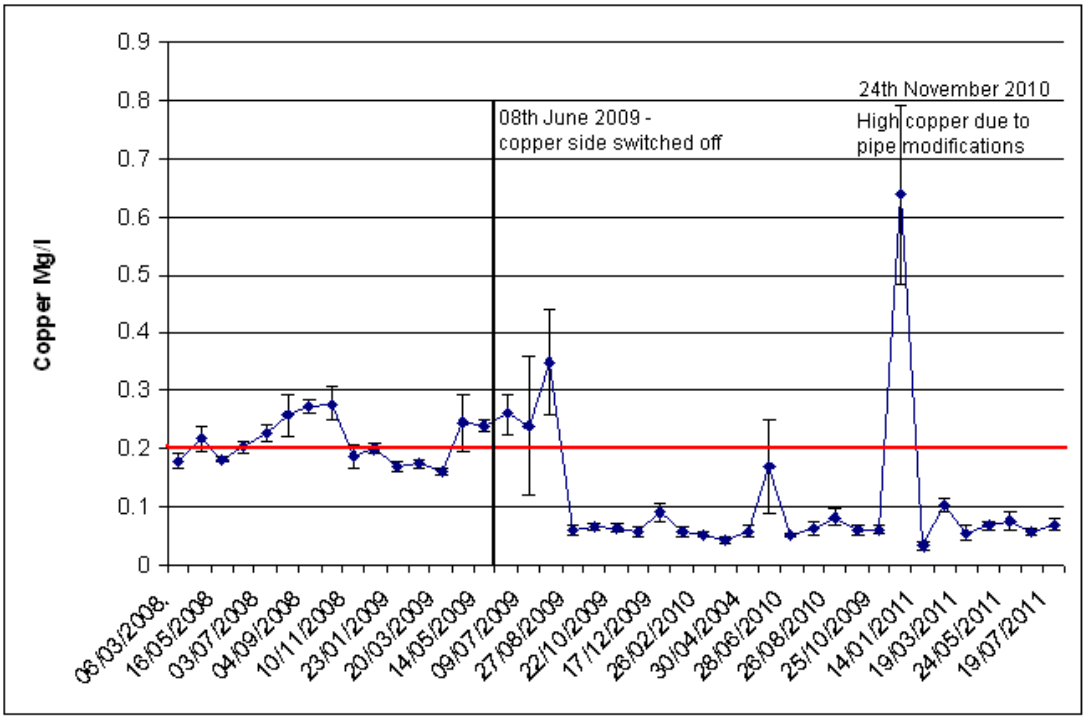


Graph 4.7 Study hospital 3 – Average *Legionella* results from the 06th March 2008 to the 19th July 2011.

Graph 4.8 shows the average copper levels found in samples taken monthly from 10 outlets from the 06th March 2008 to the 19th July 2011.

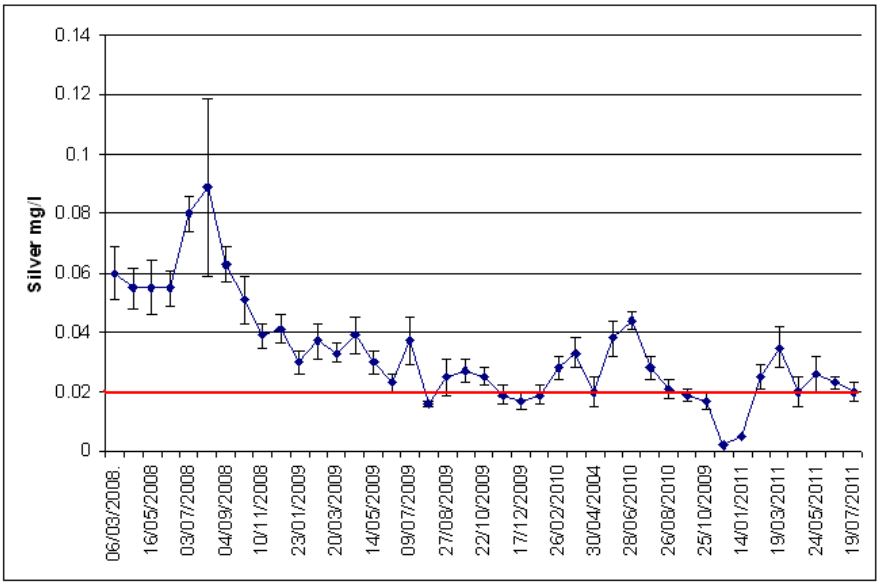
The treated water started to discolour in 2009, which may have been due to high phosphate levels in the mains water supply potentially forming precipitates with the higher levels of copper, and the copper side of the ionization system was turned off from the 08th June 2009 onwards.

The average copper levels in samples taken on the 08th June, the 09th July and the 04th August 2009 remained, however, above 0.2mg/l, at 0.26mg/l, 0.241mg/l and 0.35mg/l respectively, which was most likely residual copper from the ionization treatment as well as copper leaching from the copper pipes in the water system, but the levels reduced from the 27th August 2009 onwards. The average copper level from the 27th August to the 19th July 2011 was 0.093mg/l (± 0.025mg/l), which was solely the copper that had leached from the copper pipes.



Graph 4.8 Study hospital 3 – Average copper levels from the 06th March 2008 to the 19th July 2011 (the red line is the target level for copper - 0.2mg/l).

Graph 4.9 shows the average silver level found in samples taken monthly from 10 outlets from the 06th March 2008 to the 19th July 2011.



Graph 4.9 Study hospital 3 – Average silver levels from the 06th March 2008 to the 19th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average silver level from the 06th March 2008 to the 19th July 2011 was 0.033mg/l (± 0.003mg/l).

The copper target level at the blended outlet at which *Legionella* persisted, the APN 49 mixer tap (set on hot), was not maintained. The silver target level was also often not maintained, see Table 4.8 below.

Date	Copper mg/l	Silver mg/l	Legionella CFU/l
04/02/08	**0.043**	<0.0003	**1100 np**
06/03/08	**0.162**	0.045	<LOD
03/04/08	**0.199**	0.074	**700 np**
16/05/08	**0.172**	0.038	<LOD
03/07/08	0.243	0.079	<LOD
01/08/08	0.202	0.051	**200 np**
04/09/08	0.246	0.048	<LOD
10/10/08	0.201	0.034	<LOD
10/11/08	**0.171**	0.033	<LOD
04/12/08	**0.181**	0.036	<LOD
23/01/09	**0.145**	0.022	**100 np**
26/02/09	**0.155**	0.033	**100 np**
20/03/09	**0.152**	**0.017**	**100 np**
15/04/09	**0.183**	0.044	**200 np**
14/05/09	0.221	**0.017**	**1000 np**
08/06/09	0.2	**0.016**	**2400 np**
22/06/09	**0.115**	**0.008**	<LOD
09/07/09	**0.148**	0.029	<LOD
04/08/09	0.580	**0.0012**	<LOD
27/08/09	**0.086**	**0.017**	<LOD
05/10/09	**0.074**	0.02	<LOD
22/10/09	**0.075**	**0.017**	<LOD
26/11/09	**0.071**	**0.001**	<LOD
17/12/09	**0.098**	**0.009**	<LOD
28/01/10	**0.056**	**0.0161**	<LOD
26/02/10	**0.062**	**0.019**	<LOD
26/03/10	**0.056**	0.02	<LOD
30/04/10	**0.08**	**0.003**	<LOD
28/05/10	0.888	0.027	<LOD
26/08/10	**0.103**	0.036	<LOD
23/09/10	**0.064**	**0.011**	<LOD
25/10/10	**0.081**	**0.005**	<LOD
24/11/10	1.18	**0.004**	<LOD
14/01/11	**0.05**	**0.005**	<LOD
09/03/11	**0.056**	**0.019**	<LOD
05/04/11	**0.092**	**0.005**	<LOD
24/05/11	**0.074**	**0.012**	**600 np**
07/06/11	**0.146**	**0.003**	**400 np**
19/07/11	**0.100**	**0.009**	<LOD

np = *Legionella* non-*pneumophila,* <LOD = Below Limit of Detection (100CFU/l).

Table 4.8 Copper, silver and *Legionella* results from the 04th February 2008 to the 19th July 2011 of samples taken from the APN 49 mixer tap (set on hot) (highlighted in bold are copper and silver levels that were below the target levels).

No *Legionella* were found from June 2009 to May 2011 at this outlet, after the rubber lined flexible hoses were replaced with copper pipes in June 2009, although the

copper and silver target levels were not consistently met. *Legionella* were found in a sample taken from the outlet on the 24th May 2011 but no *Legionella* were found in the samples taken from the outlet on the 22nd June and the 19th July 2011, after the mixing valve attached to the outlet was cleaned.

The average copper found in the samples taken from the outlet was 0.185mg/l (± 0.04mg/l). The average silver level found was 0.023mg/l (± 0.003mg/l).

The average cold water temperature recorded at the cold outlets sampled from the 04th February 2008 to the 19th July 2011 was 17ºC (± 0.3ºC). The average hot water temperature recorded at the hot and blended outlets sampled was 41ºC (± 0.3ºC).

The chloride level found in the sample taken from the incoming mains on the 05th October 2009 was 24.5mg/l, the phosphorus was 1000µg/l, and the pH was 7.9.

4.4 Study hospital 4

The results of samples taken on the 08th April 2008, before the copper and silver ionization system was activated, are shown in Table 4.9 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella Cfu/l
S. Ward Male Bed HT	36	<0.003	0.135	<LOD
S11 shower hot	34	<0.003	0.169	<LOD
S. Ward Kitchen CT	13	<0.003	0.088	<LOD
B19 HT	36	<0.003	0.146	<LOD
B30 bath shower cold	13	<0.003	0.085	<LOD
B5 shower hot	41	<0.003	0.147	**100 np**
O30 CT	15	<0.003	0.094	<LOD
O15 shower hot	38	<0.003	0.177	<LOD
O32 CT	13	<0.003	0.074	<LOD
L13 CT	14	<0.003	0.109	<LOD
L37 MT hot	40	<0.003	0.227	**4100 np**
L29 CT	19	<0.003	0.291	<LOD

HT = hot tap, CT = cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, <LOD = Below Limit of Detection (100CFU/l).

Table 4.9 Study hospital 4 - Results from samples taken on the 08th April 2008, before commissioning of copper and silver ionization system.

Legionella were found in samples taken from 1 shower, at 100 CFU/L, and at 1 mixer tap (set on hot), at 4100CFU/l. Twelve outlets were tested, around 17% were, therefore, contaminated.

The average temperature at the hot water outlets was 37.5ºC (± 1ºC). Mixing valves were blending the hot water with cold water at both contaminated outlets. The temperatures recorded at the outlets were, therefore, 41ºC and 40ºC. The average temperature recorded at the cold water outlets samples were taken from was 14.5ºC (± 0.9ºC).

The average copper level found due to copper leaching from copper pipes, was 0.145mg/l (± 0.019mg/l).

The copper and silver ionization system was activated on the 08th April 2008.

Table 4.10 below shows the results of samples taken on the 07th May 2008. Samples were taken from the 2 outlets that were contaminated before the system was activated and from 3 more blended outlets that were identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella Cfu/l
L37 MT hot	43	0.034	0.327	**2300 np**
B5 shower hot t	32	0.081	0.392	<LOD
S11 shower hot	32	0.096	0.398	<LOD
O15 shower hot	34	0.092	0.417	<LOD
B32 HT	42	0.091	0.383	<LOD

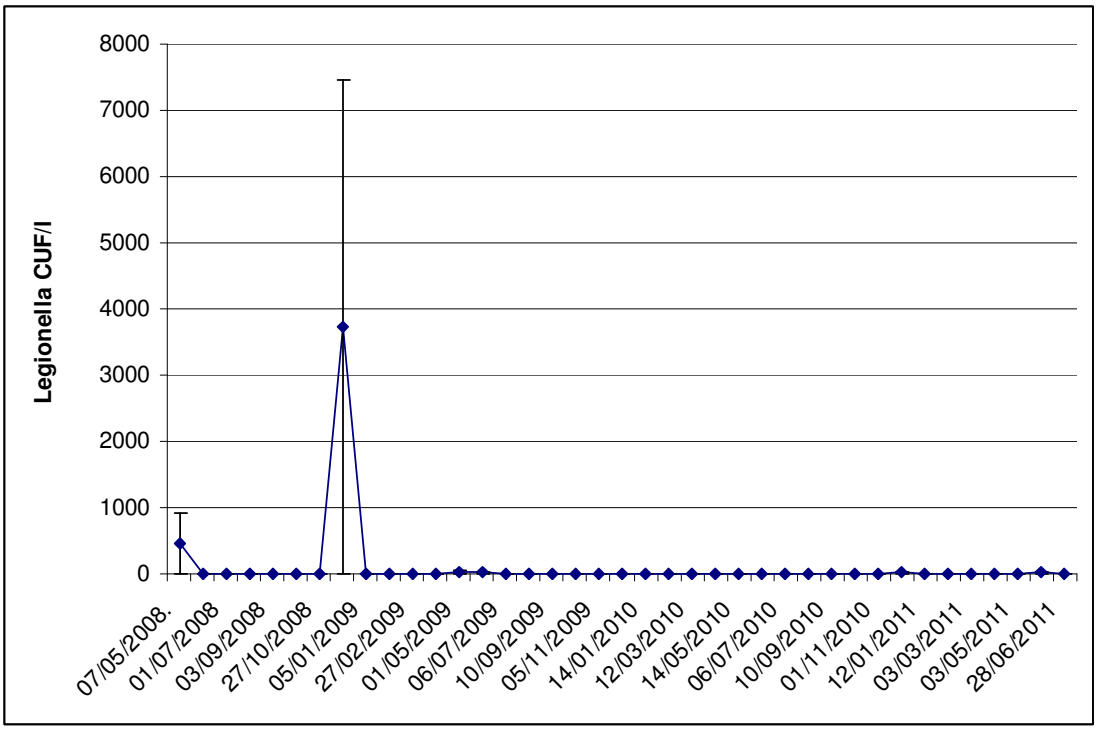
MT = Mixer tap, HT = Hot tap, np = *Legionella* non-*pneumophila,* <LOD = Below Limit of Detection (100CFU/l).

Table 4.10 Study hospital 4 - Results from samples taken on the 07th May 2008.

No *Legionella* were found in the samples taken from 4 of the 5 outlets tested. *Legionella* were found in 1 outlet that was previously contaminated but the *Legionella* colony forming unit count had dropped from 4100CFU/l to 2300CFU/l. The temperature recorded at the contaminated outlet was 43ºC, and the copper and silver levels found were 0.327mg/l copper and 0.034mg/l silver.

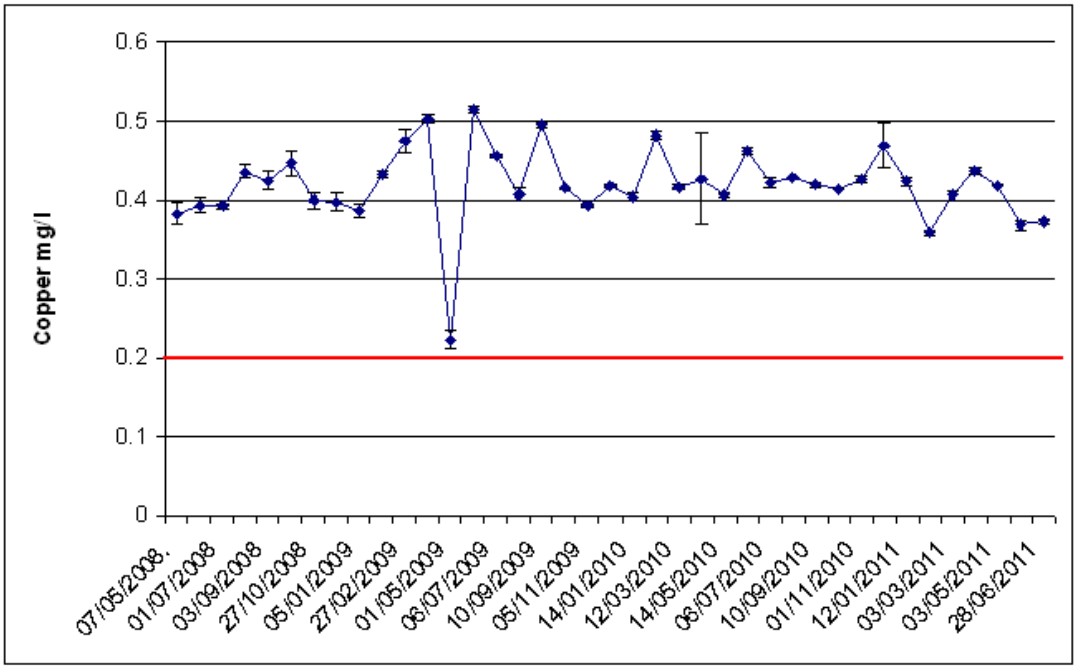
The average copper level of the 5 outlets tested was 0.383mg/l (± 0.015mg/l), and the average silver level was 0.079mg/l (± 0.011mg/l). The average hot water temperature recorded was 37ºC (± 2ºC) because hot water was blended with cold water.

No *Legionella* were found in samples taken monthly from 6 outlets from the 30th May 2008 to the 28th June 2011, see Graph 4.10 below. Except for a high count of *Legionella* non-*pneumophila*, of 22400CFU/l, which was found in a sample taken from a blended outlet (a shower, set on hot) on the 24th November 2008. No *Legionella* were found at this outlet, however, since rubber lined flexible hoses were replaced with copper pipes in December 2008. The average copper level found in samples taken from the outlet from January 2009 to July 2010 was 0.451mg/l (± 0.012mg/l) and the average silver level was 0.04mg/l (± 0.003mg/l).

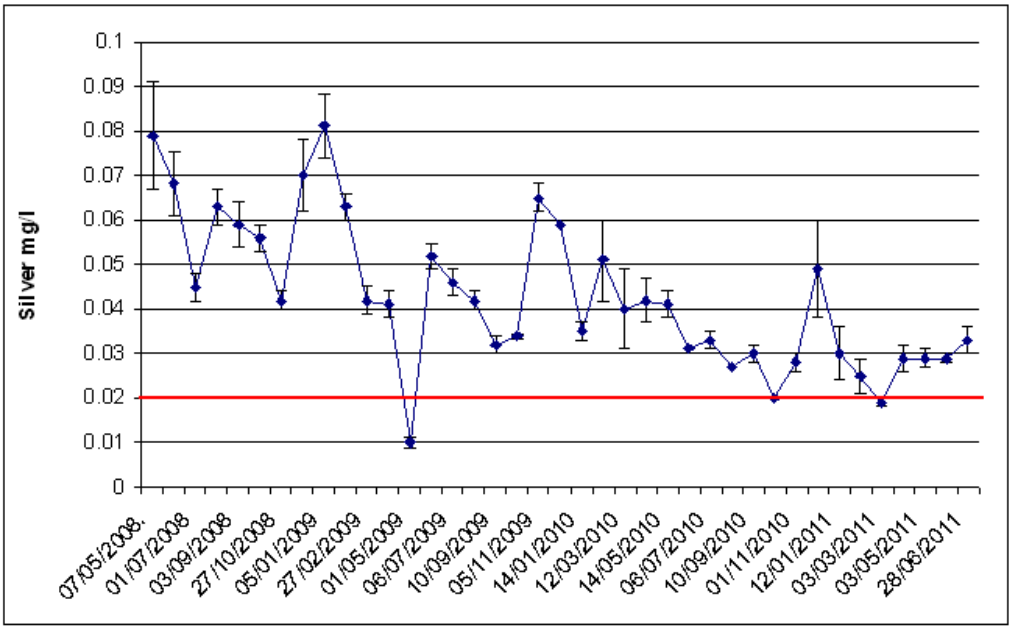


Graph 4.10 Study hospital 4 - Average *Legionella* results of all 6 outlets sampled monthly from the 07th May 2008 to the 28th June 2011.

Graphs 4.11 and 4.12 show the averages of the copper and silver levels found in samples taken monthly from 6 outlets from the 07th May 2008 to the 28th June 2011. The average copper level was 0.419mg/l (± 0.008mg/l). The average silver level was 0.043mg/l (± 0.003mg/l).



Graph 4.11 Study hospital 4 – Average copper levels from the 07th May 2008 to the 28th June 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.12. Study hospital 4 – Average silver levels from the 07th May 2008 to the 28th June 2011 (the red line is the target level for silver - 0.02mg/l).

The average hot water temperature recorded at the blended outlets sampled from the 07th May 2008 to the 28th June 2011 was 38ºC (± 0.3ºC).

The chloride level found in the sample taken from the incoming mains on the 08th October 2009 was 30.2mg/l, the phosphorus was 577µg/l, and the pH was 7.47.

4.5 Study hospital 5

The results of samples taken on the 25th July 2008, before the copper and silver ionization systems were activated, are shown in Table 4.11 below.

The samples were taken from pure cold and pure hot outlets but in order to obtain a wider view of the potential contamination of the cold and hot water system and to reduce analysis costs, samples were also taken by filling sample bottles half with hot water and half with cold water from 17 mixer taps, these outlets are abbreviated as H&C on Table 4.11 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
3.2.118 HT	60	<0.0003	0.214	<LOD
3.2.118 CT	17	<0.0003	0.067	<LOD
S1 nurse station HT	42	<0.0003	0.160	<LOD
S1.nurse station CT	17	<0.0003	0.085	<LOD
3.2.036 HT	43	<0.0003	0.176	**100 np**
3.2.036 CT	17	<0.0003	0.176	**400 np**
S2 nurse station CT	17	<0.0003	0.097	**100 np**
S2 nurse station HT	42	<0.0003	0.174	**100 np**
3.2.068 HT	40	<0.0003	0.314	**100 np**
3.2.068 CT	20	<0.0003	0.212	**200 np**
S3 nurse station HT	59	<0.0003	0.210	<LOD
S3 nurse station CT	20	<0.0003	0.272	<LOD
3.2.089.3 HT	58	<0.0003	0.209	<LOD
3.2.089.3 CT	21	<0.0003	0.251	**100 np**
S4 nurse station HT	58	<0.0003	0.200	<LOD
S4 nurse station CT	21	<0.0003	0.087	<LOD
S4 sluice H&C	39	<0.0003	0.155	<LOD
3.3.118 HT	60	<0.0003	0.205	<LOD
3.3.118 CT	18	<0.0003	0.074	**200 np**
H4 nurse station HT	60	<0.0003	0.202	<LOD
H4 nurse station CT	28	<0.0003	0.640	**600 np**
3.3.106 H&C	33	<0.0003	0.159	<LOD
H3 nurse station HT	42	<0.0003	0.232	**300 s1**
H3 nurse station CT	20	<0.0003	0.190	**100 np**
H3 cleaners H&C	34	<0.0003	0.182	**300 np**
H2 nurse station HT	54	<0.0003	0.213	<LOD
H2 nurse station CT	23	<0.0003	0.433	**200 np**
Room 68 H&C	37	<0.0003	0.450	**400 np**
3.3.032/2 H&C	30	<0.0003	0.151	**100 np**
3.3.019 H&C	28	<0.0003	0.202	**500 np**
M kitchen HT	59	<0.0003	0.050	<LOD
M kitchen CT	17	<0.0003	0.127	<LOD
M shower H&C	28	<0.0003	0.147	<LOD
M sluice HT	55	<0.0003	0.208	<LOD
M sluice CT	22	<0.0003	0.202	**2400 np**
A kitchen CT	25	<0.0003	17.9	<LOD
4.3.39 H&C	27	<0.0003	0.174	**1100 np**
F S1 H&C	31	<0.0003	4.92	**100 np**
C2094 H&C	35	<0.0003	0.187	<LOD
C2027 CT	20	<0.0003	0.100	**100 s1**
Theatres H&C	34	<0.0003	0.127	**100 s1**
Endoscopy H&C	31	<0.0003	0.208	**100 np**
Maternity sluice H&C	35	<0.0003	0.204	<LOD
D.1.12 H&C	43	<0.0003	0.178	**300 np**
6.2.142 H&C	36	<0.0003	0.448	**2600 s1**
C3152 H&C	35	<0.0003	0.168	**300 s1**
C3100 shower HT	37	<0.0003	0.149	<LOD
C3060 H&C	33	<0.0003	0.213	<LOD
C3057 HT	57	<0.0003	0.207	<LOD
C3057 CT	19	<0.0003	0.161	<LOD
C3100 after filter HT	33	<0.0003	0.136	<LOD

HT = Hot tap, CT = Cold tap, H&C = sample filled with half hot and half cold water, np = *Legionella* non-*pneumophila,* s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.11 Study hospital 5 - Results from samples taken on the 25th July 2008, before commissioning of copper and silver ionization systems.

Legionella were found in samples taken from 25 outlets. Fifty one outlets were sampled, therefore, around 49% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 2600CFU/l. The average *Legionella* count of the 51 outlets sampled was 214CFU/l (± 10CFU/l). Blended and cold outlets were contaminated and *Legionella* were found in 11 of the 17 H&C samples taken.

The water temperatures recorded at 6 of the 17 cold water outlets sampled were above 20ºC. The average at the 17 cold water outlets tested was 20ºC (± 0.8ºC).

The average hot water temperature at the 17 hot water outlets tested was 51ºC (± 2ºC). Although mixing valves were hidden and could not be seen, the hot water was, most likely, blended with cold water at 7 of the 17 hot water outlets tested because the temperatures recorded at these were ranging from 33ºC to 43ºC.

The average temperature recorded in the 17 H&C samples was 33ºC (± 1ºC).

The average copper level found at the 51 outlets, due to copper leaching from copper pipes, was 0.323mg/l (± 0.014mg/l).

The copper and silver ionization systems were activated on the 25th July 2008.

Table 4.12 below shows the results of samples taken on the 18th August 2008 from the 25 outlets that were contaminated before the systems were activated.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
3.2.036 HT	22	0.074	0.434	<LOD
3.2.036 CT	20	0.06	0.378	<LOD
S2 nurse station CT	43	0.080	0.428	<LOD
S2 nurse station HT	22	0.086	0.390	<LOD
3.2.068 HT	43	0.066	0.473	<LOD
3.2.068 CT	22	0.081	0.451	<LOD
3.2.089.3 CT	20	0.099	0.404	<LOD
3.3.118 CT	18	0.1	0.380	<LOD
H4 nurse station CT	22	0.089	0.424	<LOD
H3 nurse station HT	45	0.074	0.462	<LOD
H3 nurse station CT	18	0.094	0.444	<LOD
H3 cleaners H&C	28	0.063	0.469	<LOD
H2 nurse station CT	19	0.056	0.749	<LOD
Room 68 H&C	35	0.039	0.612	**200 np**
3.3.032/2 H&C	29	0.091	0.427	**400 np**
3.3.019 H&C	27	0.086	0.428	**800 np**
M sluice CT	18	0.089	0.472	<LOD
4.3.39 H&C	32	0.084	0.540	<LOD
F S1 H&C	34	0.076	0.565	<LOD
Theatres H&C	32	0.094	0.428	<LOD
Endoscopy H&C	29	0.1	0.410	<LOD
D.1.12 H&C	34	0.083	0.434	<LOD
6.2.142 H&C	32	0.019	0.746	**100 s1 + 200 np**
C2027 CT	22	0.09	0.421	<LOD
C3152 H&C	30	0.097	0.438	<LOD

HT = Hot tap, CT = Cold tap, H&C = sample filled with half hot and half cold water, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.12 Study hospital 5 - Results from samples taken on the 18th August 2008.

No *Legionella* were found in samples taken from 21 outlets that were previously contaminated. Only H&C samples were contaminated. The highest *Legionella* count was 800CFU/l. The temperature recorded in this sample was 27ºC.

The cold temperatures remained above 20ºC at 4 out of the 10 cold water outlets tested. The hot water temperatures were predominantly below 50ºC because it was, most likely, blended water. The hot water temperature was particularly low at 22ºC at the 3.2.036 hot tap.

The silver found in the 25 outlets tested ranged from 0.019mg/l to 0.1mg/l. The average silver level was 0.079mg/l (± 0.004mg/l). The copper ranged from 0.378mg/l to 0.749mg/l. The average copper level was 0.472mg/l (± 0.02mg/l).

Because the *Legionella* contaminated H&C samples that were taken on the 18th August 2008 were half filled with hot water and topped up with cold water, samples were taken on the 15th September 2008 from the hot and the cold sides of the 4 mixer taps. The results are shown in Table 4.13 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
Room 68 HT	42	0.038	0.553	**2500 np**
Room 68 CT	18	0.05	0.601	**500 np**
3.3.032/2 HT	39	0.06	0.513	<LOD
3.3.032/2 CT	14	0.066	0.582	<LOD
3.3.019 HT	40	0.067	0.503	<LOD
3.3.019 CT	14	0.055	0.580	**100 np**
6.2.142 HT	38	0.02	0.498	**100 np**
6.2.142 CT	20	0.021	0.869	**100 np**

HT = Hot tap, CT = Cold tap, np = *Legionella* non-*pneumophila*, <LOD = Below Limit of Detection (100CFU/l).

Table 4.13 - Study hospital 5 - Results from samples taken on the 15th September 2008 from single hot and cold taps of the previously *Legionella* contaminated outlets.

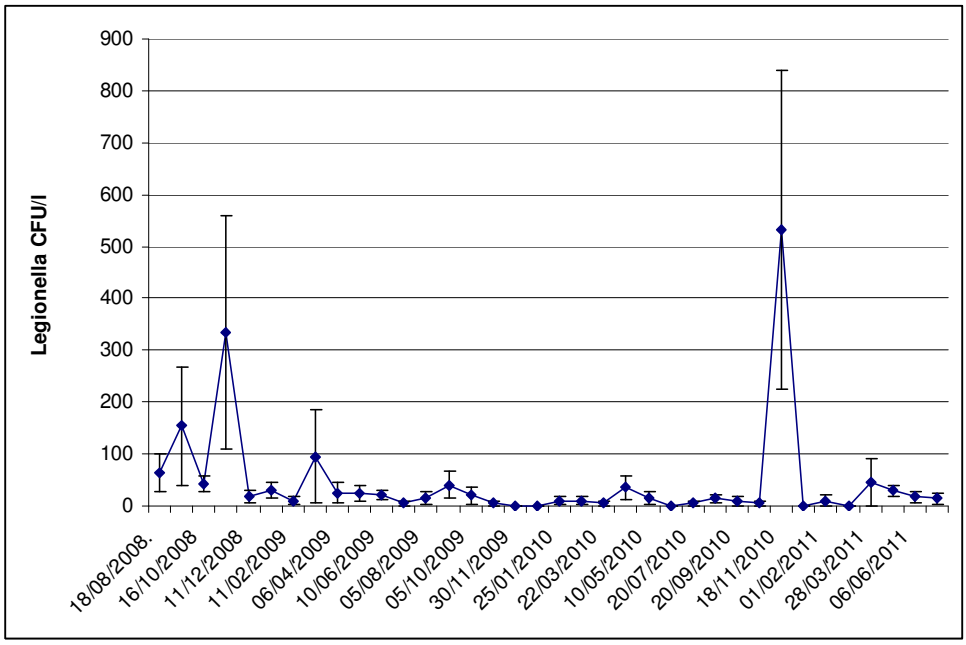
Both the hot and cold water was contaminated at 2 of the 4 outlets tested. The cold water only was contaminated at 1 of the 4 outlets tested. The highest *Legionella* count, of 2500CFU/l, was found at a hot outlet that was, most likely, blended because the temperature recorded was 42ºC.

From the 15th September 2008, the *Legionella* contamination declined in the blended and cold water system, except for at 1 hot outlet at which a *Legionella* count of 2800CFU/l was found, and at 1 cold outlet at which a count of 3700CFU/l was found in samples taken on the 13th November 2008. The samples were taken from the hot and cold outlets of the same sink. The cold water was, most likely, contaminating the hot water because the water was, probably, blended as the temperature recorded was 35ºC. Although a higher count, of 1300CFU/l, was found in a sample taken on the 14th January 2009 after running the cold outlet for 10 minutes, after the rubber lined flexible hoses were replaced in November 2008, the *Legionella* counts declined. No

Legionella were found at both outlets from February 2009 onwards. The average silver level found in the samples taken from the hot outlet from the 15th September 2008 to March 2010 was 0.026mg/l (± 0.003mg/l), and the average copper level was 0.463mg/l (± 0.02mg/l). The average silver level found in the samples taken from the cold outlet was 0.020mg/l (± 0.005mg/l), and the average copper level was 0.464mg/l (± 0.03mg/l). The average hot temperature was 39ºC (± 1ºC). The average cold temperature was 22ºC (± 1ºC).

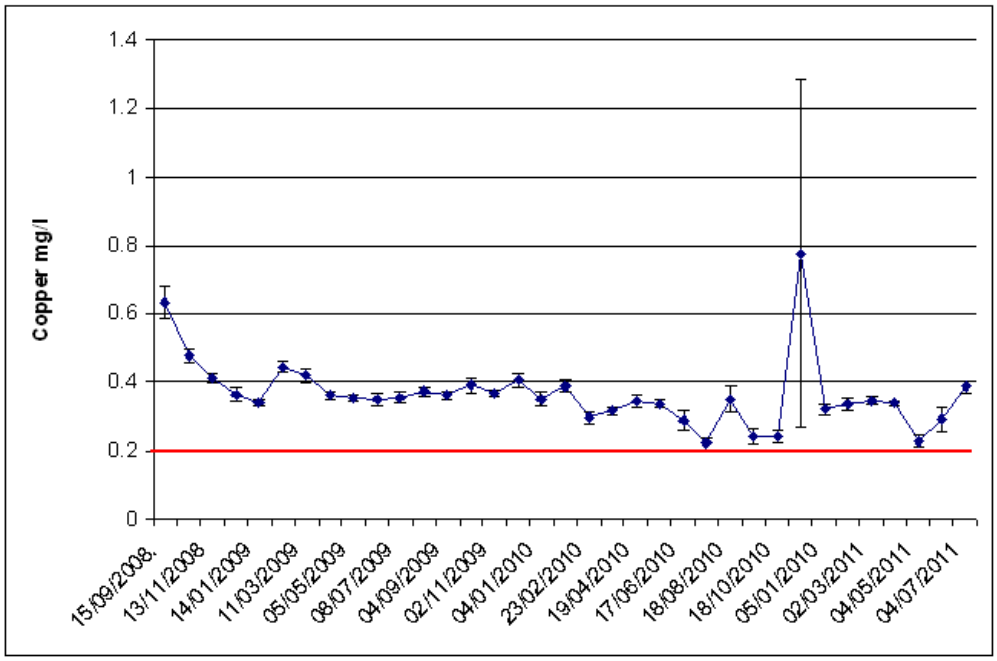
Legionella counts were found in samples taken on the 18th November 2010 from a cold outlet, at 3100CFU/l, from the hot outlet of the same sink, at 5800CFU/l, and from another cold outlet, at 1500CFU/l. The temperatures recorded at the 2 cold outlets were both 16ºC. The temperature recorded at the hot outlet was 40ºC, most likely, because it was blended water. The copper and silver targets were met in these samples but the copper and silver had been below the targets previously. The outlets were flushed regularly after the *Legionella* were found, and the copper and silver levels were kept above 0.3mg/l copper and 0.08mg/l silver in samples taken in January, February and March 2011. In samples taken on the 04th May 2011 the copper and silver levels dropped to 0.189mg/l copper and to 0.052mg/l silver in 1 of the cold outlet samples and to 0.233mg/l copper and to 0.043mg/l silver in the hot outlet sample, and *Legionella* returned, albeit at lower counts of 100CFU/l in the cold outlet sample and 200CFU/l in the hot outlet sample. The average of the temperatures recorded from January 2011 to the 04th May 2011 was 43ºC (± 2ºC) at the hot outlet and 15ºC (± 0.5ºC), 14ºC (± 0.4ºC) at the cold outlets.

Graph 4.13 below shows the average *Legionella* counts in samples taken monthly from 20 outlets from the 18th August 2008 to the 04th July 2011.

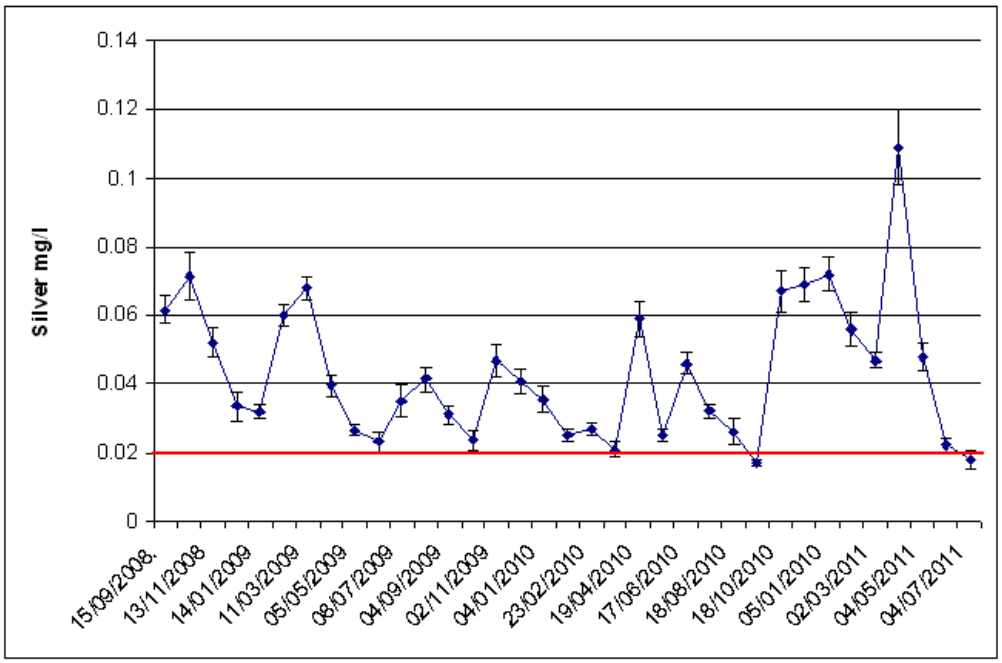


Graph 4.13 Average *Legionella* counts in samples taken monthly from 20 outlets from the 18th August 2008 to the 04th July 2011.

Graphs 4.14 and 4.15 show the averages of the copper and silver levels found in samples taken monthly from 20 outlets from the 15th September 2008 to the 04th July 2011. The average copper level was 0.366mg/l (± 0.02mg/l). The average silver level was 0.043mg/l (± 0.003mg/l).



Graph 4.14. Study hospital 5 – Average copper levels from 15th September 2008 to the 04th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.15. Study hospital 5 – Average silver levels from the 15th September 2008 to the 04th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average hot water temperature recorded from the 15th September 2008 to the 04th July 2011 was 46ºC (± 0.5ºC). The average cold water temperature was 16ºC (± 0.2ºC).

The chloride level found in the sample taken from the incoming mains on the 30th November 2010 was 60.7mg/l, the phosphorus was 701µg/l, and the pH was 7.24.

4.6 Study hospital 6

The results of samples taken on the 02nd October 2008, before the copper and silver ionization systems were activated, are shown in Table 4.14 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
92-1-010 CT	16	<0.0003	0.058	<LOD
31-01-019 CT	17	<0.0003	0.060	<LOD
32-01-027 CT	21	<0.0003	0.473	<LOD
32-01-007 HT	14	<0.0003	0.645	<LOD
39-01-004 CT	16	<0.0003	0.043	<LOD
39-01-004 HT	59	<0.0003	0.312	<LOD
10-06-051 HT	36	<0.0003	0.428	<LOD
10-01-040 HT	42	<0.0003	0.228	<LOD
10-01-059 CT	23	<0.0003	0.185	<LOD
047-2-002 CT	19	<0.0003	0.020	**400 np**
047-2-002 HT	61	<0.0003	0.156	<LOD
003-1-018 HT	55	<0.0003	0.285	<LOD
003-1-018 CT	20	<0.0003	0.091	**100 np**
075-2-006 HT	39	<0.0003	0.245	<LOD
075-2-006 CT	17	<0.0003	0.093	<LOD
074-06-041 CT	22	<0.0003	0.182	**100 np**
074-06-057 HT	37	<0.0003	0.408	<LOD
074-G-074 CT	21	<0.0003	0.203	<LOD
011-G-075 CT	16	<0.0003	0.045	**100 np**
011-06-042 HT	52	<0.0003	0.301	<LOD
011-06-042 CT	20	<0.0003	0.096	**100 np**
030-G-022 HT	31	<0.0003	0.568	**600 np**
030-G-022 CT	18	<0.0003	0.139	**300 np**
072-1-006 CT	17	<0.0003	0.057	<LOD
072-1-006 HT	52	<0.0003	0.370	<LOD
071-1-006 CT	17	<0.0003	0.047	<LOD
071-1-006 HT	52	<0.0003	0.389	<LOD
005-G-058 HT	37	<0.0003	0.399	<LOD
007-1-025 CT	24	<0.0003	0.340	**4000 s1**
067-G-001 CT	21	<0.0003	0.122	<LOD
067-G-001 HT	36	<0.0003	0.412	**100 s1**
004-G-037 CT	21	<0.0003	0.122	<LOD
004-2-012 HT	54	<0.0003	0.213	<LOD
004-2-012 CT	26	<0.0003	0.225	<LOD
009-G-042 HT	41	<0.0003	0.422	<LOD
009-G-042 CT	22	<0.0003	0.374	<LOD
001-G-062 HT	42	<0.0003	0.522	<LOD
001-G-062 CT	21	<0.0003	0.116	**800 np**
095-G-B027 CT	18	<0.0003	0.086	<LOD
095-G-B021 CT	25	<0.0003	1.28	<LOD
095-G-B021 HT	40	<0.0003	1.14	<LOD
095-1-H038 HT	39	<0.0003	0.784	<LOD
095-1-H038 CT	21	<0.0003	0.658	<LOD
095-G F21 CT	17	<0.0003	0.213	<LOD
095-G-F21 HT	39	<0.0003	0.347	<LOD
030-G-079 HT	40	<0.0003	0.414	<LOD
030-G-079 CT	18	<0.0003	0.102	<LOD
021-1-037 CT	20	<0.0003	0.159	**200 np**
021-G-032 CT	21	<0.0003	0.241	<LOD
021-B-014 CT	20	<0.0003	0.285	<LOD

HT = Hot tap, CT = Cold tap, np = *Legionella* non-*pneumophila,* s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.14 Study hospital 6 - Results from samples taken on the 02nd October 2008, before commissioning of copper and silver ionization systems.

Legionella were found in samples taken from 11 outlets. Fifty outlets were sampled, therefore, 22% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 4000CFU/l. The average *Legionella* count of the 50 outlets sampled was 136CFU/l (± 82CFU/l). Hot and cold outlets were contaminated.

The average hot water temperature at the 21 hot water outlets tested was 43ºC (± 2ºC). Although mixing valves were hidden and could not be seen, the hot water could have been blended with cold water at 13 of the 21 hot water outlets tested because the temperatures recorded at these were ranging from 36ºC to 42ºC.

The water temperatures recorded at 13 of the 29 cold water outlets sampled were above 20ºC. The average at the 29 cold water outlets tested was 20ºC (± 0.5ºC).

The average copper level found at the 50 outlets, due to copper leaching from copper pipes, was 0.302mg/l (± 0.037mg/l).

The copper and silver ionization systems were activated on the 02nd October 2008.

Table 4.15 below shows the results of samples taken on the 27th November 2008 from the 11 outlets that were contaminated before the systems were activated and from 14 outlets that were identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
10-06-051 HT	40	0.0218	0.325	<LOD
10-01-059 HT	40	0.0146	0.435	<LOD
047-2-002 CT	10	< 0.0003	0.011	<LOD
003-1-018 CT	13	0.0396	0.207	<LOD
075-2-006 HT	41	0.0027	0.293	<LOD
075-2-006 CT	10	0.0007	0.413	<LOD
074-06-041 CT	15	0.0312	0.252	<LOD
074-06-057 HT	40	0.0058	0.404	<LOD
011-G-075 CT	10	0.0139	0.138	<LOD
011-06-042 CT	14	0.0283	0.197	<LOD
030-G-022 HT	46	0.0103	0.519	<LOD
030-G-022 CT	14	0.0238	0.162	<LOD
072-1-006 CT	13	0.0124	0.124	<LOD
071-1-006 CT	11	0.0125	0.138	<LOD
005-G-058 HT	32	0.0130	0.428	<LOD
007-1-025 CT	18	0.0116	0.207	**700 s1**
007-1-025 HT	47	0.0100	0.506	<LOD
067-G-001 HT	40	0.0115	0.444	<LOD
004-G-037 CT	13	0.0058	0.145	<LOD
009-G-042 HT	44	0.0130	0.391	<LOD
001-G-062 HT	41	0.0086	0.532	<LOD
001-G-062 CT	14	0.0157	0.198	<LOD
095-G-B021 HT	39	0.0047	1.48	<LOD
095-1-H038 HT	40	0.0048	0.964	<LOD
021-1-037 CT	16	0.0357	0.273	<LOD

HT = Hot tap, CT = Cold tap, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.15 Study hospital 6 - Results from samples taken on the27th November 2008.

No *Legionella* were found in samples taken from 10 outlets that were previously contaminated. The *Legionella* persisted only at 1 cold outlet, the 007-1-025 CT, but the count had dropped from 4000CFU/l to 700CFU/L. The silver target, of 0.02mg/l, was not met in the contaminated sample, only 0.012mg/l was found. The temperature recorded at the contaminated outlet was 18ºC.

The cold temperatures recorded at the 13 cold outlets tested were below 20ºC. The hot water temperatures at the 12 hot outlets tested were below 50ºC because it was, most likely, blended water.

The silver found in the 25 outlets tested ranged from <0.0003mg/l to 0.036mg/l. The average silver level was 0.014mg/l (± 0.002mg/l). The silver target level, of 0.02mg/l,

was not met at 19 of the 25 outlets tested. The copper ranged from 0.011mg/l to 1.480mg/l. The average copper level was 0.367mg/l (± 0.061mg/l. The copper target, of 0.2mg/l, was not met at 8 of the 25 outlets tested.

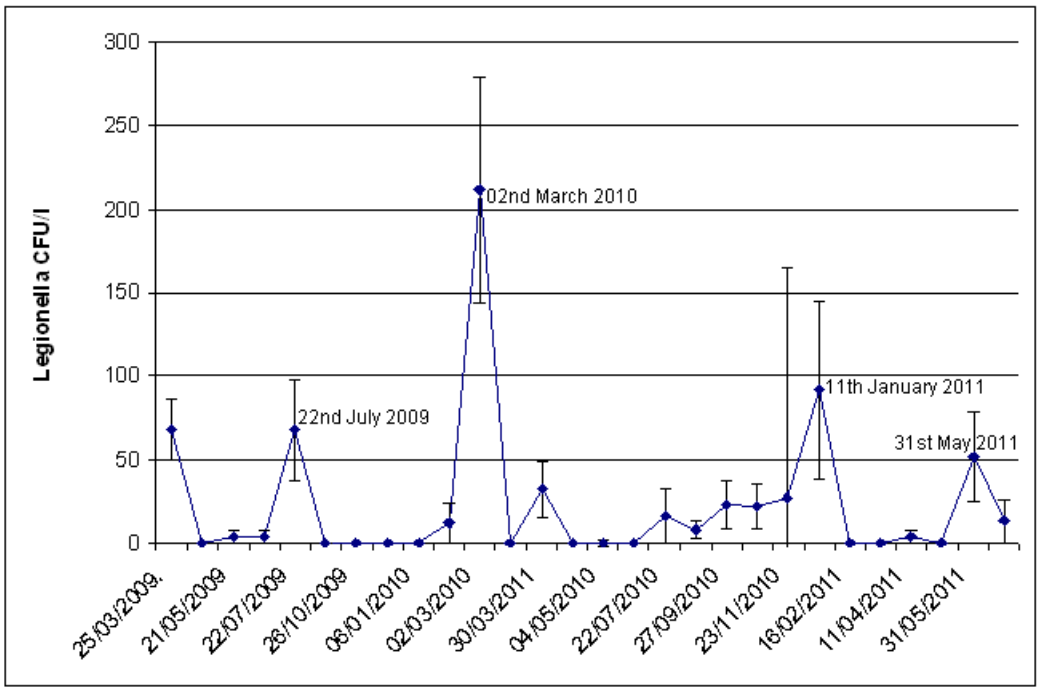
Re-samples for *Legionella*, copper and silver analysis were taken from the 007-1-025 cold outlet on the 19th December 2008. 2700CFU/l were found in the sample taken immediately after opening the outlet but the count dropped, to 300CFU/l, in the sample that was taken after running the outlet for 10 minutes. The silver target was not met in both samples and the copper target was also not met in the sample that was taken after running the outlet for 10 minutes.

It was difficult to maintain the copper and silver target levels at outlets because they were not regularly used, and although the *Legionella* declined from the 07th January 2009 onwards, it persisted at outlets at which very low copper and silver levels were found.

A *Legionella* count of 4500CFU/l was found in the brushed through rubber lined hose that was removed on the 25th March 2009 from the 007-1-025 outlet. After the hose was removed, the contamination dropped.

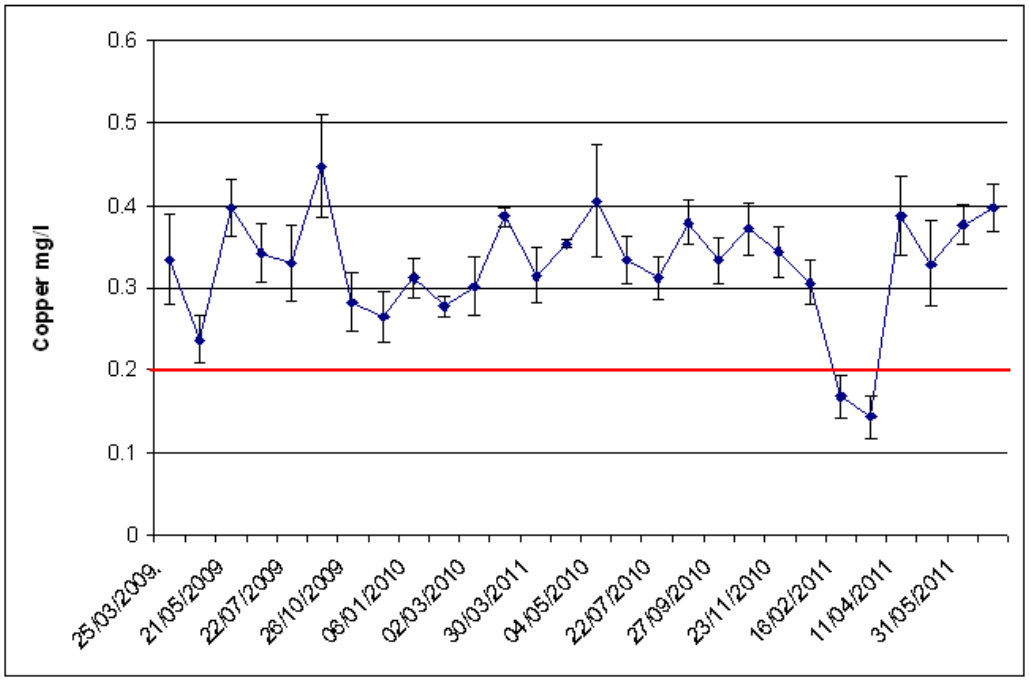
The copper and silver target levels were not consistently maintained at some outlets, and the contamination worsened. *Legionella* were found, albeit at low counts, in samples taken from 8 outlets on the 22nd July 2009, from 10 outlets on the 02nd March 2010, from 5 outlets on the 11th January 2011 and from 5 outlets on the 31st May 2011. However, after the copper and silver levels increased and the targets were consistently met at the outlets, no *Legionella* were detected.

Graph 4.16 below shows the average *Legionella* counts in samples taken monthly from 25 outlets from the 25th March 2009 to the 08th July 2011.

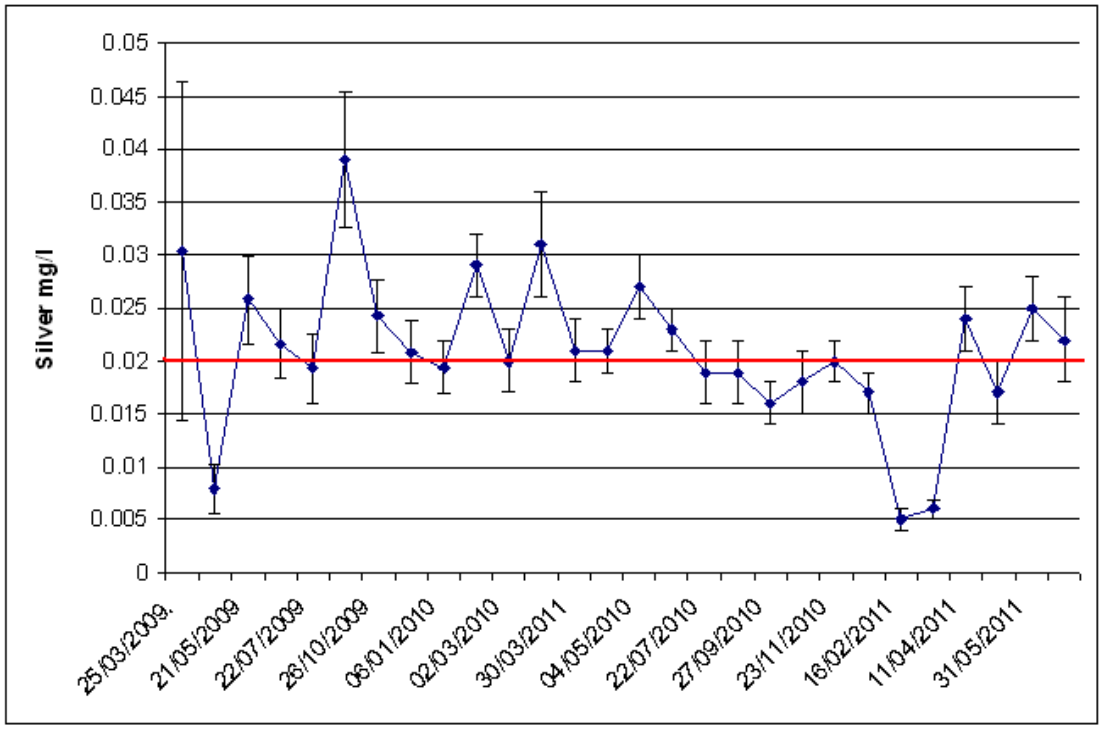


Graph 4.16 Study hospital 6 - Average *Legionella* counts in samples taken monthly from 25 outlets from the 25th March 2009 to the 08th July 2011.

Graphs 4.17 and 4.18 show the averages of the copper and silver levels found in samples taken monthly from the 25 outlets from the 25th March 2009 to the 08th July 2011. The average copper level was 0.328mg/l (± 0.013mg/l). The average silver level was 0.021mg/l (± 0.001mg/l).



Graph 4.17 Study hospital 6 – Average copper levels from 25th March 2009 to the 08th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.18 Study hospital 6 – Average silver levels from the 25th March 2009 to the 08th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average hot water temperature recorded from the 02nd October 2008 to the 08th July 2011 was 44ºC (± 0.4ºC). The average cold water temperature was 15ºC (± 0.1ºC).

The chloride level found in the sample taken from the incoming mains on the 26th October 2009 was 46.7mg/l, the phosphorus was 1500µg/l, and the pH was 7.2.

The chloride level found in the sample taken from the borehole water on the 26th October 2009 was 32.9mg/l, the phosphorus was 2100µg/l, and the pH was 6.9.

4.7 Study hospital 7

The results of samples taken on the 25th November 2008, before the copper and silver ionization systems were activated, are shown in Table 4.16 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
FB21 HT	35	< 0.0003	0.135	**300 np**
FA91 HT	37	< 0.0003	0.387	**1300 s1**
FA73 CT	15	< 0.0003	0.106	<LOD
GM24 HT	23	< 0.0003	0.602	**44800 s1**
GG16 HT	51	< 0.0003	0.265	**300 s1 + 200 np**
FA116 CT	17	< 0.0003	0.123	**1400 np**
DE03 MT	34	< 0.0003	0.313	**12100 np**
DE39 HT	36	< 0.0003	0.333	**200 np**
DA43 MT	31	< 0.0003	0.346	**7200 s1 + 6600 np**
CE101 CT	14	< 0.0003	0.101	<LOD
CE83 HT	42	< 0.0003	0.265	**3600 s1 + 1200 np**
CE66 HT	61	< 0.0003	0.391	<LOD
FA2 CT	19	0.0020	11.8	**10600 s1 + 76800 np**
AJ12 HT	28	< 0.0003	0.745	**300 s1 + 100 np**
AE05 CT	14	< 0.0003	0.087	**100 s1 + 3200 np**
AK 16 HT	39	0.0007	1.41	**100 s2-14**
AX40 shower hot	33	< 0.0003	0.425	**500 np**
AX12 CT	18	< 0.0003	0.201	**3200 np**
AN04 HT	37	< 0.0003	0.527	**100 np**
AN44 CT	23	< 0.0003	0.339	**11000 np**
CY16 CT	13	< 0.0003	0.127	**1100 np**
AWT Kitchen HT	64	< 0.0003	0.387	<LOD
CE118 CT	15	< 0.0003	0.299	**200 s1**
D Nurse Station HT	60	< 0.0003	0.600	<LOD
BG11 MT	13	< 0.0003	0.160	**100 s1 + 300 np**
BG23 HT	50	< 0.0003	0.488	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

Table 4.16 Study hospital 7. Results from samples taken on the 25th November 2008, before commissioning of copper and silver ionization systems.

Legionella were found in samples taken from 20 outlets. Twenty six outlets were sampled, therefore, around 77% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 87400CFU/l. The average *Legionella* count of the 26 outlets sampled was 7188CFU/l (± 3681CFU/l). Hot, blended and cold outlets were contaminated. The highest

Legionella count, of 87400CFU/l, was found in a sample taken from a cold outlet. The temperature recorded at this outlet was 19ºC.

The average hot water temperature at the 16 hot water outlets tested was 41ºC (± 3ºC). Although mixing valves were hidden and could not be seen, the hot water could have been blended with cold water at 11 of the 16 hot water outlets tested because the temperatures recorded at these were ranging from 23ºC to 42ºC.

The water temperatures recorded at 9 cold water outlets were below 20ºC. The cold water temperature was high at 1 outlet at 23ºC. The average at the 10 cold water outlets tested was 16ºC (± 1ºC).

The average copper level found at the 26 outlets, due to copper leaching from copper pipes, was 0.398mg/l (± 0.062mg/l). Copper levels exceeded 1mg/l in samples taken from 2 outlets.

The copper and silver ionization systems were activated on the 25th November 2008.

Table 4.17 below shows the results of samples taken on the 05th January 2009 from the 20 outlets that were contaminated before the systems were activated.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
FB21 HT	33	0.0168	0.323	<LOD
FA91 HT	33	0.0434	0.644	**100 np**
GM24 HT	17	0.0173	3.23	**33600 s1 + 11600 np**
GG16 HT	58	0.0184	0.308	<LOD
FA116 CT	19	0.146	0.360	<LOD
DE03 MT	34	0.0983	0.509	**700 np**
DE39 HT	33	0.0963	0.515	<LOD
DA43 MT	32	0.0635	0.609	**600 s1**
CE83 HT	33	0.0771	0.343	**1500 s1+ 1600 np**
FA2 CT	17	0.0467	0.395	**100 s1 + 10800 np**
AJ12 HT	17	0.0236	2.16	**100 s1 + 400 np**
AE05 CT	11	0.115	0.250	<LOD
AK 16 HT	37	0.0243	1.13	**100 s1**
AX40 shower hot	31	0.0903	0.625	<LOD
AX12 CT	16	0.126	0.481	<LOD
AN04 HT	34	0.0350	0.581	**300 np**
AN44 CT	23	0.0861	0.439	**400 np**
CY16 CT	17	0.123	0.427	<LOD
CE118 CT	15	0.141	0.397	**100 np**
BG11 MT	11	0.113	0.402	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.17 Study hospital 7. Results from samples taken on the 05th January 2009.

No *Legionella* were found in samples taken from 9 outlets that were previously contaminated. The *Legionella* persisted at 11 outlets.

The *Legionella* colony forming unit counts had dropped in places. The average *Legionella* count of the 20 outlets sampled was 3085CFU/l (± 2284CFU/l). Hot, blended and cold outlets were again contaminated. The silver target level was not met in the sample taken from a hot outlet in which 45200CFU/l was found, only 0.017mg/l silver was found. The temperature recorded at this outlet was very low at 17ºC.

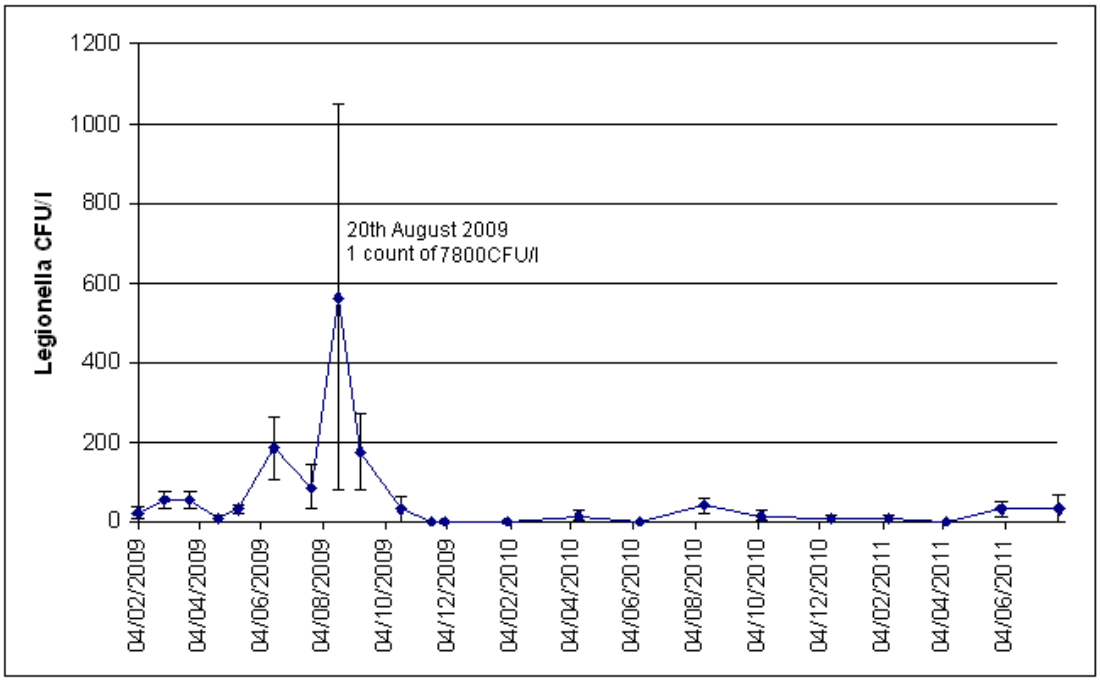
The cold temperatures recorded at the 8 cold outlets tested were below 20ºC, except for at 1 cold outlet at which the temperature was 23ºC. The copper found in this sample was 0.439mg/l, the silver was 0.086mg/l, and the *Legionella* count had dropped from 11000CFU/l to 400CFU/l.

The hot water temperatures at the 12 hot outlets tested were below 50ºC, except for at 1 hot outlet at which the temperature was 58ºC.

The silver found at the 20 outlets tested ranged from 0.017mg/l to 0.146 mg/l. The average silver level was 0.075mg/l (± 0.01mg/l). The silver target level, of 0.02mg/l, was not met at 3 of the 20 outlets tested. The copper ranged from 0.25mg/l to 3.23mg/l. The average copper level was 0.706mg/l (± 0.16mg/l). The copper target, of 0.2mg/l, was met at all outlets tested.

From the 05th January 2009 the *Legionella* contamination declined. *Legionella* counts of 100CFU/l and 200 CFU/l only persisted at some outlets tested.

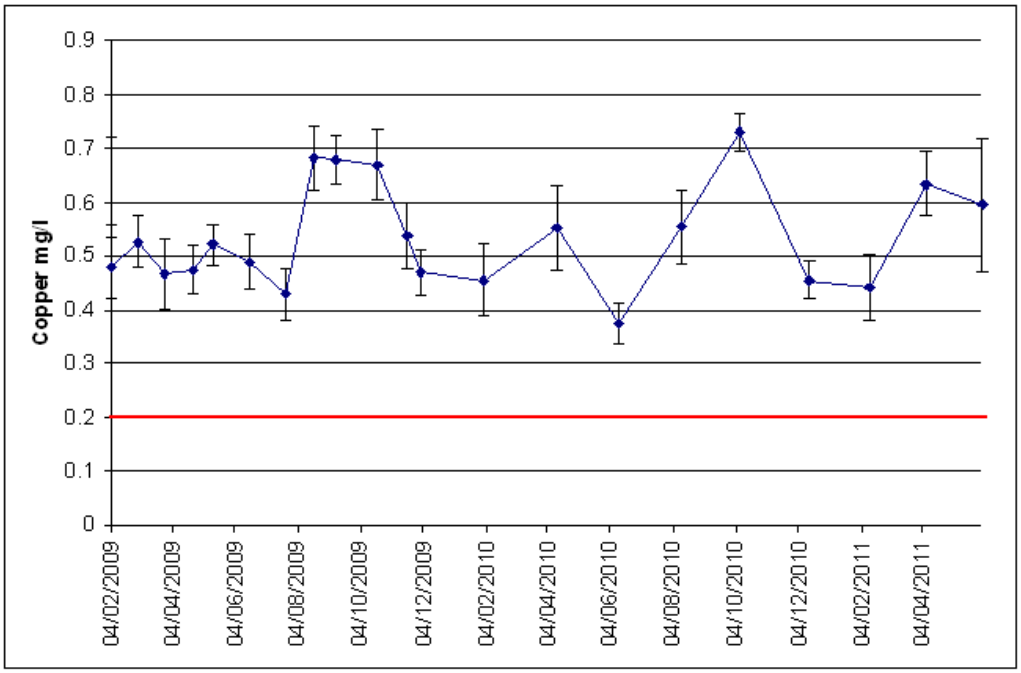
Graph 4.19 below shows the average *Legionella* counts in samples taken monthly from 13 outlets from the 04th February 2009 to the 26th July 2011.



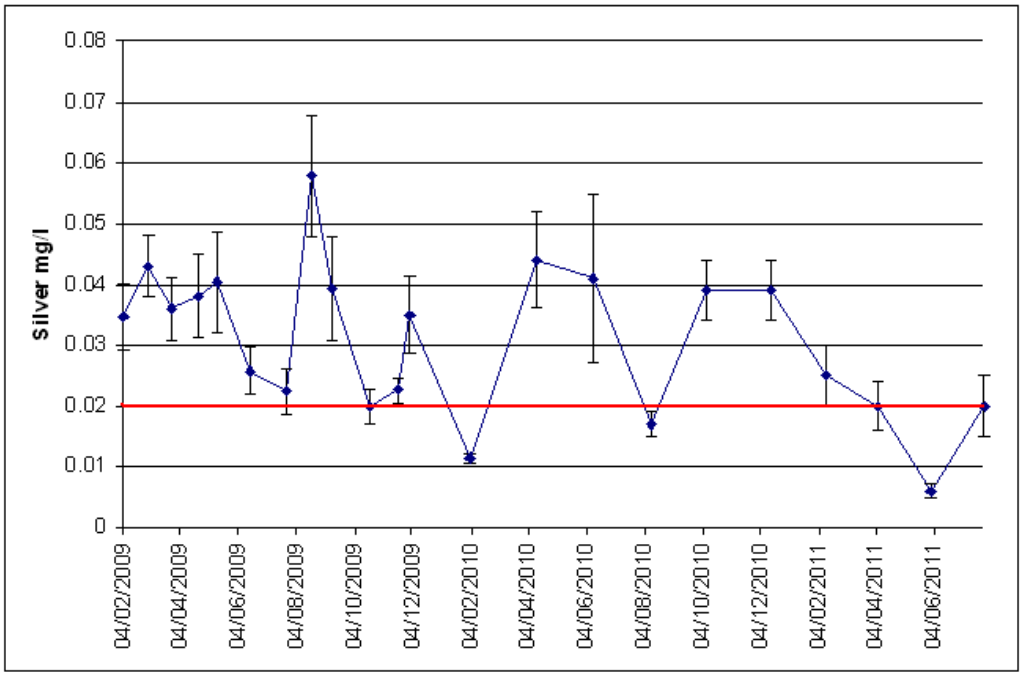
Graph 4.19. Study hospital 7 - Average *Legionella* counts in samples taken monthly from 13 outlets from the 04th February 2009 to the 26th July 2011.

A *Legionella* count of 7800CFU/l was found in a sample taken from a hot outlet on the 20th August 2009, which increased the average level. The copper found in this sample was above the target at 0.654mg/l, however, the silver target was not met as only 0.008mg/l silver was found. The temperature recorded was 35ºC, indicating that the hot water may have been blended with cold water.

Graphs 4.20 and 4.21 show the averages of the copper and silver levels found in samples taken monthly from the 13 outlets from the 04th February 2009 to the 26th July 2011. The average copper level was 0.539mg/l (± 0.02mg/l). The average silver level was 0.031mg/l (± 0.003mg/l).



Graph 4.20 Study hospital 7 – Average copper levels from the 04th February 2009 to the 26th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.21 Study hospital 7 – Average silver levels from the 04th February 2009 to the 26th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average hot water temperature recorded from the 25th November 2008 to the 26th July 2011 was 40ºC (± 0.5ºC). The average cold water temperature was 18ºC (± 0.4ºC).

The chloride level found in the sample taken from the incoming mains on the 20th October 2009 was 58.5mg/l, the phosphorus was 237µg/l, and the pH was 7.6.

4.8 Study hospital 8

The results of samples taken on the 27th April 2009, before the copper and silver ionization system was activated, are shown in Table 4.18 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
RO PR2 CT	13.0	< 0.0003	0.005	<LOD
RO 002 HT	59.0	< 0.0003	0.067	<LOD
R1 018 CT	12.0	< 0.0003	0.019	**500 np**
R1 149 HT	43.0	< 0.0003	0.060	**100 s1 + 400 np**
R2 204 CT	12.0	< 0.0003	0.015	<LOD
R2 017 HT	54.0	< 0.0003	0.049	**300 s1**
R3 122 CT	12.0	< 0.0003	0.010	**100 np**
R3 022 MT	31.0	< 0.0003	0.061	**100 np**
P3 012 HT	61.0	< 0.0003	0.066	<LOD
G3 021 MT	32.0	< 0.0003	0.033	<LOD
N3 015 CT	16.0	< 0.0003	0.014	<LOD
M3.023 HT	58.0	< 0.0003	0.063	<LOD
L3.001CT	15.0	< 0.0003	0.033	<LOD
D3.012 HT	59.0	< 0.0003	0.067	<LOD
E3 019 MT	27.0	< 0.0003	0.025	<LOD
F3 010 HT	58.0	< 0.0003	0.041	<LOD
N5 010 CT	16.0	< 0.0003	0.026	<LOD
F5.006 MT	25.0	< 0.0003	0.025	<LOD
G4.023 HT	63.0	< 0.0003	0.061	<LOD
P4.012 CT	14.0	< 0.0003	0.019	<LOD
N4.010 MT	42.0	< 0.0003	0.070	<LOD
M4.017 CT	13.0	< 0.0003	0.015	<LOD
N2.002 HT	45.0	< 0.0003	0.046	<LOD
N1.012 CT	12.0	< 0.0003	0.007	<LOD
E4.022 HT	61.0	< 0.0003	0.045	<LOD
D4.034 CT	12.0	< 0.0003	0.043	<LOD
L4.011 HT	57.0	< 0.0003	0.091	<LOD
K2.001 CT	12.0	< 0.0003	0.011	<LOD
D221 MT	42.0	< 0.0003	0.095	<LOD
E2.023 HT	61.0	< 0.0003	0.055	<LOD
M2.013 CT	14.0	< 0.0003	0.022	<LOD
F2.050 HT	45.0	< 0.0003	0.033	<LOD
G2.001 CT	12.0	< 0.0003	0.024	<LOD
P1.031 HT	49.0	< 0.0003	0.043	<LOD
P2.056 HT	58.0	< 0.0003	0.135	<LOD
F1.034 CT	12.0	< 0.0003	0.010	<LOD
F1.019 HT	60.0	< 0.0003	0.098	<LOD
G1.017 CT	12.0	< 0.0003	0.004	<LOD
GX HT	42.0	< 0.0003	0.149	<LOD
X1.005 CT	12.0	< 0.0003	0.014	<LOD
J3.006 CT	12.0	< 0.0003	0.010	<LOD
AX002 HT	59.0	< 0.0003	0.042	<LOD
A2.024 HT	42.0	< 0.0003	0.073	**300 s1**
A2.005 CT	15.0	< 0.0003	0.138	**100 s1 + 200 np**
A2.011 MT	44.0	< 0.0003	0.391	**100 s1 + 500 np**

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.18 Study hospital 8. Results from samples taken on the 27th April 2009, before commissioning of copper and silver ionization system.

Legionella were found in samples taken from 8 outlets. Forty five outlets were sampled, therefore, around 18% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 600CFU/l. The average *Legionella* count of the 45 outlets sampled was 60CFU/l (± 23CFU/l). Hot, blended and cold outlets were contaminated. The highest *Legionella* count, of 600CFU/l, was found in a sample taken from a blended outlet. The temperature recorded at this outlet was 44ºC.

The average hot water temperature at the 26 hot water outlets tested was 49ºC (± 2ºC). Although mixing valves were hidden and could not be seen, the hot water could have been blended with cold water at 12 of the 26 hot water outlets tested because the temperatures recorded at these were ranging from 25ºC to 45ºC.

The water temperatures recorded at the 19 cold water outlets were below 20ºC. The average temperature at the 19 cold water outlets tested was 13ºC (± 0.3ºC). Three of the 8 outlets that were contaminated with *Legionella* were cold outlets.

The average copper level found at the 45 outlets, due to copper leaching from copper pipes, was 0.054mg/l (± 0.009mg/l).

The copper and silver ionization system was activated on the 27th April 2009.

Table 4.19 below shows the results of samples taken on the 27th May 2009 from the 8 outlets that were contaminated before the system was activated and from 14 outlets that had been identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
R1 018 CT	14.0	0.0306	0.191	**100 np**
R1 149 HT	43.0	0.0194	0.152	<LOD
R2 017 HT	58.0	0.0115	0.094	<LOD
R3 122 CT	15.0	0.0325	0.209	**100 np**
R3 022 MT	19.0	0.0299	0.342	**2000 np**
G3 021 MT	32.0	0.0229	0.184	<LOD
N4.010 MT	37.0	0.0211	0.187	<LOD
A2.024 HT	40.0	0.0110	0.141	<LOD
A2.005 CT	15.0	0.0323	0.387	**100 s1 + 200 np**
A2.011 MT	32.0	0.0190	0.176	<LOD
N5 010 CT	16.0	0.0314	0.233	<LOD
M4.017 CT	14.0	0.0156	0.230	<LOD
E4.022 HT	59.0	0.0096	0.104	<LOD
K2.001 CT	14.0	0.0187	0.187	<LOD
P4.012 CT	15.0	0.0317	0.230	<LOD
D3.012 HT	58.0	0.0052	0.098	<LOD
M3.023 HT	57.0	0.0095	0.125	<LOD
RO.002 HT	55.0	0.0155	0.143	<LOD
X1.005 CT	14.0	0.0297	0.193	<LOD
P1.031 HT	43.0	0.0182	0.142	<LOD
G2.001 CT	14.0	0.0318	0.910	<LOD
D221 MT	38.0	0.0114	0.153	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (CFU/l).

Table 4.19 Study hospital 8. Results from samples taken on the 27th May 2009.

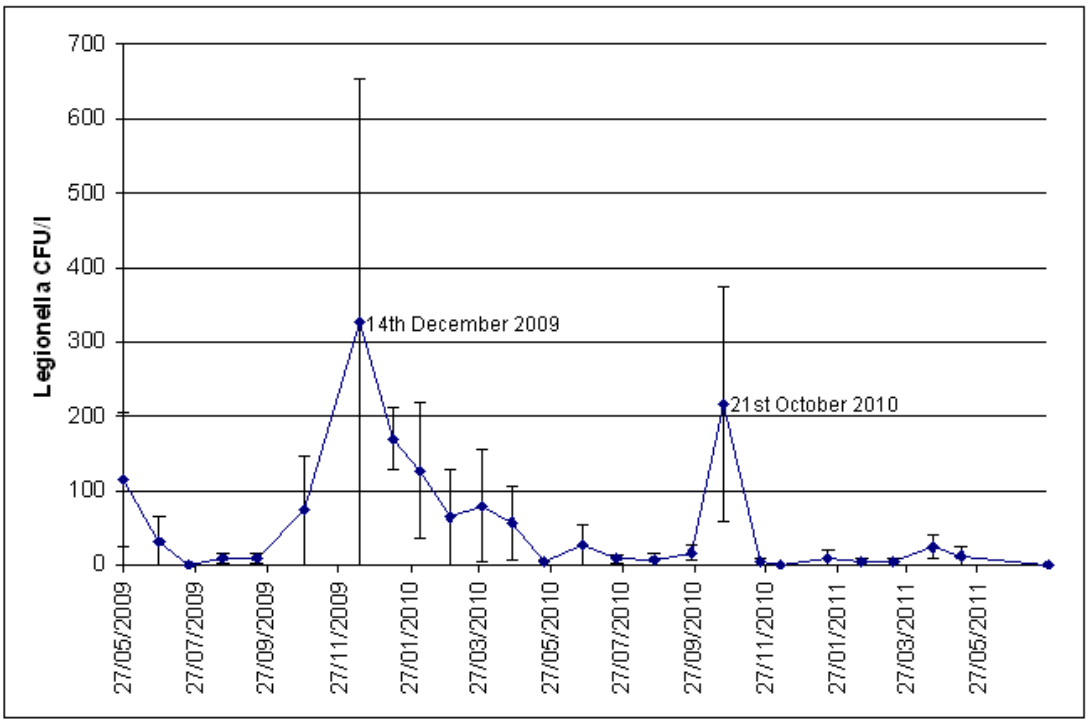
No *Legionella* were found in samples taken from 4 outlets that were previously contaminated. The average *Legionella* count of the 22 outlets sampled was 114CFU/l (± 91CFU/l). Three cold outlets and 1 blended outlet were contaminated. The copper and silver targets were met in the contaminated samples.

The *Legionella* colony forming unit count had increased at 1 blended outlet from 100 CFU/l to 2000CFU/l. However, no *Legionella* were found in the sample taken from this outlet on the 21st July 2009, only 100CFU/l were found in the sample taken on the 17th September 2009, and from the 17th September 2009 onwards no *Legionella* were found.

The cold temperatures recorded at the 9 cold outlets tested were below 20ºC. The hot water temperatures at 8 of the 13 hot outlets tested were below 50ºC.

The silver found at the 22 outlets tested ranged from 0.005mg/l to 0.032mg/l. The average silver level was 0.021mg/l (± 0.002mg/l). The copper ranged from 0.094mg/l to 0.91mg/l. The average copper level was 0.219mg/l (± 0.036mg/l). The copper target, of 0.2mg/l, was not met at 15 outlets.

Graph 4.22 below shows the average *Legionella* counts in samples taken monthly from 22 outlets from the 27th May 2009 to the 25th July 2011.

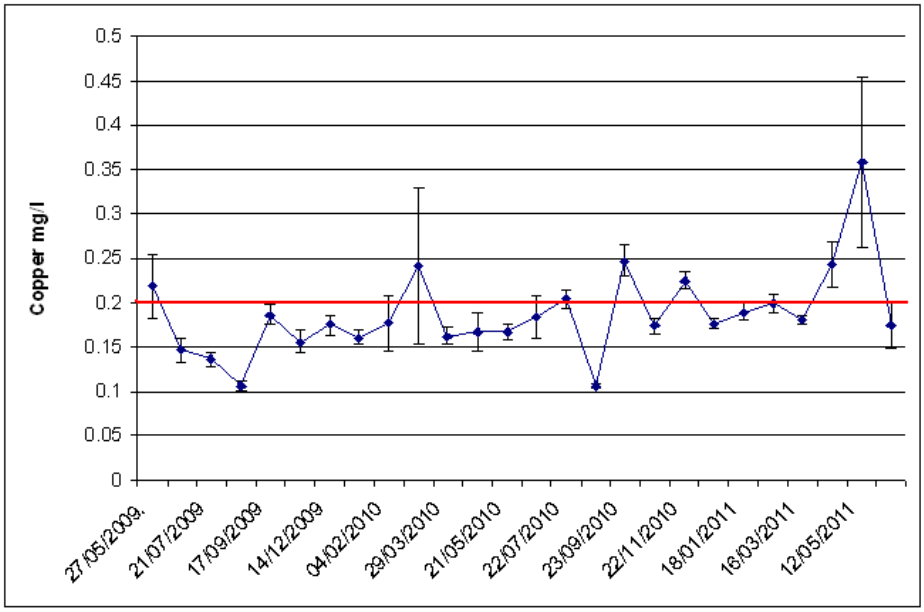


Graph 4.22 Study hospital 8 - Average *Legionella* counts in samples taken monthly from 22 outlets from the 27th May 2009 to the 25th July 2011.

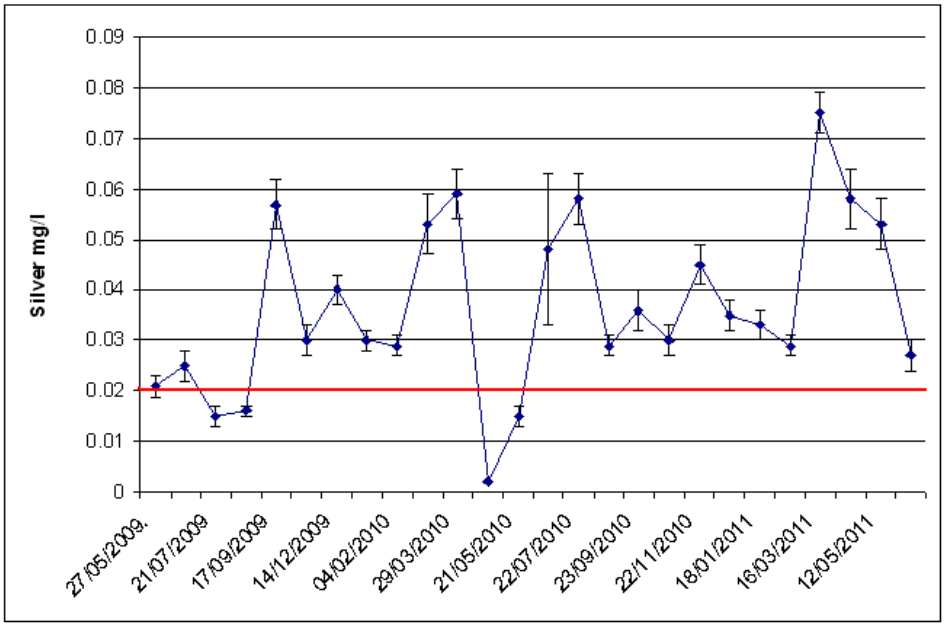
The *Legionella* contamination declined from the 27th May 2009 onwards but it persisted at 1 cold outlet that was first tested on the 17th September 2009. The copper and silver targets were met in the sample taken on the 17th September 2009, which indicated that a potentially large source, too large for the available copper and silver to completely control, could be seeding the bacteria into the water. Hospital estates personnel were, therefore, advised to trace the pipework upstream the outlet with the view to isolate and remove any potential *Legionella* sources. This was not done and the *Legionella* contamination persisted even though the copper and silver targets were met. High *Legionella* colony forming unit counts were found in samples taken from the outlet on the 14th December 2009 – 7200CFU/l – and on the 21st October 2010 - 3500CFU/l in the sample taken immediately after opening outlet and 500CFU/l in the

sample taken after running outlet for 10 minutes. These counts increased the average *Legionella* levels.

Graphs 4.23 and 4.24 show the averages of the copper and silver levels found in samples taken monthly from the 22 outlets from the 27th May 2010 to the 25th July 2011. The average copper level was 0.187mg/l (± 0.01mg/l). The average silver level was 0.036mg/l (± 0.003mg/l).



Graph 4.23 Study hospital 8 – Average copper levels from the 27th May 2009 to the 25th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.24 Study hospital 8 – Average silver levels from the 27th May 2009 to the 25th July 2011 (the red line is the target level for silver - 0.02mg/l).

The copper release by the copper and silver ionization system was too low, which resulted in less copper being available at the outlets. *Legionella* was, however, controlled, which suggested that the higher silver levels at the outlets compensated for the lower copper levels.

There was a substantial drop in silver found in the samples taken on the 24th April 2010. This was explained by the samples not being pre-treated properly by another UKAS accredited laboratory that had to be used to analyze the samples.

The average hot water temperature recorded from the 27th April 2009 to the 25th July 2011 was 45ºC (± 0.4ºC). The average cold water temperature was 15ºC (± 0.2ºC).

The chloride level found in the sample taken from the incoming mains on the 15th November 2010 was 8.4mg/l, the phosphorus was 452µg/l, and the pH was 8.5.

4.9 Study hospital 9

The results of samples taken on the 29th July 2009, before the copper and silver ionization system was activated, are shown in Table 4.20 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F HT	47	< 0.0003	0.153	**500 s1**
1F CT	18	< 0.0003	0.022	<LOD
1F WC MT	39	< 0.0003	0.129	**1500 s2-14**
D6 HT	34	< 0.0003	0.115	<LOD
D3 shower set on hot	39	< 0.0003	0.122	**300 s2-14**
Staff WC HT	46	< 0.0003	0.2	**6400 s2-14**
Staff WC CT	23	< 0.0003	0.118	<LOD
D10 shower set on hot	37	< 0.0003	0.124	**600 s1**
D11 CT	20	< 0.0003	0.053	<LOD
B1 HT	40	< 0.0003	0.179	**100 s1**
Day room CT	19	< 0.0003	0.168	<LOD
B5 HT	49	< 0.0003	0.205	<LOD
Physio CT	22	< 0.0003	0.15	<LOD
Physio HT	53	< 0.0003	1.3	**200 s2-14**
Red CT	17	< 0.0003	0.029	<LOD
Treatment room CT	18	< 0.0003	0.053	<LOD
Theatre shower set on hot	40	< 0.0003	0.173	<LOD
TSSU CT	18	< 0.0003	0.053	<LOD
Anaesthetic room 1 HT	51	< 0.0003	0.181	<LOD
Recovery room CT	22	< 0.0003	0.159	<LOD
T3 HT	46	0.0003	0.357	<LOD
T3 CT	20	< 0.0003	0.04	<LOD
Joyce shower set on hot	33	< 0.0003	0.126	**100 np**
Cillia shower set on cold	19	< 0.0003	0.068	<LOD
Surgical HT	51	< 0.0003	0.138	**600 s2-14**
C16 shower set on hot	34	< 0.0003	0.125	**700 s2-14**
X-Ray CT	32	< 0.0003	0.221	**300 s2-14**
OP HT	49	< 0.0003	0.16	**700 s2-14**
Disabled WC CT	24	< 0.0003	0.052	**600 s2-14**
Hydro WC HT	47	< 0.0003	0.144	**1000 s2-14**

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

Table 4.20 Study hospital 9. Results from samples taken on the 29th July 2009, before commissioning of copper and silver ionization system.

Legionella were found in samples taken from 14 outlets. Thirty outlets were sampled, therefore, around 47% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 6400CFU/l. The average *Legionella* count of the 30 outlets sampled was 453CFU/l (± 216CFU/l). Hot, blended and cold outlets were contaminated. The highest *Legionella* count, of 6400CFU/l, was found in a sample taken from a blended outlet. The temperature recorded at this outlet was 46ºC.

The average hot water temperature at the 17 hot water outlets tested was 43ºC (± 1.6ºC). Although mixing valves were hidden and could not be seen, the hot water could have been blended with cold water at 12 of the 17 hot water outlets tested because the temperatures recorded at these were ranging from 33ºC to 47ºC. Nine of these outlets were contaminated with *Legionella*. The temperatures recorded at 3 of the contaminated pure hot outlets were 53ºC, 51ºC, and 49ºC.

The average water temperature at the 13 cold water outlets tested was 21ºC (± 1.1ºC). The temperatures recorded at the 2 contaminated cold outlets were high at 32ºC and 24ºC.

The average copper level found at the 30 outlets, due to copper leaching from copper pipes, was 0.171mg/l (± 0.04mg/l). This figure included a high copper level of 1.3 mg/l found in a sample taken from a pure hot outlet. Excluding this level the average copper level found was 0.132mg/l (± 0.01mg/l).

The copper and silver ionization system was activated on the 03rd August 2009.

Table 4.21 below shows the results of samples taken on the 02nd and 04th September 2009 from the 14 outlets that were contaminated before the system was activated and from 2 outlets that had been identified as being at risk of *Legionella* contamination.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F HT	51	0.0382	0.318	<LOD
1F WC MT	37	0.0322	0.385	**500 np**
D3 shower set on hot	39	0.0958	0.367	**400 np**
Staff WC HT	49	0.0369	0.316	<LOD
D10 shower set on hot	37	0.0946	0.292	**300 s1**
B1 CT	24	0.0060	0.380	<LOD
B1 HT	47	0.031	0.31	<LOD
Physio HT	52	0.0309	0.279	<LOD
TSSU CT	20	0.0363	0.142	<LOD
Joyce shower set on hot	39	0.0757	0.359	**200 s1 + 100 np**
Surgical HT	53	0.0283	0.323	<LOD
C16 shower set on hot	38	0.0407	0.466	<LOD
X-Ray CT	28	0.0075	0.428	<LOD
OP HT	50	0.0269	0.323	<LOD
Disabled WC CT	24	0.0618	0.431	**3300 np**
Hydro WC HT	54	0.0389	0.327	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.21 Study hospital 9. Results from samples taken on the 02nd September 2009.

No *Legionella* were found in samples taken from 9 outlets that were previously contaminated. The water temperatures recorded at 4 hot outlets, at which 500CFU/l, 400CFU/l, 300CFU/l and 300CFU/l *Legionella* were found, were below 40ºC, which indicated that these outlets may have been blended.

The cold temperatures recorded at the 4 cold outlets tested were above 20ºC. The temperature of the cold outlet, the disabled WC cold tap, at which *Legionella* persisted, was 24ºC. The *Legionella* colony forming unit count increased at this outlet from 300CFU/l found in the sample taken on the 29th July to 3300CFU/l. The *Legionella* non-*pneumophila* species that were found instead of the *Legionella pneumophila* serogroup 2 to 14 found previously must be an evidence of a new contamination source.

The average *Legionella* count of the 16 outlets sampled was 300CFU/l (± 205CFU/l).

The silver found at the 16 outlets ranged from 0.006mg/l to 0.096mg/l. The average silver level was 0.043mg/l (± 0.007mg/l). The silver target, of 0.02mg/l, was met at 14 of the 16 outlets tested. The copper ranged from 0.142mg/l to 0.477mg/l. The average

copper level was 0.34mg/l (± 0.018mg/l). The copper target, of 0.2mg/l, was met at 15 of the 16 outlets tested.

No *Legionella* were found in the samples taken from 15 outlets, including the previously contaminated 5 outlets, on the 01st October 2009.

Legionella persisted at the cold outlet of the disabled WC, albeit at lower counts, from the 29th October 2009 onwards.

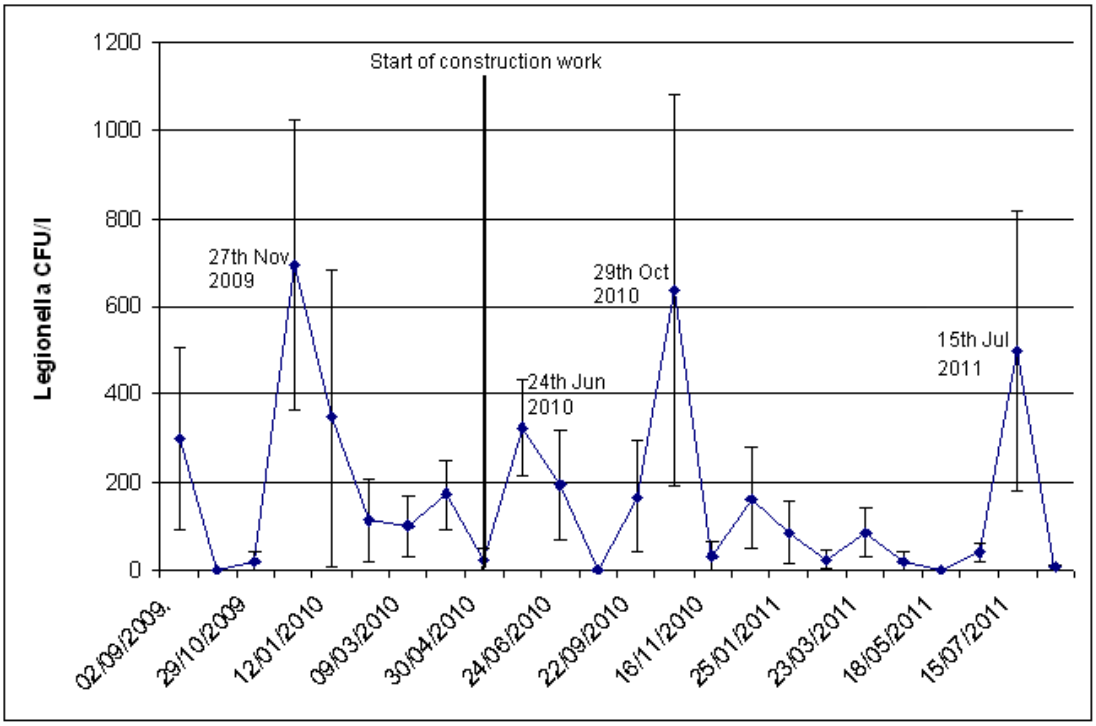
Legionella were found in samples and re-samples taken from 15 outlets on the 27th November 2009, see Table 4.22 below. The copper and silver targets were met in these samples.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F HT	57	0.0289	0.295	<LOD
1F WC MT	41	0.0475	0.29	<LOD
D3 shower set on hot	41	0.0398	0.312	**2600 np**
Staff WC HT	42	0.0344	0.339	**4500 np**
D10 shower set on hot	43	0.0574	0.28	**200 s1**
B1 HT	43	0.0565	0.32	<LOD
Therapy HT	41	0.0551	0.297	<LOD
TSSU CT	14	0.0891	0.105	<LOD
Joyce shower set on hot	56	0.0059	0.256	<LOD
Surgical HT	50	0.0239	0.309	**100 np**
C16 shower set on hot	42	0.0772	0.365	<LOD
X-Ray CT	19	0.0035	0.383	<LOD
OP HT	52	0.0309	0.552	<LOD
Disabled WC CT – pre-flush	15	0.0955	0.268	**1800 np**
Disabled WC CT – post-flush	14	0.0917	0.265	**1900 np**
Hydro WC HT	42	0.0417	0.301	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

Table 4.22 Study hospital 9. Results from samples and re-samples taken on the 27th November 2009.

Graph 4.25 below shows the average *Legionella* counts in samples and re-samples taken monthly from the 02nd September 2009 to the 15th July 2011.



Graph 4.25 Study hospital 9 - Average *Legionella* counts in samples and re-samples taken monthly from the 02nd September 2009 to the 15th July 2011.

After the construction work was started, higher *Legionella* colony forming unit counts were found in re-samples taken on the 24th June 2010, in samples and re-samples taken on the 29th October 2010, and in re-samples taken on the 15th July 2011, see Tables 4.23, 4.24, and 4.25 below.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F HT - pre-flush	44	0.0145	0.309	<LOD
1F HT – post-flush	47	0.0171	0.295	<LOD
1F WC MT – pre-flush	40	0.0215	0.359	**1100 np**
1F WC MT – post-flush	40	0.0286	0.301	**100 np**
Staff WC HT – pre-flush	40	0.0094	0.41	<LOD
Staff WC HT – post-flush	40	0.0254	0.308	<LOD
D3 shower set on hot – pre-flush	20	0.0037	0.563	<LOD
D3 shower set on hot – post-flush	49	0.0185	0.302	<LOD
B1 HT – pre-flush	32	0.0131	0.278	<LOD
B1 HT – post-flush	42	0.0211	0.285	<LOD
Disabled WC MT – pre-flush	41	0.0061	0.266	**2600 np**
Disabled WC MT – post-flush	41	0.0153	0.282	**400 np**
Consulting room 5 HT – pre-flush	48	0.0094	0.373	**100 s2-14**
Consulting room 5 HT – post-flush	53	0.0131	0.351	<LOD
C16 shower set on hot	41	0.0172	0.378	<LOD
TSSU CT	17	0.0257	0.228	<LOD
Hydro WC HT	56	0.0088	0.292	<LOD
OP HT	50	0.008	0.292	<LOD
X-Ray CT	19	0.02	0.337	<LOD
Joyce shower set on hot	45	0.0088	0.397	<LOD
D8 shower set on hot	40	0.0137	0.267	<LOD
D10 shower set on hot	50	0.0073	0.245	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

Table 4.23 Study hospital 9. Results from re-samples taken on the 24th June 2010. The copper target was met in all the samples taken from the contaminated outlets.

Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F HT	48	0.0176	0.378	<LOD
1F WC MT	35	0.0537	0.363	**100 np**
Disabled WC MT – pre-flush	35	0.0581	0.367	**5800 np**
Disabled WC MT – post-flush	38	0.0583	0.369	**1000 np**
Consulting room 5 MT	50	0.0300	0.673	**1300 s2-14**
TSSU CT	15	0.0462	0.187	<LOD
OP HT	39	0.0307	0.549	<LOD
X-Ray CT	22	0.0539	0.457	<LOD
Joyce shower set on hot	38	0.0527	0.510	<LOD
D7 shower set on hot – pre-flush	34	0.0314	0.340	<LOD
D7 shower set on hot – post-flush	39	0.0491	0.312	<LOD
D10 shower set on hot – pre-flush	30	0.0158	0.324	**100 s1**
D10 shower set on hot – post-flush	52	0.0245	0.285	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

Table 4.24 Study hospital 9. Results from samples and re-samples taken on the 29th October 2010.

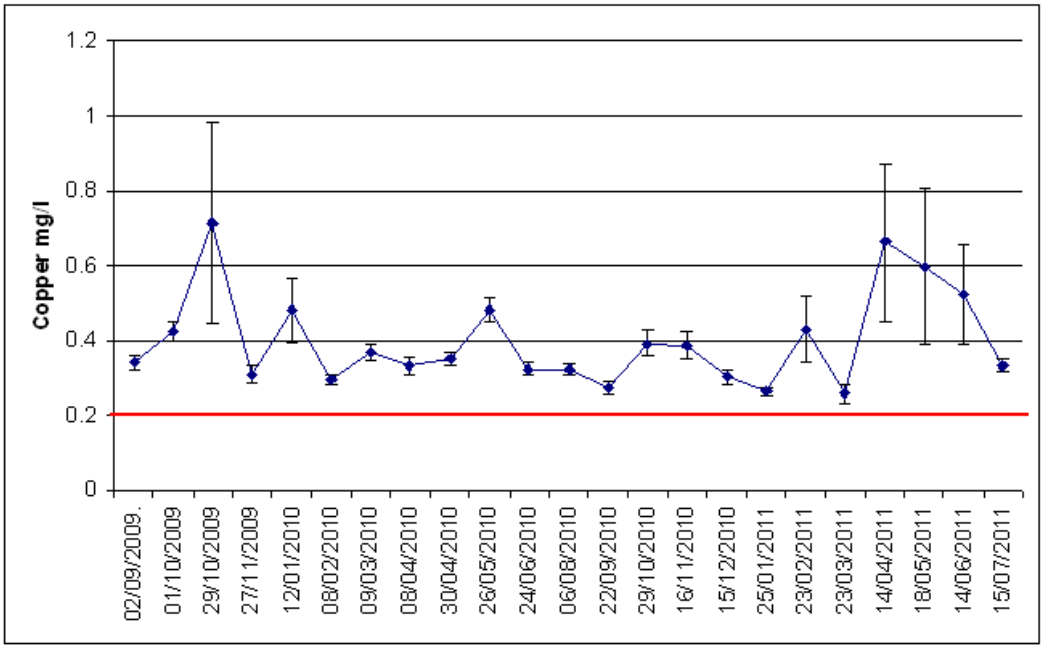
Sample Point	Temp. ºC	Silver Mg/l	Copper Mg/l	Legionella CFU/l
1F WC MT – pre-flush	36	0.037	0.399	<LOD
1F WC MT – post-flush	38	0.0492	0.3237	<LOD
Cilla shower set on hot – pre-flush	34	0.0158	0.3349	**1600 s1**
Cilla shower set on hot – post-flush	36	0.0572	0.3101	<LOD
Joyce shower set on hot – pre-flush	41	0.0123	0.3293	**1400 np**
Joyce shower set on hot – pre-flush	41	0.047	0.3096	<LOD

MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, <LOD = Below Limit of Detection (100CFU/l).

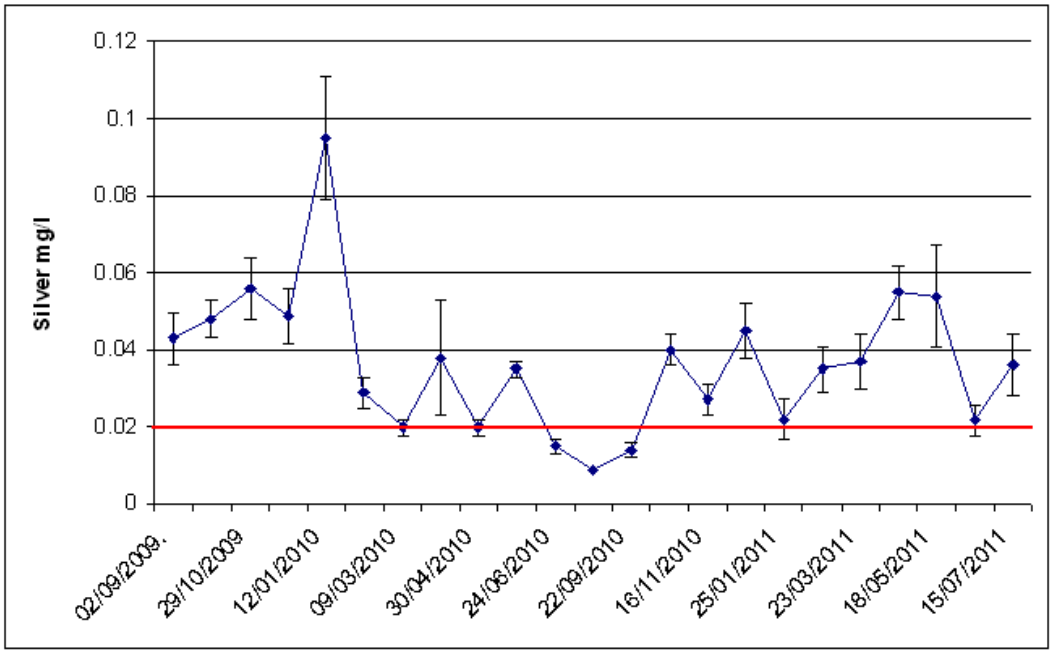
Table 4.25 Study hospital 9. Results from re-samples taken on the 15th July 2011.

The silver target was not met in samples taken from 2 contaminated outlets on the 24th June 2010, also not in a sample taken from 1 contaminated outlet on the 29th October 2010, and also not in the samples taken from the 2 contaminated outlets on the 15th July 2011.

Graphs 4.26 and 4.27 show the averages of the copper and silver levels found in samples and re-samples taken monthly from the 02nd September 2009 to the 15th July 2011. The average copper level was 0.398mg/l (± 0.026mg/l). The average silver level was 0.037mg/l (± 0.004mg/l).



Graph 4.26 Study hospital 9 – Average copper levels from the 02nd September 2009 to the 15th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.27 Study hospital 9 – Average silver levels from the 02nd September 2009 to the 15th July 2011 (the red line is the target level for silver - 0.02mg/l).

The results suggested that interruptions to the water system, due to the extensive construction work, had increased microbial growth and biofilm formation due to water pipes being exposed to the environment and wards being temporarily unoccupied, which may have resulted in an influx of organisms entering the water system from outside and water stagnation. Modifications to the pipework may also have dislodged biofilms and may have caused biofilms to disperse, colonizing other parts of the water system. Although the copper and silver target levels to control *Legionella*, of more than 0.2mg/l copper and more than 0.02mg/l silver, at outlets were mainly met, not enough was available at the *Legionella* contaminated outlets to completely control the microbial growth and *Legionella* contamination.

The average hot water temperature recorded from the 29th July 2009 to the 15th July 2011 was 43ºC (± 0.4ºC). The average cold water temperature was 17ºC (± 0.5ºC).

The chloride level found in the sample taken from the incoming mains on the 01st October 2009 was 54.9mg/l, the phosphorus was 191µg/l, and the pH was 7.5.

4.10 Study hospital 10

The results of samples taken on the 15th December 2009, before the copper and silver ionization systems were activated, are shown in Table 4.26 below.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
10th FR305 CT	15	< 0.0003	0.064	<LOD
10th DRB Shower	39	< 0.0003	0.08	<LOD
10th DRB HT	61	< 0.0003	0.159	<LOD
Ward 9A Kitchen HT	42	< 0.0003	0.144	<LOD
Ward 9A Sluice CT	13	< 0.0003	0.013	<LOD
Ward 9A Room 6 HT	38	< 0.0003	0.17	**100 np**
Ward 9B Sluice HT	53	< 0.0003	0.124	<LOD
Ward 9B Nurse Station CT	20	< 0.0003	0.038	<LOD
Ward 9B Male WC Shower	40	< 0.0003	0.099	**2000 s2-14 + 200 np**
Ward 8A Female Shower	40	< 0.0003	0.079	<LOD
Ward 8A Sluice HT	58	< 0.0003	0.118	<LOD
Ward 8A Kitchen CT	15	< 0.0003	0.035	<LOD
Ward 8A Kitchen HT	54	< 0.0003	0.12	<LOD
Ward 8A Sluice CT	12	< 0.0003	0.01	<LOD
Ward 8A Bay A MT	36	< 0.0003	0.187	**1700 s1**
Ward 7 Kitchen HT	56	< 0.0003	0.117	<LOD
Ward 7 Sluice CT	12	< 0.0003	0.01	<LOD
Ward 7A Bay A MT	34	< 0.0003	0.123	**1900 s1 + 100 np**
Ward 7B Kitchen CT	11	< 0.0003	0.008	<LOD
Ward 7B Female Shower	39	< 0.0003	0.08	<LOD
Ward 7B Sluice HT	54	< 0.0003	0.115	<LOD
Ward 6A Kitchen CT	24	< 0.0003	0.043	<LOD
Ward 6A Sluice HT	53	< 0.0003	0.12	<LOD
Ward 6A Shower Room MT	38	< 0.0003	0.089	<LOD
Ward 6B Arjo MT	40	< 0.0003	0.215	**2900 s2-14 + 1200 np**
Ward 6B Sluice CT	33	< 0.0003	0.136	**100 s1 + 1300 np**
Ward 6B Bay A MT	40	< 0.0003	0.173	**2100 s2-14 + 300 np**
Ward 5A Arjo MT	38	< 0.0003	0.14	<LOD
Ward 5A Sluice CT	13	< 0.0003	0.019	<LOD
Ward 5A Bay A MT	38	< 0.0003	0.069	**400 s1**
Ward 5B Sluice HT	57	< 0.0003	0.119	<LOD
Ward 5B Shower	40	< 0.0003	0.129	**100 s1 + 400 np**
Ward 5B Kitchen CT	13	< 0.0003	0.008	<LOD
Ward 5B Corridor CT	25	< 0.0003	0.096	<LOD
Ward 4A Sluice HT	53	< 0.0003	0.114	<LOD
Ward 4A Kitchen CT	15	< 0.0003	0.023	<LOD
Ward 4A Shower	33	< 0.0003	0.126	<LOD
Ward 4B Sluice HT	56	< 0.0003	0.119	<LOD
Ward 4A Bay A MT	40	< 0.0003	0.085	**2500 s1**
Ward 4B Kitchen CT	14	< 0.0003	0.025	<LOD
Ward 3A Sluice HT	56	< 0.0003	0.116	<LOD
Ward 3A Bay A MT	40	< 0.0003	0.091	**400 s1 + 400 np**
Ward 3A Kitchen CT	19	< 0.0003	0.052	<LOD

Ward 3B Shower MT	18	< 0.0003	0.039	<LOD
Ward 3B Sluice CT	12	0.0133	0.021	<LOD
Ward 3B Kitchen HT	59	< 0.0003	0.119	<LOD
Ward 2A Bathroom HT	36	0.0025	0.077	<LOD
Ward 2A Kitchen CT	11	< 0.0003	0.011	<LOD
Ward 2A Bay C HT	40	< 0.0003	0.089	**1600 s2-14**
Ward 2B Sluice HT	55	< 0.0003	0.147	<LOD
Ward 2B Kitchen CT	14	0.0093	0.028	**100 s1 + 100 np**
Ward 2B Shower MT	41	0.0016	0.141	**7200 s1**
Ward 1 Waiting Room HT	53	< 0.0003	0.155	<LOD
Ward 1 Pantry CT	18	< 0.0003	0.191	<LOD
Ward 1 Dirty Utility HT	59	0.0008	0.125	<LOD
Ward 1 Clinical Area MT	42	0.0007	0.054	<LOD
Pathology WC 46 MT	41	0.0006	0.081	<LOD
Path Lab 124 HT	51	0.0011	0.797	<LOD
Pathology Pan Reception CT	20	< 0.0003	0.223	**800 s2-14**
Pathology Sluice HT	44	0.0005	0.162	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

Table 4.26 Study hospital 10. Results from samples taken on the 15th December 2009, before commissioning of copper and silver ionization systems.

Legionella were found in samples taken from 15 outlets. Sixty outlets were sampled, therefore, 25% of the outlets were contaminated.

The *Legionella* colony forming unit counts found ranged from 100CFU/l to 7200CFU/l. The average *Legionella* count of the 60 outlets sampled was 465CFU/l (± 156CFU/l). Blended and cold outlets were contaminated. The highest *Legionella* count, of 7200CFU/l, was found in a sample taken from a blended outlet. The temperature recorded at this outlet was 41ºC.

The average hot water temperature at the 40 hot water outlets tested was 45ºC (± 1.5ºC). Although mixing valves were hidden and could not be seen, the hot water could have been blended with cold water at 24 of the 40 hot water outlets tested because the temperatures recorded at these were ranging from 18ºC to 44ºC. Thirteen of these outlets were contaminated with *Legionella*.

The average water temperature at the 20 cold water outlets tested was 16ºC (± 1ºC). The temperature recorded at 1 of the 3 contaminated cold outlets was high at 33ºC.

The average copper level found at the 60 outlets, due to copper leaching from copper pipes, was 0.108mg/l (± 0.01mg/l).

The copper and silver ionization systems were activated on the 21st December 2009.

Table 4.27 below shows the results of samples taken on the 13th January 2010 from the 15 outlets that were contaminated before the systems were activated and from 15 outlets that had been identified as being at risk of *Legionella* contamination.

Sample Point	Temp. °C	Silver Mg/l	Copper Mg/l	Legionella CFU/l
9A Room 6 HT	37	0.0357	0.234	<LOD
9B Shower	39	0.0363	0.226	<LOD
8A Bay A MT	39	0.0416	0.236	**200 s1**
8B Bay B MT	34	0.0284	0.243	**12800 np**
7A Bay A MT	39	0.0384	0.233	<LOD
7A Bay B MT	39	0.0396	0.227	<LOD
7B WC CT	10	0.0677	0.237	<LOD
7B Bay A MT	43	0.036	0.229	<LOD
6A Bay B MT	39	0.0432	0.238	**500 np**
6B Arjo MT	39	0.0442	0.288	<LOD
6B Sluice CT	9	0.0661	0.234	<LOD
6B Bay A MT	40	0.028	0.242	<LOD
5A Bay A MT	40	0.043	0.243	<LOD
5A Bay B MT	35	0.0524	0.511	**200 s1**
5B WC MT	42	0.0356	0.232	**12800 np**
5B Shower	39	0.033	0.233	<LOD
4A Bay D MT	39	0.0393	0.232	<LOD
4A Bay A MT	38	0.0413	0.23	<LOD
4B Bay B MT	40	0.0403	0.342	<LOD
4B Bay A MT	40	0.0454	0.446	**500 np**
3A Bay A MT	40	0.039	0.233	<LOD
3A Bay B MT	44	0.0367	0.225	<LOD
3B Shower	35	0.0348	0.234	<LOD
3B WC CT	8	0.069	0.234	<LOD
2A Bay B MT	39	0.0393	0.228	<LOD
2A Bay C HT	38	0.0393	0.227	**100 s2-14**
2B Kitchen CT	52	0.0328	0.226	<LOD
2B Shower	39	0.0376	0.229	**4700 s1**
Path Pan Reception CT	19	0.0594	0.294	<LOD
Path Pan Reception HT	42	0.0337	0.247	<LOD

HT = Hot tap, CT = Cold tap, MT = Mixer tap, np = *Legionella* non-*pneumophila*, s1 = *Legionella pneumophila* serogroup 1, s2-14 = *Legionella pneumophila* serogroup 2 to 14, <LOD = Below Limit of Detection (100CFU/l).

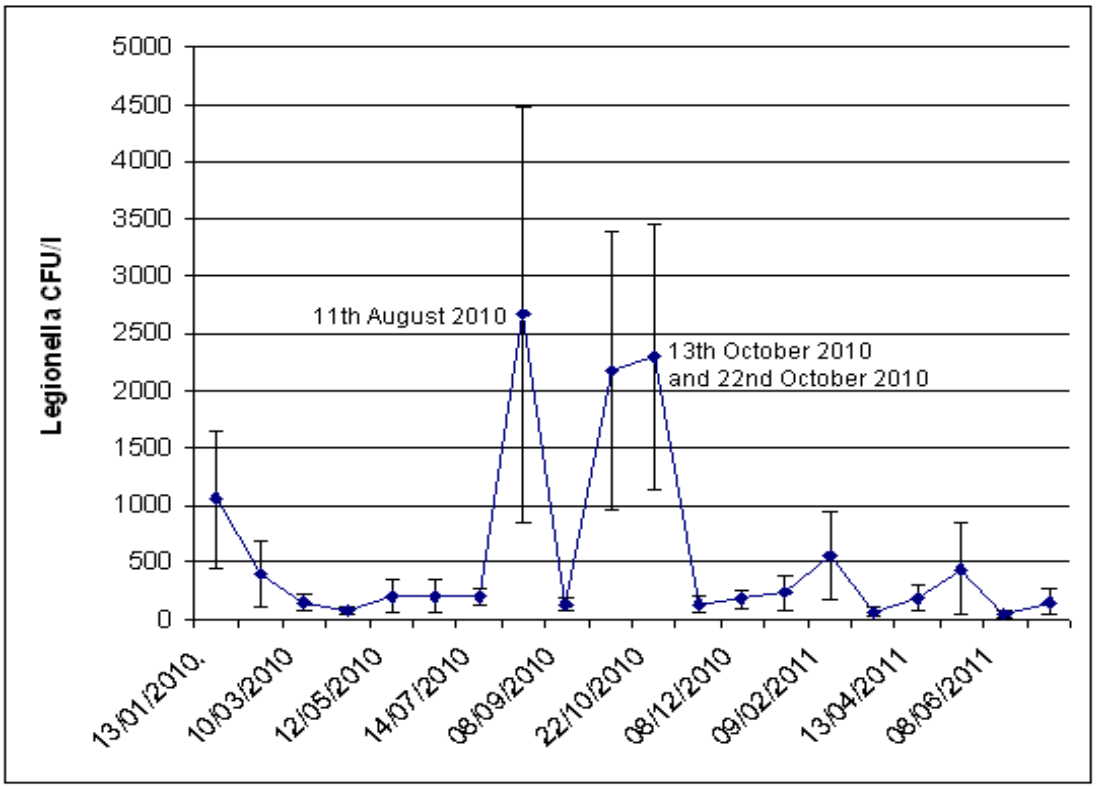
Table 4.27 Study hospital 10. Results from samples taken on the 13th January 2010.

No *Legionella* were found in samples taken from 11 outlets that were previously contaminated. The *Legionella* colony forming unit counts reduced in samples taken from the 4 outlets that were previously contaminated; from 1700CFU/l to 200CFU/l, from 2400CFU/l to 200CFU/l, from 1600CFU/l to 100CFU/l, and from 7200CFU/l to 4700CFU/l. *Legionella* were found at 4 outlets from which samples were not taken before the copper and silver ionization systems were activated.

The hot water temperatures of the 8 outlets at which *Legionella* were found were all below 50ºC, indicating that these were blended outlets.

The silver found at the 30 outlets ranged from 0.028mg/l to 0.069mg/l. The average silver level was 0.042mg/l (± 0.002mg/l). The silver target, of 0.02mg/l, was met at all the 30 outlets tested. The copper ranged from 0.225mg/l to 0.511mg/l. The average copper level was 0.257mg/l (± 0.012mg/l). The copper target, of 0.2mg/l, was met at all the 30 outlets tested.

Graph 4.28 below shows the average *Legionella* counts in samples taken monthly from 30 outlets from the 13th January 2010 to the 13th July 2011.



Graph 4.28 Study hospital 10 - Average *Legionella* counts in samples taken monthly from the 13th January 2010 to the 13th July 2011.

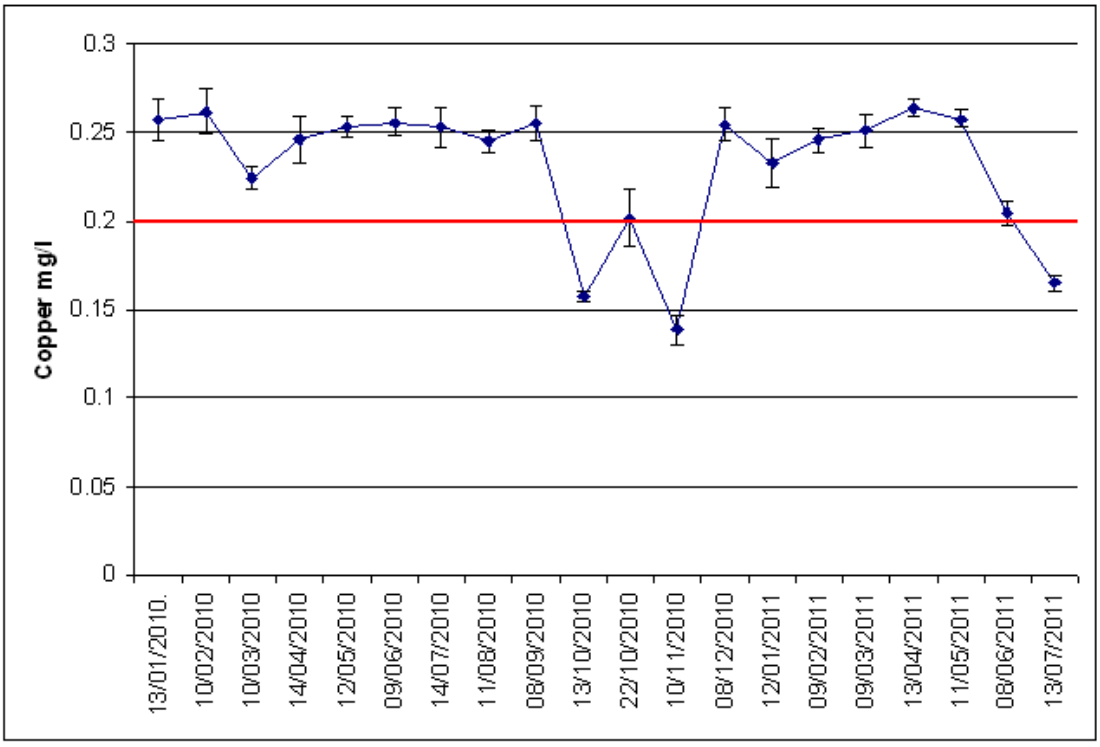
Legionella were found at high colony forming unit counts in samples taken on the 11th August 2010 from a blended outlet, 36200CFU/l, and from a cold outlet, 42000CFU/l, which increased the average *Legionella* count. The copper target was met in both contaminated samples but the silver found in the sample taken from the cold outlet was low at 0.0003mg/l.

Legionella were found at high counts in samples taken on the 13th October 2010. The copper had dropped substantially and its target was not met in samples taken from the 15 contaminated outlets. The average copper level found in the contaminated samples was 0.158mg/l (± 0.005mg/l).The silver had also dropped and its target was not met at 10 of the 15 contaminated outlets. The average silver level found at the contaminated outlets was 0.019mg/l (± 0.002mg/l).

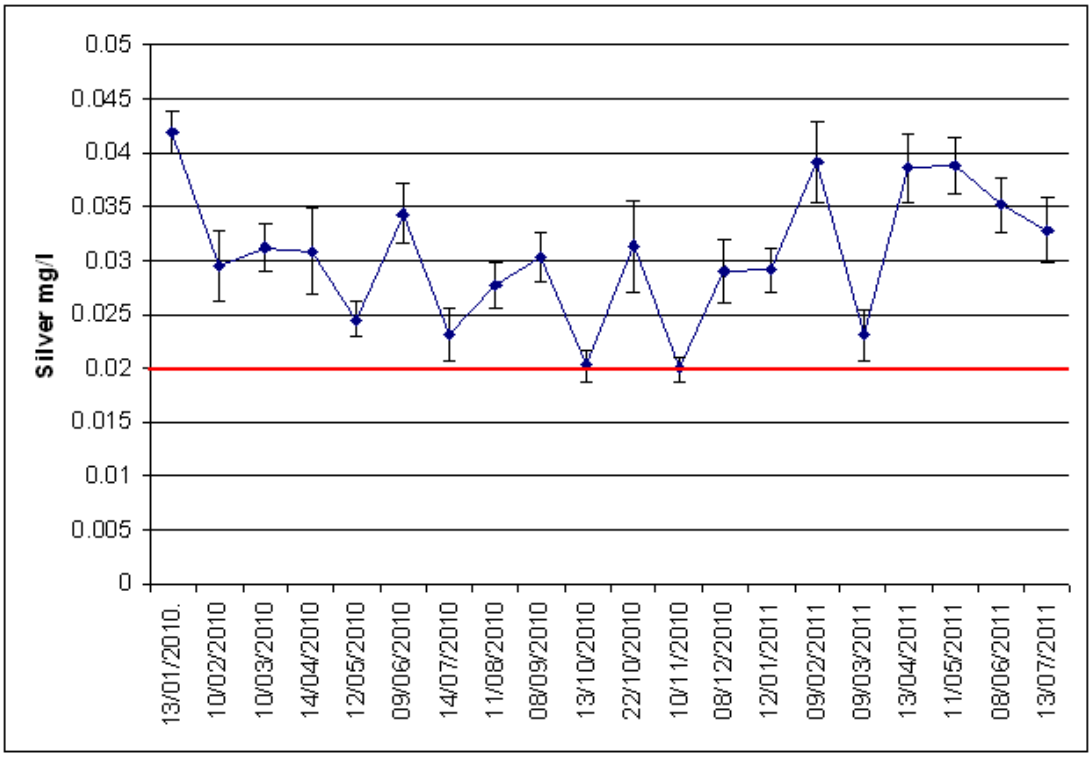
Of the 15 contaminated outlets sampled on the 13th October, 7 were found to have no *Legionella* when sampled on the 22nd October. At these outlets the copper and silver levels had improved. At 3 outlets the copper and silver targets were not met and *Legionella* were still found. At 2 outlets the copper and silver levels had improved but *Legionella* were still found. A point of use filter was attached to 1 outlet and a mixing valve was attached to the other outlet. At 3 outlets the *Legionella* counts had increased in spite of improved copper and silver levels.

When the copper and silver targets were consistently maintained, the *Legionella* contamination declined and the *Legionella* colony forming unit counts reduced.

Graphs 4.29 and 4.30 show the averages of the copper and silver levels found in samples taken from 30 outlets from the 13th January 2010 to the 13th July 2011. The average copper level was 0.231mg/l (± 0.008mg/l). The average silver level was 0.031mg/l (± 0.001mg/l).



Graph 4.29 Study hospital 10 – Average copper levels from the 13th January 2010 to the 13th July 2011 (the red line is the target level for copper - 0.2mg/l).



Graph 4.30 Study hospital 10 – Average silver levels from the 13th January 2010 to the 13th July 2011 (the red line is the target level for silver - 0.02mg/l).

The average hot water temperature recorded from the 15th December 2009 to the 13th July 2009 was 41ºC (± 0.3ºC). The average cold water temperature was 16ºC (± 0.4ºC).

The chloride level found in the sample taken from the incoming mains on the 05th July 2011 was 57.3mg/l, the phosphorus was 1030µg/l, and the pH was 7.5.

4.11 Summary of results of study hospitals

Table 4.28 below shows the number of outlets that were contaminated with *Legionella* before the copper and silver ionization systems were activated in the 10 hospitals as well as 3 months after activation of the systems and at the end of the studies.

Study hospital	Number of contaminated outlets before activation	Number of contaminated outlets 3 months after activation	Number of contaminated outlets at end of study
1	21 out of 46	1 out of 21	0 out of 21
2	7 out of 30	2 out of 15	0 out of 15
3	4 out of 21	1 out of 10	0 out of 10
4	2 out of 12	0 out of 6	0 out of 6
5	25 out of 51	6 out of 20	2 out of 20
6	11 out of 50	2 out of 25	1 out of 25
7	20 out of 26	4 out of 13	1 out of 13
8	8 out of 45	0 out of 21	0 out of 21
9	14 out of 30	1 out of 15	1 out of 14
10	15 out of 60	13 out of 29	6 out of 30

Table 4.28 Study hospitals - Number of *Legionella* contaminated outlets before activation of copper and silver ionization systems as well as 3 months after activation and at end of studies.

Table 4.29 below shows the average *Legionella* colony forming unit counts found before the copper and silver ionization systems were activated in the 10 hospitals as well as 3 months after activation of the systems and at the end of the studies.

Study hospital	Average *Legionella* counts before activation CFU/l	Average *Legionella* counts 3 months after activation CFU/l	Average *Legionella* counts at end of study CFU/l
1	983 (± 331)	19 (± 19)	0
2	290 (± 20)	53 (± 41)	0
3	138 (± 77)	90 (± 90)	0
4	350 (± 356)	0	0
5	214 (± (10)	42 (± 16)	14 (± 10)
6	136 (± 82)	16 (± 11)	13 (± 13)
7	7188 (± 3681)	54 (± 24)	33 (± 33)
8	60 (± 23)	0	0
9	453 (± 216)	20 (± 20)	7CFU/l (± 7CFU/l)
10	465 (± 156)	155 (± 66)	157 (± 112)

Table 4.29 Study hospitals - Average *Legionella* colony forming unit counts before activation of copper and silver ionization systems as well as 3 months after activation and at end of studies.

Table 4.30 below shows the averages of the copper and silver levels of the samples taken after the copper and silver ionization systems were activated in the 10 hospitals as well as the averages of the cold and hot water temperatures, and the chloride, phosphorus and pH values.

Study Hospital	Copper mg/l	Silver mg/l	Chloride mg/l	Phosphorus µg/l	pH	Hot temp. ºC	Cold temp. ºC	Borehole Chloride Mg/l	Borehole Phosphorus µg/l	Borehole pH
1	0.201	0.04	15.1	455	8.5	41	17			
2	0.235	0.037	17	390	8.5	44	15			
3	0.151	0.033	24.5	1000	7.9	41	17			
4	0.419	0.043	30.2	577	7.5	38	*			
5	0.366	0.043	60.7	701	7.2	46	16			
6	0.328	0.021	46.7	1500	7.2	44	15	32.9	2100	6.9
7	0.539	0.031	58.5	237	7.6	40	18			
8	0.187	0.036	8.4	452	8.5	45	15			
9	0.398	0.037	54.9	191	7.5	43	17			
10	0.231	0.031	57.3	1030	7.5	41	16			

Table 4.30 Study hospitals - Average copper and silver results. Chloride, phosphorus and pH values. Average temperatures.

* The cold water temperatures were not recorded at study hospital 4.

4.12 Initial experiment - Rigs

4.12.1 Rigs A

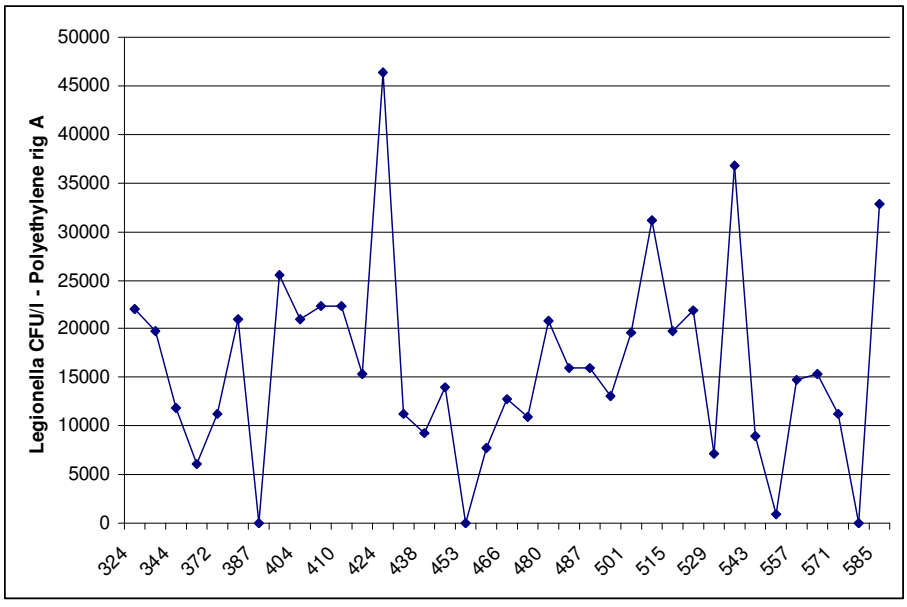
The TVC results of the 67 samples taken from each rig were variable.

The TVC results of the samples taken from the polyethylene rig, incubated at 37ºC for 2 days, ranged from 0CFU/ml to 11900CFU/ml. The average of the 67 results was 1892CFU/ml (± 361CFU/ml).

The TVC results of samples incubated at 22ºC for 3 days ranged from 7CFU/ml to 7430CFU/ml. The average of the 67 results was 1001CFU/ml (± 194CFU/ml). The average temperature of the polyethylene's rig tank water was 44ºC (± 0.3ºC).

The results of the samples taken from the copper rig, incubated at 37ºC for 2 days, ranged from 0CFU/ml to 9410CFU/ml. The average of the 67 results was 1810CFU/ml (± 264CFU/ml). The results of samples incubated at 22ºC for 3 days ranged from 0CFU/ml to 10400CFU/ml. The average of the 67 results was 1417CFU/ml (± 279CFU/ml). The average temperature of the copper rig's tank water was 42ºC (± 0.3ºC).

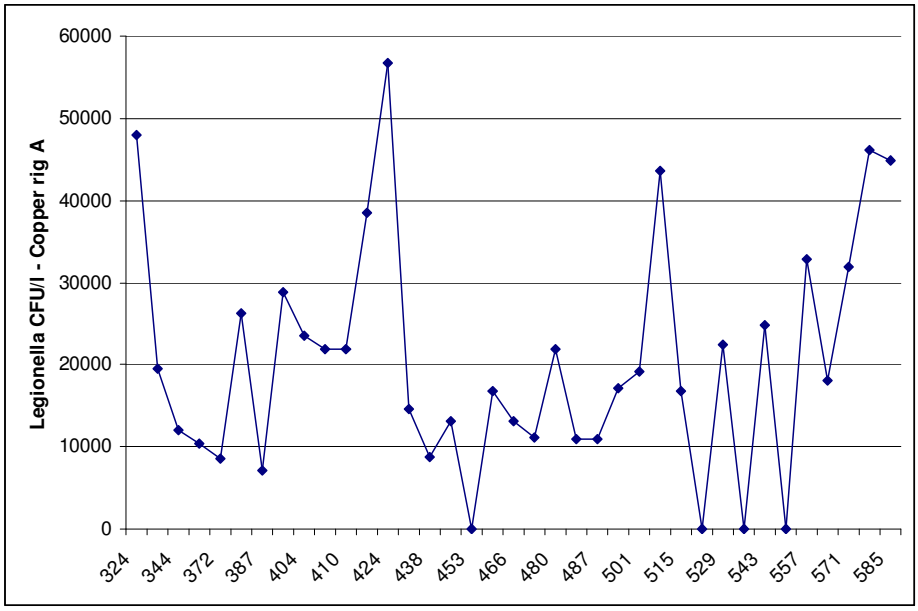
Graph 4.31 below shows the planktonic *Legionella* results of samples taken from the polyethylene rig from first detection, at 324 days, to 585 days.



Graph 4.31 Initial experiment - *Legionella* results polyethylene A

The *Legionella* results were variable and ranged from 0CFU/l to 46400CFU/l. The average *Legionella* count found in the 37 samples was 16146CFU/l (± 1667CFU/l). The average temperature was 44ºC (± 0.4ºC).

Graph 4.32 below also shows the planktonic *Legionella* results of samples taken from the copper rig from first detection, at 324 days, to 585 days.



Graph 4.32 Initial experiment - *Legionella* results copper rig A.

The *Legionella* results were again variable and ranged from 0CFU/l to 56800CFU/l. The average *Legionella* count found in the 37 samples taken was 20611CFU/l (± 2352CFU/l). The average temperature was 42ºC (± 0.4ºC).

The *Legionella* species found in the rigs, *L. pneumophila* serogroup 2 to 14, were different from the species that were detectable in the original inoculum, *L. pneumophila* serogroup 1 and *Legionella* non-*pneumophila*. This may have been because the environment within the rigs supported the growth of *Legionella pneumophila* serogroup 2 to 14 and the original inoculum may have contained a mixture of species including serotypes that were not readily detectable.

Tables 4.31 and 4.32 below show the results of the methods applied to sections 1 to 16 of the rigs, as an initial experiment, to establish the best way to remove and quantify biofilms. The highest weight was found in the samples from the brushed through sections, although this was not necessarily reflected in some viable cell

results, for instance the TVC results obtained from section 11 of the copper rig at 211 days, where there appears to be a significant biolayer (in weight) but few viable cells. This could indicate that much of the biolayer consisted of non-viable material.

Tables 4.31 and 4.32 also show the results of the analysis for viable biological material within the sonified, shaken and brushed samples. The results were variable and did not indicate a steady build-up of biofilm with the length of time that sections were in place. The results also suggested that within 478 days a substantial biofilm did not form as the TVC density was calculated to be below 70/mm^2, which is generally considered to be insignificant.

Section	Days in rig	Procedure	TVC @37ºC cfu/ml	TVC @22ºC cfu/ml	Weight grams	Biolayer @ 37ºC per mm^2	Biolayer @ 22ºC per mm^2
1	19	Shaken	0	0	0	0	0
2	27	Shaken	0	0	0	0	0
3	70	Shaken	10	6	0	0.7	0.4
4	80	Shaken	1	1	0.004	0.07	0.07
5	89	Shaken	0	1	0	0	0.07
6	128	Shaken	9	7	0	0.6	0.5
7	128	Sonicated	880	204	0	63	15
8	211	Dried	4	11	0.001	0.3	0.8
9	211	Sonicated	1	119	0.0008	0.07	9
10	211	Shaken	2	0	0.007	0.1	0
11	211	Brushed	2	1	0.02	0.1	0.07
12	284	Brushed	640	126	0.016	46	9
13	387	Brushed	50	1	0	4	0.07
14	387	Brushed	14	4	0	1	0.3
15	409	Brushed	60	3	0	4	0.2
16	478	Brushed	1	1	0	0.07	0.07

Table 4.31 Initial experiment - Biofilm results in weight and biolayer per mm^2 from TVC results at 37ºC and at 22ºC (TVC result x 500ml/7000mm^2) - copper rig.

Section	Days in rig	Procedure	TVC @37ºC cfu/ml	TVC @22ºC cfu/ml	Weight grams	Biolayer @ 37ºC per mm^2	Biolayer @ 22ºC per mm^2
1	19	Shaken	605	0	0	43	0
2	27	Shaken	0	4	0	0	0.3
3	70	Shaken	0	0	0	0	0
4	80	Shaken	0	1	0.004	0	0.07
5	89	Shaken	0	3	0.0004	0	0.2
6	128	Shaken	5	5	0	0.4	0.4
7	128	Sonicated	80	5	0	6	0.4
8	211	Dried	1	165	0.001	0.07	12
9	211	Sonicated	1	2	0.001	0.07	0.1
10	211	Shaken	0	1	0.0006	0	0.07
11	211	Brushed	3	91	0.006	0.2	7
12	284	Brushed	16	0	0.005	1	0.07
13	387	Brushed	29	1	0	2	0.07
14	387	Brushed	30	7	0	2	0.5

15	409	Brushed	1	0	0	0.07	0
16	478	Brushed	330	4	0	24	0.3

Table 4.32 Initial experiment - Biofilm results in weight and biolayer per mm^2 from TVC results at 37ºC and at 22ºC (TVC result x 500ml/7000mm^2) – polyethylene rig.

Analysis for sessile *Legionella* was carried out on the brushed samples of sections 13, 14, 15 and 16 removed from the rigs on days 387, 409 and 478. The results are shown in Table 4.33 below. This table also shows the corresponding planktonic levels of *Legionella.*

Section	**Days in Rigs**	***Legionella* Copper Rig Sections**	***Legionella* Polyethylene Rig Sections**	**Planktonic *Legionella* Copper Rig**	**Planktonic *Legionella* Polyethylene Rig**
13	387	4300 cfu/l	0	7200 cfu/l	0
14	387	15200 cfu/l	0	7200 cfu/l	0
15	409	21000 cfu/l	4800 cfu/l	21800 cfu/l	22400 cfu/l
16	478	70000 cfu/l	40800 cfu/l	21800 cfu/l*	20800 cfu/l*

* - Results from samples taken from rigs on day 480.

Table 4.33 Initial experiment - Sessile and planktonic *Legionella* results of copper and polyethylene rigs.

More than 10000CFU/l were found in sections 14, 15 and 16 of the copper rig and in section 16 of the polyethylene rig. *Legionella pneumophila* serogroup 2 to 14 were only found.

Sections 13, 14, 15 and 16 were again simultaneously removed from each rig, as well as sections 1 to 13, on day 564 for *Legionella* analysis, representing residence times of between 86 and 547 days.

The results of the copper rig sections are shown in Figure 4.1 below.

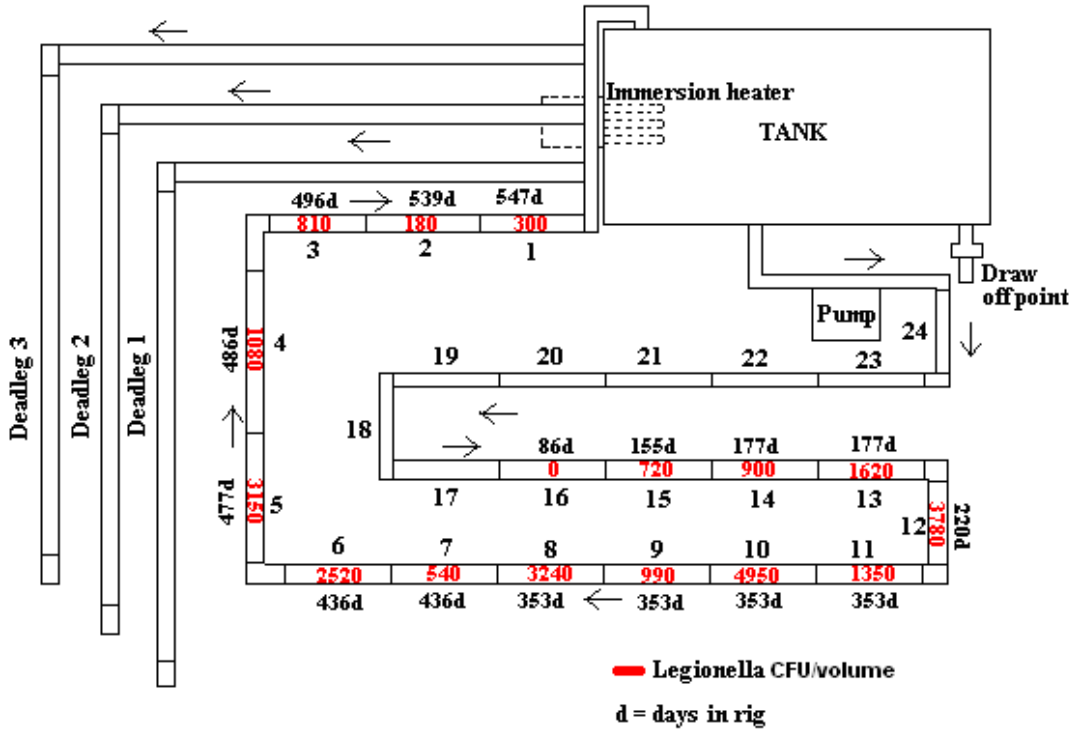


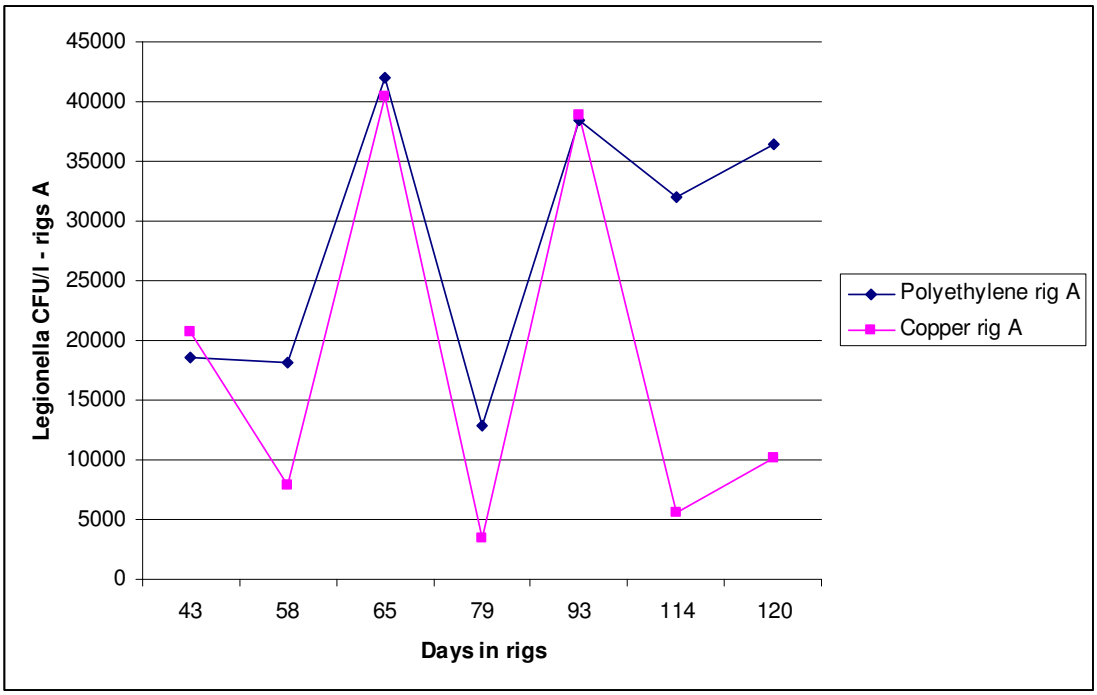
Figure 4.1 *Legionella* results of sections 1 to 16 of the copper rig – removed simultaneously on day 564.

Legionella were found in sections 1 to 15 of the copper rig. No *Legionella* were found in section 16, which had been in the copper rig for 86 days. The results indicated that *Legionella* multiplied in biofilm in pipe sections that were in the copper rig for more than 86 days and that multiplication peaked in pipe sections that had been in the rig for 353 days after which multiplication dropped to only 300CFU found in the pipe section that had been in the copper rig for 547 days.

Very surprisingly, no *Legionella* were found in the polyethylene rig sections 1 to 15 but a colony forming unit count of 1800CFU/500ml was found in section 16.

Legionella non-*pneumophila* and *L. pneumophila* serogroup 2 to 14 were found in the copper rig sections. Only *L. pneumophila* serogroup 2 to 14 were found in the polyethylene section samples.

Legionella results of samples taken from rigs A, after rigs B and C were inoculated with water and sections from rigs A (day 0), and before treatment was started (day 120), are shown in Graph 4.33 below.



Graph 4.33 *Legionella* results of samples taken from rigs A after inoculation of rigs B and C (day 0) and before treatment (day 120).

The *Legionella* results of the samples taken from the polyethylene rig were variable and ranged from 12800CFU/l to 42000CFU/l. The average *Legionella* count found in the 7 samples was 28329CFU/l (± 4384CFU/l).The temperature of the rigs was kept below 45ºC.

The *Legionella* results of the samples taken from the copper rig were also variable and ranged from 3500CFU/l to 40400CFU/l. The average *Legionella* count found in the 7 samples was 18129CFU/l (± 5920CFU/l).

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig A brushed through sections, which were removed after inoculation of rigs B and C and before the comparison experiment, were:
Section 19, in place in rig since design stage, 5600CFU/l
Section 18, in place in rig for 79 days, 0CFU/l
Section 17, in place in rig for 114 days, 13600CFU/l.

The *Legionella* colony forming unit counts found in the samples of copper rig A brushed through sections, which were removed after inoculation of rigs B and C and before the comparison experiment, were:

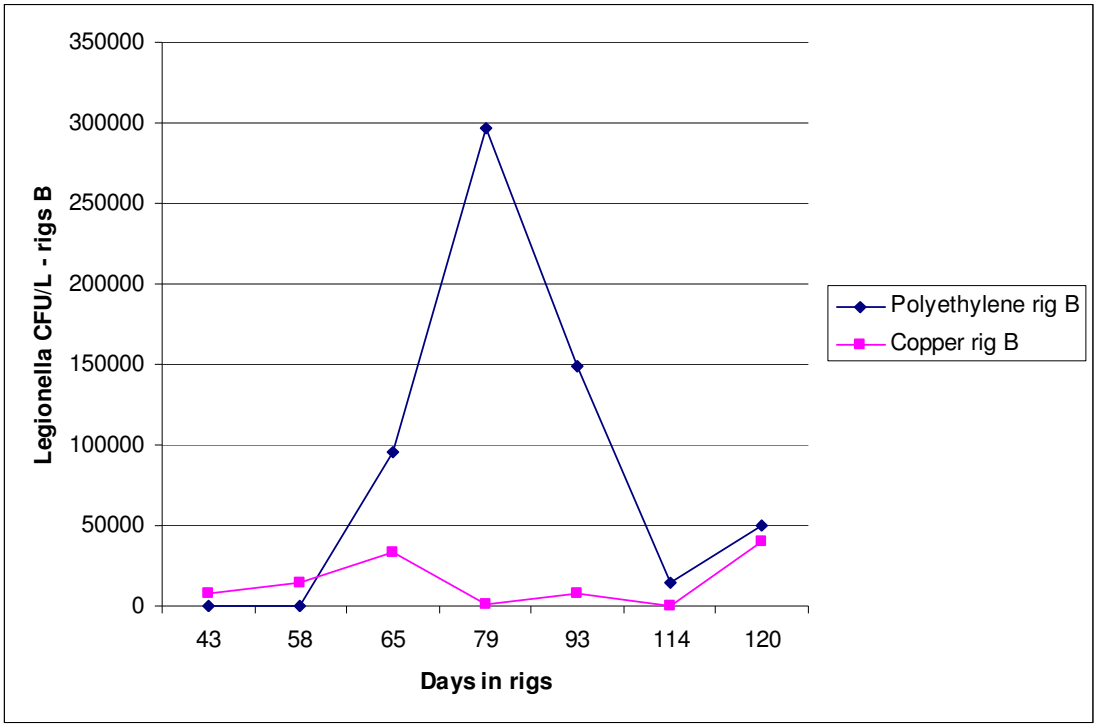
Section 19, in place in rig for 58 days, 6700CFU/l

Section 17, in place in rig for 79 days, 1500CFU/l

Section 16, in place in rig for 114 days, 800CFU/l.

4.12.2 Rigs B and C

Graph 4.34 below shows the *Legionella* colony forming unit counts found in samples taken from rigs B after inoculation (day 0) and before treatment was started (day 120).



Graph 4.34 *Legionella* results of samples taken from rigs B after inoculation and before treatment.

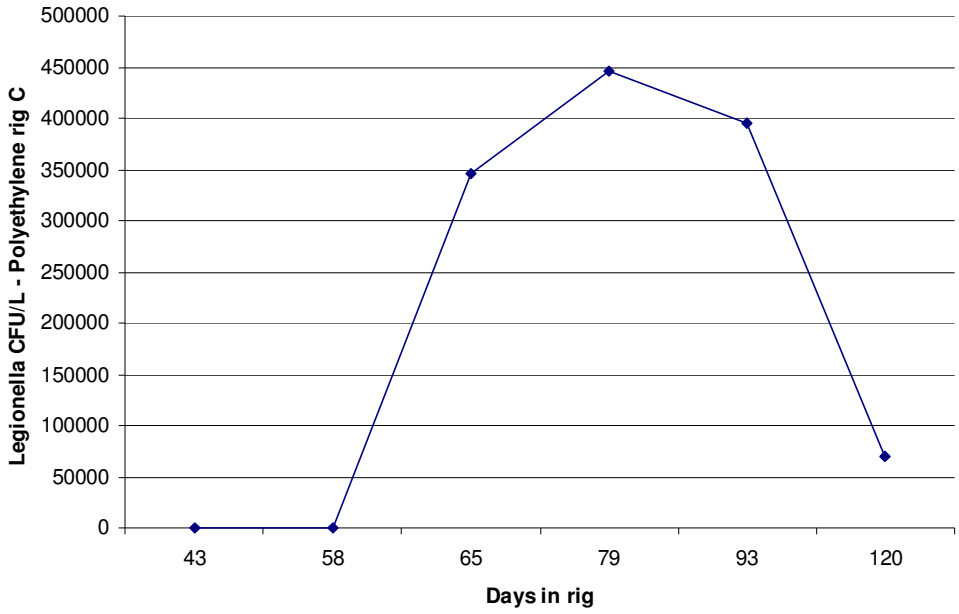
The *Legionella* results of the samples taken from the polyethylene rig were variable and ranged from 0CFU/l to 297000CFU/l. The average *Legionella* count found in the 7 samples was 86486CFU/l (± 40760CFU/l). The temperature of the rigs was kept below 45ºC.

The *Legionella* results of the samples taken from the copper rig were also variable and ranged from 0CFU/l to 40400CFU/l. The average *Legionella* count found in the 7 samples was 14986CFU/l (± 6006CFU/l).

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig B brushed through sections were:
Section 19, in place in rig for 58 days, 0CFU/l
Section 18, from rig A, in place in rig for 79 days, 594000CFU/l
Section 17, from rig A, in place in rig for 114 days, 89600CFU/l.

The *Legionella* colony forming unit counts found in the samples of copper rig B brushed through sections, before the comparison experiment was started, were:
Section 19, from rig A, in place in rig for 58 days, 4500CFU/l
Section 18, from rig A, in place in rig for 79 days, 300CFU/l
Section 17, from rig A, in place in rig for 114 days, 21600CFU/l.

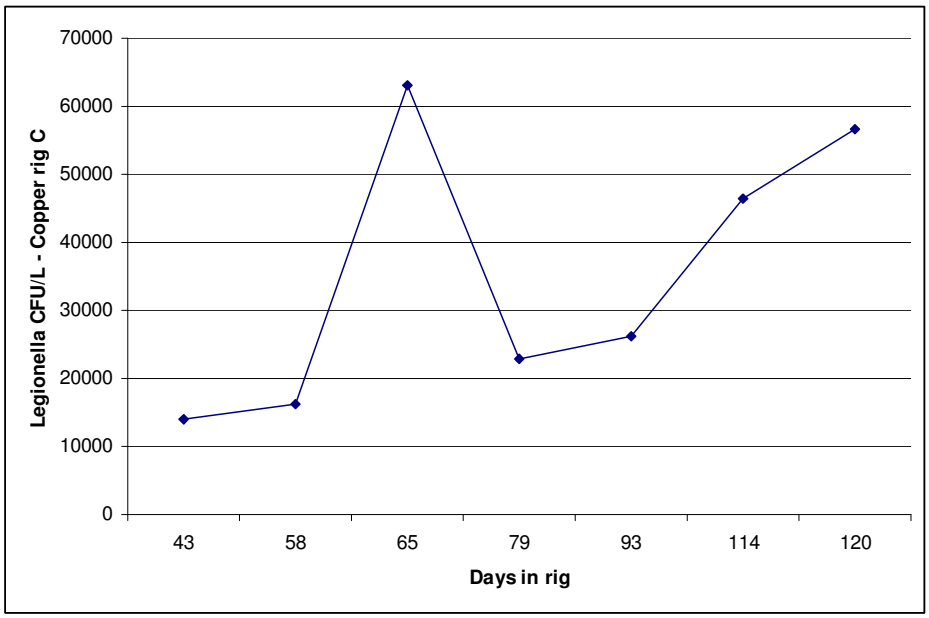
Graph 4.35 below shows the *Legionella* colony forming unit counts found in samples taken from the polyethylene rig C after inoculation (day 0) and before treatment (day 120).



Graph 4.35 *Legionella* results of samples taken from the polyethylene rig C after inoculation and before treatment.

The *Legionella* results in the samples taken from the polyethylene rig C *r*anged from 0CFU/l to a very high count of 18150000CFU/l, which was not included in Graph 4.35. The average *Legionella* count found including the high count was 2772471CFU/l (± 2563928CFU/l). The average *Legionella* count excluding the high count was 209550CFU/l ((±78683CFU/l). The temperature of the rig was kept below 45ºC.

Graph 4.36 below shows the *Legionella* colony forming unit counts found in samples taken from the copper rig C after inoculation (day 0) and before treatment (day 120).



Graph 4.36 *Legionella* results of samples taken from the copper rig C after inoculation and before treatment.

The *Legionella* results of the samples taken from the copper rig ranged from 14000CFU/l to 63200CFU/l. The average *Legionella* count found in the samples was 35071CFU/l (±7573CFU/l). The temperature of the rig was also kept below 45ºC.

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig C brushed through sections were:
Section 19, in place in rig for 58 days, 0CFU/l
Section 18, in place in rig for 79 days, 0CFU/l
Section 6, from rig A, in place in rig for 114 days, 69600CFU/l.

The *Legionella* colony forming unit counts found in the samples of copper rig C brushed through sections, before the comparison experiment was started, were:
Section 19, in place in rig for 58 days, 5100CFU/l
Section 18, in place in rig for 79 days, 4300CFU/l
Section 8, from rig A, in place in rig for 114 days, 18800CFU/l.

4.13 Comparison experiment - Rigs

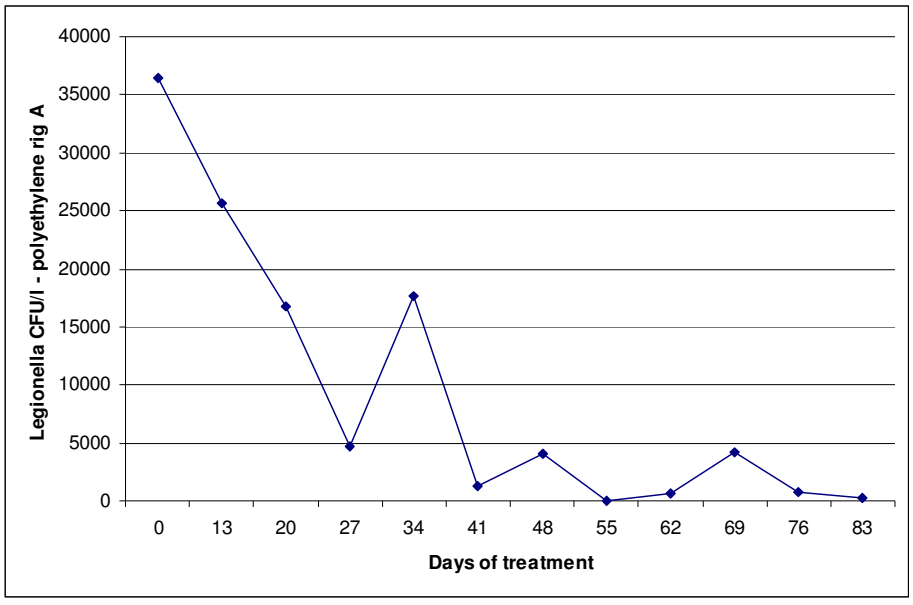
4.13.1 Rigs A

Three litres of copper and silver ionized water produced by the copper and silver ionization system was added to rigs A daily for 83 days in a separate operation.

The average silver level found in samples taken daily from the copper and silver ionization system over the 83 days was 0.196mg/l. The average copper level was 3.31mg/l.

The average silver level found in samples taken daily from the draw off point of the polyethylene rig was 0.042mg/l (± 0.011mg/l). The average copper level was 0.866mg/l (± 0.127mg/l).

Graph 4.37 shows the *Legionella* results of samples taken once a week from the polyethylene rig draw off point from when treatment started to the end of treatment.



Graph 4.37 *Legionella* results of polyethylene rig A.

At day 0, before copper and silver ionized water was added, a *Legionella* colony forming unit count of 36400CFU/l was found in the sample taken from the draw off point of the polyethylene rig. The *Legionella* contamination declined to a *Legionella* colony forming unit count of 200CFU/l found in the sample taken on day 83 (~ 99%

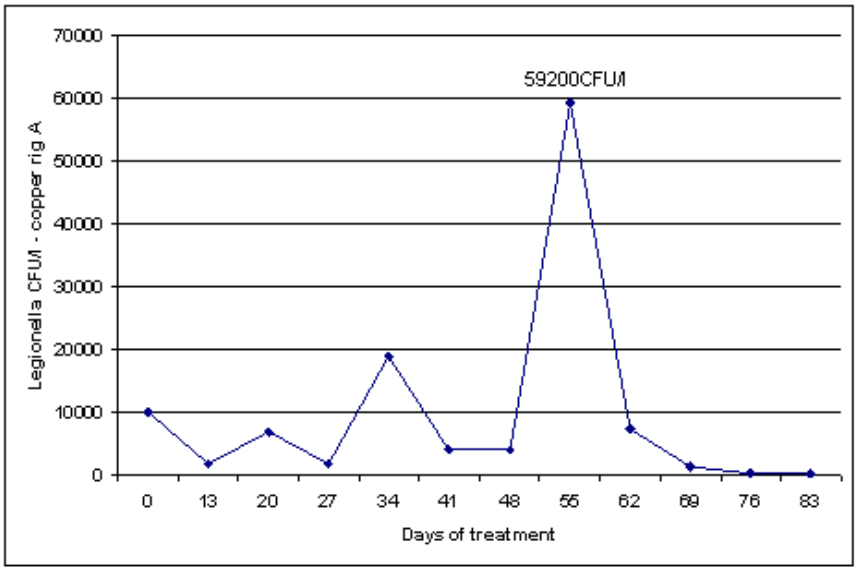
reduction). The average *Legionella* count of samples taken from day 13 to day 83 was 6900CFU/l (± 2668CFU/l).

The temperatures of the polyethylene rig tank recorded daily for 83 days ranged from 37ºC to 47ºC. The most common temperature recorded was 42ºC. The average temperature recorded in the polyethylene rig A tank during treatment was 44ºC (± 0.4ºC).

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig brushed through sections during treatment were:
Section 16, in place in rig for 133 days, day 13 of trial, 2900CFU/l
Section 14, in place in rig for 175 days, day 55 of trial, 30400CFU/l
Section 13, in place in rig for 203 days, day 83 of trial, 0CFU/l.
All these sections had been in the rig for 120 days before treatment was started.

The average silver level found in samples taken daily from the draw off point of the copper rig was 0.031mg/l (± 0.007mg/l). The copper level found, due to copper leaching from the copper pipes, before copper and silver ionized water was added to the rig was 0.325mg/l. The average copper level for the duration of treatment was 0.874 mg/l (± 0.116 mg/l).

Graph 4.38 shows the *Legionella* results of samples taken ones a week from the copper rig draw off point from when treatment started to the end of treatment.



Graph 4.38 *Legionella* results of copper rig A.

At day 0 a *Legionella* colony forming unit count of 10100CFU/l was found in the sample taken from the draw off point of the copper rig. A high *Legionella* colony forming unit count of 59200CFU/l was found in the sample taken from the copper rig on day 55. The silver found was 0.062mg/l and the copper was 0.664mg/l. The count reduced in a sample taken one week later, day 62, to 7400CFU/l, and the *Legionella* contamination declined from day 62 onwards to a count of 200CFU/l found in a sample taken on day 83 (~ 98% reduction). The average *Legionella* count of samples taken from day 13 to day 83 was 9636CFU/l (± 5207CFU/l).

The temperatures of the copper rig tank recorded daily for 83 days ranged from 33ºC to 50ºC. The most common temperature recorded was 44ºC. The average temperature recorded in the copper rig A tank during treatment was 42ºC (± 0.3ºC).

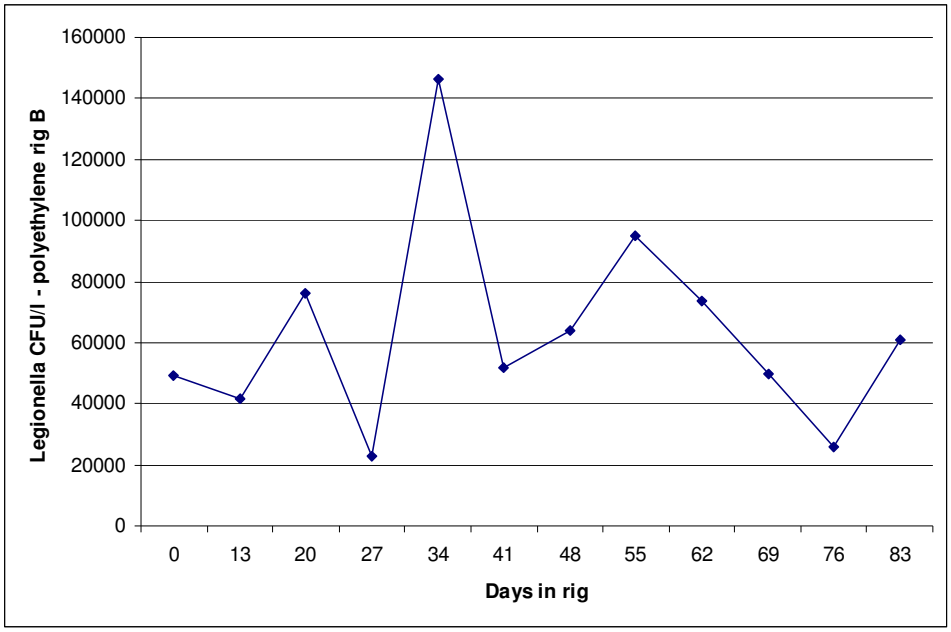
The *Legionella* colony forming unit counts found in the samples of the copper rig brushed through sections during treatment were:
Section 15, in place in rig for 133 days, day 13 of trial, 200CFU/l
Section 14, in place in rig for 175 days, day 55 of trial, 6600CFU/l
Section 13, in place in rig for 203 days, day 83 of trial, 900CFU/l.
All these sections had been in the rig for 120 days before treatment started.

4.13.2 Rigs B

The temperatures in rigs B were maintained below 45ºC, simulating a typical hot water circulating system in which mixing valves are used to blend water to a temperature below 45ºC to avoid scalding. This was effectively the 'control' rig in this set of experiments.

The temperatures of the polyethylene rig tank recorded daily for 83 days ranged from 36ºC to 49ºC. The most common temperature recorded was 45ºC. The average temperature in the polyethylene rig tank recorded over the 83 days was 43ºC (± 0.4ºC).

Graph 4.39 shows the *Legionella* results of samples taken ones a week from the polyethylene rig draw off point.



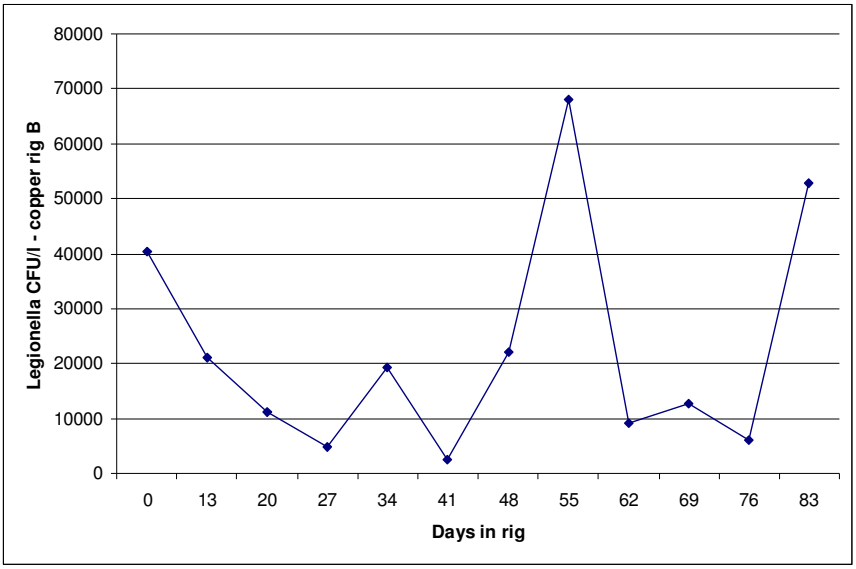
Graph 4.39 *Legionella* results of polyethylene rig B.

At day 0 a *Legionella* colony forming unit count of 49500CFU/l was found in the sample taken from the draw off point of the polyethylene rig. The *Legionella* contamination was variable but increased to a *Legionella* colony forming unit count of 60800CFU/l found in the sample taken on day 83 (~ 23% increase).The average *Legionella* count of samples taken from day 13 to day 83 was 64309CFU/l (± 10421CFU/l).

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig brushed through sections were:
Section 16, from rig A, in place in rig for 133 days, day 13 of trial, 8800CFU/l
Section 14, from rig A, in place in rig for 175 days, day 55 of trial, 96800CFU/l
Section 13, from rig A, in place in rig for 203 days, day 83 of trial, 32600CFU/l.
All these sections had been removed from the polyethylene rig A to inoculate rig B and had been in rig B for 120 days at day 0.

The temperatures of the copper rig tank recorded daily for 83 days ranged from 37ºC to 50ºC. The most common temperature recorded was 43ºC. The average temperature recorded in the copper rig tank was 44ºC (± 0.3ºC).

Graph 4.40 shows the *Legionella* results of samples taken ones a week from the copper rig draw off point.



Graph 4.40 *Legionella* results of copper rig B.

At day 0 a *Legionella* colony forming unit count of 40400CFU/l was found in a sample taken from the draw off point of the copper rig. The *Legionella* contamination increased to a *Legionella* colony forming unit count of 52800CFU/l found in the sample taken on day 83 (~31% increase).The average *Legionella* count of samples taken from day 13 to day 83 was 20873CFU/l (± 6294CFU/l).

The *Legionella* colony forming unit counts found in the samples of the copper rig brushed through sections were:

Section 15, in place in rig for 133 days, day 13 of trial, 200CFU/l

Section 14, in place in rig for 175 days, day 55 of trial, 25200CFU/l

Section 13, in place in rig for 203 days, day 83 of trial, 3400CFU/l.

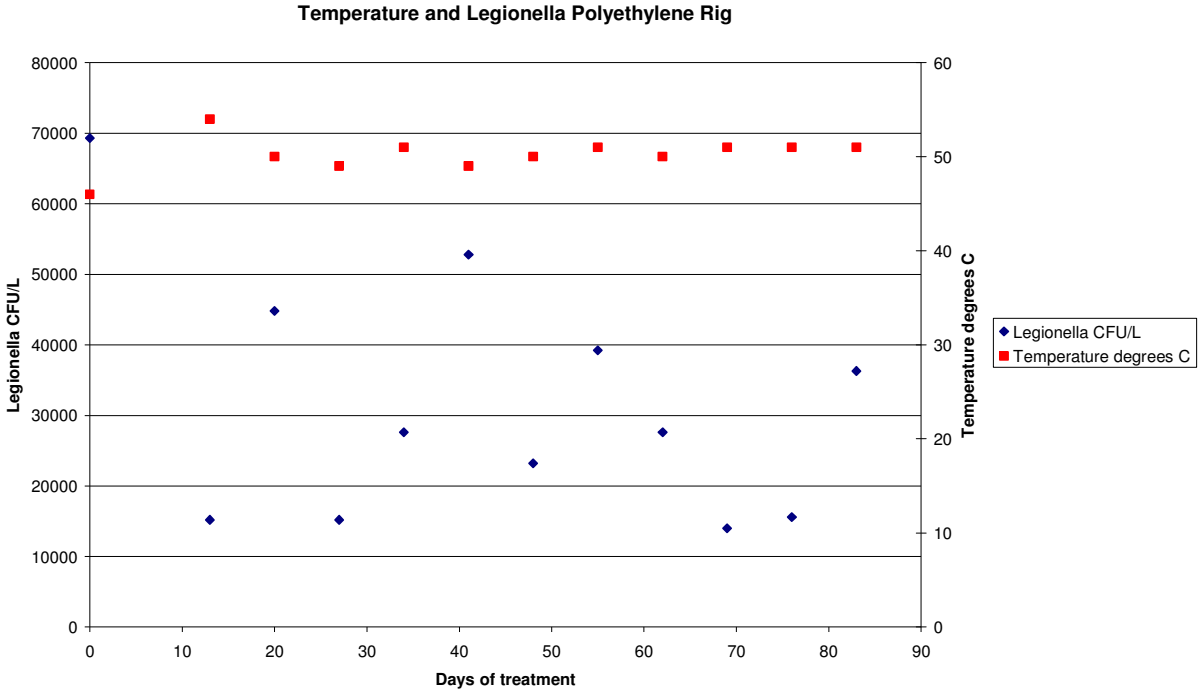
All these sections had been in the rig for 120 days at day 0.

4.13.3 Rigs C

The temperatures in rigs C were maintained ~50ºC, simulating a pure hot water circulating system to which the temperature control regime, as recommended in the ACoP (L8) and the HTM 04-01 documents, is applied.

The temperatures of the polyethylene rig tank recorded daily for 83 days ranged from 42ºC to 60ºC. The most common temperatures recorded were 45ºC, 46ºC, and 48ºC. The average temperature recorded was 49ºC (±0.6ºC).

Graph 4.41 shows the *Legionella* results of samples taken once a week from the polyethylene rig draw off point as well as the temperatures that were recorded on the day the *Legionella* samples were taken.



Graph 4.41 *Legionella* results and temperature recordings of polyethylene rig C.

At day 0 a *Legionella* colony forming unit count of 69300CFU/l was found in the sample taken from the draw off point of the polyethylene rig. The *Legionella* contamination declined to a *Legionella* colony forming unit count of 36300CFU/l found in the sample taken on day 83 (~48% reduction).The average *Legionella* count of samples taken from day 13 to day 83 was 28318CFU/l (± 4035CFU/l).

The *Legionella* colony forming unit counts found in the samples of the polyethylene rig brushed through sections were:
Section 7, from rig A, in place in rig for 133 days, day 13 of trial, 700CFU/l
Section 17, in place in rig for 175 days, day 55 of trial, 26400CFU/l
Section 16, in place in rig for 203 days, day 83 of trial, 500CFU/l.
Sections 17 and 16 had been in the rig for 120 days at day 0. Section 7 was removed from the polyethylene rig A to inoculate rig C and had been in rig C for 120 days at day 0

The temperatures of the copper rig tank recorded daily for 83 days ranged from 40ºC to 58ºC. The most common temperature recorded was 46ºC. .The average temperature recorded in the copper rig tank was 48ºC (± 0.5ºC).

Graph 4.42 shows the *Legionella* results of samples taken ones a week from the copper rig draw off point as well as the temperatures that were recorded on the day the *Legionella* samples were taken.

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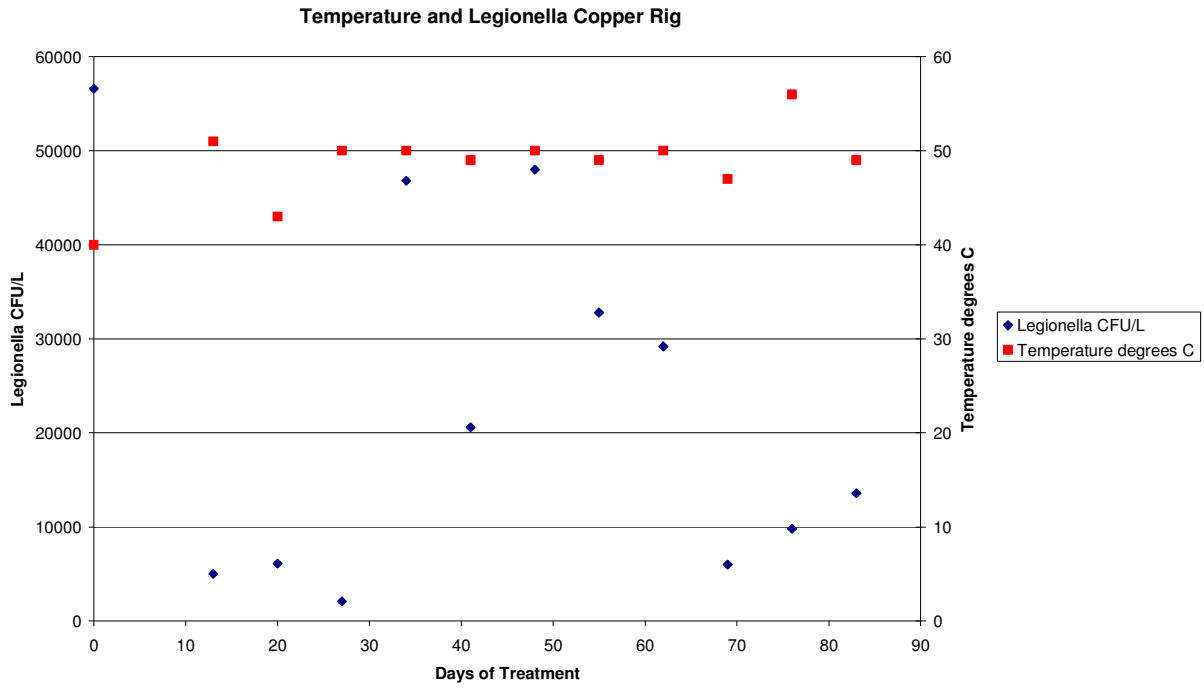

Graph 4.42 *Legionella* results and temperature recordings of copper rig C.

At day 0 a *Legionella* colony forming unit count of 56600CFU/l was found in the sample taken from the draw off point of the copper rig. The *Legionella* contamination declined to a *Legionella* colony forming unit count of 13600CFU/l found in the

sample taken on day 83 (~76% reduction).The average *Legionella* count of samples taken from day 13 to day 83 was 20000CFU/l (± 5072CFU/l).

The *Legionella* count dropped to below 10000CFU/l in samples taken on day 13, the temperature was 51ºC, on day 20, the temperature was 43ºC, on day 27, the temperature was 50ºC, on day 69, the temperature was 47ºC, and on day 76, the temperature was 56ºC.

The *Legionella* colony forming unit counts found in the samples of the copper rig brushed through sections were:
Section 9, in place in rig for 133 days, day 13 of trial, 0CFU/l
Section 16, from rig A, in place in rig for 175 days, day 55 of trial, 0CFU/l
Section 13, in place in rig for 203 days, day 83 of trial, 200CFU/l.
Sections 9 and 13 had been in the rig for 120 days at day 0. Section 16 was removed from the copper rig A to inoculate rig C.

4.14 The Robbins device

Tables 4.34, 4.35, and 4.36 below show the results of the 3 tests carried out with the Robbins device by releasing the rubber, copper, and polyethylene discs in 10ml distilled water and shaking the sample for 30 seconds.

Test	Disc	10^{-2} CFU/ml	10^{-2} CFU/ml
Test 1	A	350000	340000
Test 1	B	2800000	2400000
Test 1	C	2200000	3600000
Test 1	D	1500000	1200000
Test 1	E	1800000	1300000
Test 1	F	380000	460000
Test 2	A	1400000	550000
Test 2	B	1200000	720000
Test 2	C	1400000	870000
Test 2	D	560000	750000
Test 2	E	1500000	1100000
Test 2	F	720000	540000
Test 3	A	750000	840000
Test 3	B	610000	560000
Test 3	C	560000	600000
Test 3	D	1330000	1430000
Test 3	E	920000	800000
Test 3	F	710000	700000

Table 4.34 The Robbins device - rubber discs results.

Test	Disc	10^{-2} CFU/ml	10^{-2} CFU/ml
Test 1	A	210000	170000
Test 1	B	130000	200000
Test 1	C	1300000	1000000
Test 1	D	880000	1160000
Test 1	E	1640000	1240000
Test 1	F	1410000	740000
Test 2	A	1900000	1500000
Test 2	B	1900000	2100000
Test 2	C	900000	920000
Test 2	D	570000	580000
Test 2	E	810000	920000
Test 2	F	1200000	1100000
Test 3	A	140000	180000
Test 3	B	280000	270000
Test 3	C	70000	210000
Test 3	D	250000	450000
Test 3	E	300000	300000
Test 3	F	300000	450000

Table 4.35 The Robbins device - copper discs results.

Test	Disc	10^{-2} CFU/ml	10^{-2} CFU/ml
Test 1	A	260000	50000
Test 1	B	230000	200000
Test 1	C	120000	380000
Test 1	D	500000	70000
Test 1	E	110000	280000
Test 1	F	140000	200000
Test 2	A	110000	90000
Test 2	B	60000	110000
Test 2	C	390000	100000
Test 2	D	270000	110000
Test 2	E	80000	50000
Test 2	F	230000	190000
Test 3	A	140000	140000
Test 3	B	140000	240000
Test 3	C	140000	190000
Test 3	D	160000	150000
Test 3	E	90000	210000
Test 3	F	260000	50000

Table 4.36 The Robbins device - polyethylene discs results.

The average results of the 3 tests of the rubber, copper and polyethylene disc samples were:

Rubber 1.1×10^6 CFU/ml

Copper 7.7×10^5 CFU/ml

Polyethylene 1.7×10^5 CFU/ml

Table 4.37 below shows the results of the tests carried out with the Robbins device by brushing the discs in 10ml distilled water.

Test	Disc	10^{-2} CFU/ml	10^{-2} CFU/ml	10^{-3} CFU/ml	10^{-3} CFU/ml
Test 4	C- Rubber	TNTC	TNTC	11100000	16900000
Test 4	F Rubber	TNTC	TMTC	13900000	8300000
Test 4	I Rubber	TNTC	TNTC	5600000	5600000
Test 4	L Rubber	TNTC	TNTC	12300000	13200000
Test 4	B Copper	310000	150000	0	300000
Test 4	E Copper	100000	80000	100000	300000
Test 4	H Copper	890000	850000	500000	900000
Test 4	K Copper	370000	670000	400000	500000
Test 4	A Polyethylene	570000	480000	600000	300000
Test 4	D Polyethylene	1890000	1380000	1100000	1700000
Test 4	G Polyethylene	660000	520000	800000	700000
Test 4	J Polyethylene	770000	330000	700000	300000

TNTC = Too Numerous To Count

Table 4.37 The Robbins device - results brushed rubber, copper and polyethylene discs

The 10^{-2} samples of the rubber discs were too numerous to count. The average result of the rubber disc 10^{-3} samples was 1.1 x 10^{7} CFU/ml. The average results of the highest result of the two 10^{-2} and the two 10^{-3} copper and polyethylene disc samples were 4.6 x 10^{5} CFU/ml for the copper discs and 8.7 x 10^{5} CFU/ml for the polyethylene discs.

5. DISCUSSION

5.1 Evaluation study of 10 hospitals

Legionella contaminate water systems from public water supplies and borehole waters, and colonize these systems from biofilms, and protozoa cells where they are protected from disinfection. Preventing *Legionella* contamination of these systems is, therefore, a difficult technological challenge. However, because *Legionella* can cause devastating disease in humans, it is important to prevent these systems from becoming contaminated and to control the risk of exposure.

Disturbing findings during this study were that many of the UK Health and Safety Executive's and Department of Health's recommendations may not result in control and prevention of Legionnaires' disease. Furthermore, several of their recommended practices can place an undue burden on UK national health estates departments to perform costly, labour intensive, and hazardous tasks with uncertain benefit.

In the US, routine monitoring for *Legionella* in water distribution systems is recommended as it is considered the only factor known with certainty to be predictive of the risk of legionellosis. Routine monitoring also allows for evaluation of the control modalities applied and, therefore, for effective prevention (Lin *et al.,* 2011).

In the UK, however, monitoring for *Legionella* in water distribution systems is only recommended when storage and distribution temperatures are not consistently maintained at the levels recommended in the ACoP (L8) and HTM 04-01 documents or when an outbreak is suspected or has been identified (UK Approved Code of Practice (L8), 2000, UK Health Technical Memorandum 04-01, 2006). This approach has resulted in a lack of evidence of control by the recommended control modalities.

The temperature regime described in the ACoP (L8) and HTM 04-01 documents recommends that hot water is stored at 60ºC and distributed, so that it reaches a temperature of 50ºC within 1 minute at outlets. The cold temperature should be below 20ºC after running outlets for up to 2 minutes. Whilst the ACoP (L8) advises that this regime is the 'traditional' approach to *Legionella* control for hot water and cold water systems, and that it is not necessary to apply it as long as proliferation of *Legionella* is

prevented, the HTM 04-01 documents recommend the regime, however, as the 'preferred' strategy, and advises using alternative techniques, such as copper- and silver ionization and chlorine dioxide, only together with but not instead of the temperature regime (UK Approved Code of Practice (L8), 2000, UK Health Technical Memorandum 04-01, 2006). The temperature regime is, therefore, still seen by UK national health trusts as the main control method even though it has not been validated.

None of the scientific papers evaluated by the UK Department of Health to compose the HTM 04-01 documents supported the control of *Legionella* at 50ºC after 1 minute or at 20ºC after 2 minutes (House of Commons Hansard debates for 22 May 2007, www.parliament.uk).

In this study, although some control was afforded by maintaining hot water temperatures above 55ºC, the results of the study with the 10 hospitals demonstrated that, when water systems are complex, the temperature regime, described in the ACoP (L8) and HTM 04-01 documents, does not achieve complete control.

Nine of the 10 hospitals studied relied solely on the recommended temperature regime for the control of *Legionella* in their hot and cold water distribution systems. The results of the samples taken from the hospitals before the copper and silver ionization systems were activated, therefore, demonstrate the lack of complete control by the temperature regime as *Legionella* were found. The results of the samples taken from study hospital 10, before the copper and silver ionization systems were activated, not only highlight the lack of complete control by the temperature regime but also by chlorine dioxide as chlorine dioxide was applied as well as the temperature regime because the temperature regime had failed to completely control the *Legionella* by itself. Furthermore, the results of the samples taken from the 10 hospitals also suggested the temperature regime actually promoted *Legionella* proliferation because of the requirement to reduce hot water temperatures to below 46ºC to avoid scalding.

Also supporting this are the temperature recordings of 3073 outlets of other sites at which *Legionella* were found. The hot water was deliberately blended with cold water at 1344 of these outlets (~43.8%) to temperatures ranging from 38ºC to 46ºC. The average *Legionella* colony forming unit count was the highest of all the 3073 outlets

tested at these outlets, at 8339CFU/l (± 889CFU/l), with the highest *Legionella* colony forming unit count, of 445500CFU/l, found at 38ºC.

The temperatures of 671 of the 3073 outlets (~21.8%) were between 21ºC and 38ºC with an average *Legionella* colony forming unit count of 6213CFU/l (± 996CFU/l). The hot water at most of these outlets was blended deliberately, which highlighted another issue with the temperature regime as the mixing valves that were fitted to these outlets may not have been working properly.

That *Legionella* are not eradicated by a hot water temperature of above 50ºC is supported by the temperatures at 199 of the 3073 outlets at which *Legionella* were found (~6.5%) being 50ºC and above. The average count was 2941CFU/l (± 474CFU/l). A *Legionella* colony forming unit count of 100CFU/l was found at the highest temperature of 76ºC and the highest *Legionella* colony forming unit count, of 45000CFU/l, was found at 55ºC.

The temperatures of 165 of the 3073 outlets (~5.4%) were between 47ºC and 50ºC. The average *Legionella* colony forming unit count was 5320CFU/l (± 1256CFU/l). Unblended hot water serviced these outlets, which highlighted another issue with the temperature regime as the water heating system may not have been working properly.

That *Legionella* are eradicated by a cold water temperature of below 20ºC is also supported by the temperatures of 694 of the 3073 outlets at which *Legionella* were found (~22.6%) being below 20ºC. The average count was 1829CFU/l (± 289CFU/l). A *Legionella* colony forming unit count of 200CFU/l was found at the lowest temperature of 6ºC. The highest *Legionella* colony forming unit count, of 130400CFU/l, was found at 20ºC.

Previous studies have also demonstrated that the recommended temperatures are ineffective in controlling *Legionella* in hospital water systems. *Legionella pneumophila* was isolated from taps and showers of 2 buildings in Holland when the hot water temperature was circulated at 55ºC. The hot water system could also not be completely decontaminated even by raising the hot water temperature to 70ºC (Groothuis *et al.,* 1985).

Maintaining hot water at 50ºC to 55ºC at peripheral outlets of a university hospital in Belgium, removing deadends wherever possible, and improving maintenance on the hot water storage tanks, by draining, rinsing, refilling them with potable water, and increasing the temperatures to 95ºC before decreasing temperatures to 60ºC, showed only a reduction of the number of positive samples but these positive samples still contained between 10^4 and 5 x 10^5CFU/l *L. pneumophila* serogroups 6 and 10 (Ezzeddine *et al.,* 1989).

Legionella pneumophila serogroup 6, associated with 2 cases of legionellosis, persisted in a hot water system of a hospital in Italy at temperatures of 55ºC and 56ºC (Visca *et al.,* 1999).

Darelid *et al.,* (2002), found that maintaining water temperatures above 55ºC was associated with 12% of water samples being positive during a 10 year period in which 4 patients developed Legionnaires' disease (Darelid *et al.,* 2002).

After an outbreak of Legionnaires' disease in August 2010, control of *Legionella*, present in the hot water and cold water system of a nursing home in Slovenia, by maintaining hot and cold water temperatures at 55°C and below 20°C proved to be inadequate. The *Legionella* results of sample testing after these interventions still were above 10^4 CFU/l (Skaza *et al.,* 2010).

The results of the research project by BSRIA in 1996 also showed that no *Legionella* were found in only 13% of the results of the rig to which the temperature regime was applied. This study also showed that the hot water storage calorifier, which transferred heat to water indirectly, did not provide complete protection against *Legionella* being drawn into the hot water circuit even when the flow water temperature was maintained at 60ºC (BSRIA TN6/96).

Studies have even demonstrated that elevated temperatures can promote biofilm re-growth and that it creates dead organic material, inducing shifts in the prokaryotic community, which is potentially beneficial for the growth of *L. pneumophila* (Saby *et al.,* 2005, van der Kooij *et al.,* 2005, Vervaeren *et al.,* 2006, Temmerman *et al.,* 2006).

Legionella control by the recommended temperatures as in the ACoP (L8) and HTM 04-01 documents in hospitals is basically not possible due to:

- *Legionella* being ubiquitous, entering hot and cold water distribution systems through mains water. Restrictions to having *Legionella* in water supplies do not exist (Water Supply (Water Quality) Regulations, 2000, UK Health Technical Memorandum 04-01, 2006).
- *Legionella* surviving temperatures of 0ºC to 63ºC (Fliermans *et al.,* 1981), and the extensive contact time at 50ºC necessary to reduce *Legionella* populations. Dennis *et al.,* (1984) reported that *L. pneumophila* serogroup 1 had a long decimal reduction time of 111 minutes at 50ºC. Muller, (1981) previously also demonstrated that *L. pneumophila* serogroups 1 and 4 were only killed when exposed to 58ºC for as long as 30 minutes (Muller, 1981, Dennis *et al.,* 1984).
- Hot water being blended with cold water at outlets to avoid scalding to temperatures of 38ºC (for bidets), 41ºC (for basins), 43ºC (for baths), and 46ºC (for assisted baths) (UK Health Technical Memorandum 04-01, 2006), and water temperatures between 20ºC and 46ºC being the range in which *Legionella* proliferates most rapidly (Fliermans *et al.,* 1981, Nguyen *et al.,* 1991, Lee and West, 1991, Newsome, 2001). Makin, (1998), also found that a mixing valve, feeding blended water to a heavily colonized shower, contained large numbers of *Legionella* (Makin, 1998), which highlighted that mixing valves can become a niche for *Legionella* proliferation and require to be inspected and cleaned regularly.
- The complexity of water systems, where there can be 4km of pipework, making the engineering requirements to maintain the recommended temperatures consistently for 24 hours a day, 365 days a year, impossible. Kusnetsov *et al.,* (2003), demonstrated that it was difficult to maintain 50ºC in the whole length of circulation in a hot water system of a hospital in Finland. The authors suggested that this probably reflected a common situation in large buildings and that the main disadvantage of maintaining such high temperatures is the lack of eradication of *Legionella* (Kusnetsov *et al*., 2003).
- The pressures on UK national health trusts to reduce expenditure. A review, completed in 2009 of healthcare premises in the UK by the Department of Health , highlighted not only that it is difficult to maintain the recommended hot temperature at the same time in all parts of the system, which frequently results in eradication failure, but it also emphasized the considerable energy cost associated with maintaining the recommended hot water temperature (UK Independent Review, 2009).

- The pressures on UK national health trusts to reduce their carbon footprint (www.sdu.nhs.uk).

It was impracticable to collect samples from all outlets in the 10 hospitals studied because of the number of outlets and the cost of *Legionella* analysis. The probability of contamination at outlets at which *Legionella* were found before the copper and silver ionization systems were activated in the 10 hospitals would be higher than at outlets at which no *Legionella* were found. The *Legionella* contamination at outlets at which *Legionella* were found before activation was, therefore, primarily monitored after activation. The water system near to outlets at which *Legionella* persisted after activation was also investigated by taking samples for *Legionella* analysis from outlets close to the *Legionella* contaminated outlets.

The number of contaminated outlets before the copper and silver ionization systems were activated in the 10 hospitals reduced considerably within 3 months after the systems were activated. The averages of *Legionella* colony forming unit counts also reduced considerably in the samples taken within 3 months after the systems were activated. The number of outlets contaminated and the averages of *Legionella* colony forming units continued to drop in all the hospitals and in 4 hospitals no *Legionella* were found at the end of the studies. This suggested significant control of *Legionella* by the copper and silver ionization systems.

The average hot water temperatures of the 10 hospitals during operation of copper and silver ionization ranged from 38ºC to 46ºC, see Table 4.30. This indicated that *Legionella* were controlled by copper and silver ionization at temperatures below 50ºC.

That *Legionella* is inactivated and controlled in water systems solely by copper and silver ionization has been demonstrated by several studies (Liu *et al.,* 1994, Miuetzner *et al*, 1997, Liu *et al*, 1998, Stout *et al.,* 1998, Rohr *et al.,* 1999, Biurrun *et al.,* 1999, Kusnetsov *et al*, 2001, Stout and Yu, 2003, Chen *et al.,* 2008, Lin *et al.,* 2011).

The ACoP (L8) and HTM 04-01 documents only recommend concentrations for *Legionella* control of 0.4mg/l copper and 0.04mg/l silver in hot water systems, and 0.4mg/l copper and 0.02mg/l silver in soft water systems. Copper and silver concentrations for cold water systems are, however, not given (UK Approved Code of Practice (L8), 2000, UK Health Technical Memorandum 04-01, 2006). This is due to lack of data being available of the copper and silver levels needed to achieve *Legionella* control in cold water systems at the time of publication of the ACoP (L8) in 2000.

The copper and silver levels necessary to maintain control in hot and cold water systems vary from site to site and depend on the intensity of the contamination and the conditions available within the water system that promote *Legionella* growth. The quality of the incoming water is also important, such as the levels of anions (mainly chloride and phosphorus) and the alkalinity of the water as these can reduce the biocidal efficacy of the copper and silver ions and more copper and silver would be needed to maintain control.

The conductivity of the water is also important as it determines the voltage required to drive the electrical current between the copper and silver electrodes. High conductivity requires less and low conductivity requires more.

The average copper and silver results of the samples taken after the copper and silver ionization systems were activated in the 10 hospitals showed that control was attained by copper levels ranging from 0.151mg/l to 0.539mg/l and silver levels ranging from 0.021mg/l to 0.043mg/l.

Legionella persisted, albeit at reduced counts, in samples taken from outlets from the 10 hospitals at which either less than 0.2mg/l copper or less than 0.02mg/l silver was found, and at outlets at which less than both these copper and silver levels were found. On the other hand, no *Legionella* were found in samples taken from outlets at which the copper and silver levels were not consistently maintained above 0.2mg/l copper and 0.02mg/l silver, indicating that the conditions at these outlets were less favourable

for *Legionella* growth. However, the average copper and silver levels at these outlets were mainly above 0.2mg/l copper and 0.02mg/l silver.

The results of study hospital 2 suggested that both the copper and silver target levels needed to be consistently maintained at the outlet at which *Legionella* persisted to control the *Legionella,* but that, especially, more silver was needed.

Legionella control was achieved solely by the silver produced by the ionization system of study hospital 3 as the copper side of this system was deliberately turned off. Control was achieved by an average of 0.033mg/l silver combined with an average of 0.151mg/l copper from the copper pipes. This suggests that *Legionella* control is possible solely by ionized silver but is accelerated in the presence of copper whether copper ions are produced or leaching from copper pipes.

The copper release by the copper and silver ionization system of study hospital 5 was low. This was because the electrical current between the copper electrodes could not be increased due to low conductivity of the water (148.5µS/cm^2). The installation of more copper electrode chambers or increasing the surface area of the copper electrodes could have overcome this lack of release but the hospital did not take this up as *Legionella* were controlled by an average copper level of 0.187mg/l and an average silver level of 0.036mg/l at the outlets tested.

The peaks in the average *Legionella* colony forming unit counts in samples taken from hospital 9 after construction work was started indicated that when construction work is carried out more copper and silver is needed to maintain control to cope with heavier invasion of *Legionella* from dislodged biofilms. Microbial growth and biofilm formation can also be higher when construction work is carried out because of microorganisms entering water systems through open pipes from contaminated water and soil, and settling on the surfaces of water pipes, as well as because of poor water flows. Although a reduction from 14 contaminated outlets before activation of the copper and silver ionization system to 1 at the end of the study as well as a reduction from a 453CFU/l (± 216CFU/l) average *Legionella* count before activation to a 7CFU/l (± 7CFU/l) average count at the end of the study was reached, higher copper and silver levels had to be maintained at the outlets, averages of 0.398mg/l copper and

0.037mg/l silver, to achieve this control. This is an important finding when considering control at other sites where maintenance work is being carried out.

Low copper and silver levels at outlets highlight poor water flow to these outlets because the copper and silver is not arriving at the outlets, which indicated that these outlets were either not used or the water flow was obstructed at the outlets by point of use filters or the copper and silver was trapped upstream the outlets, for instance in unserviced water heating systems (calorifiers, plate heat exchangers). Low used outlets were identified in study hospital 10 because of low levels of copper and silver found in the samples taken from them. The study with hospital 10 indicated that remedial work, such as regular running of the identified little used outlets, the replacement of old rubber lined flexible hoses, and regular inspection and cleaning of mixing valves, would have aided in completely controlling the *Legionella* in this hospital. Such remedial work was, however, not always possible because of lack of staff, which resulted in a slower reduction in the number of contaminated outlets from the beginning to the end of the study. The average *Legionella* colony forming unit count still reduced however from 465CFU/l (± 156CFU/l) before activation of the copper and silver ionization systems to 157CFU/l (± 112CFU/l) at the end of the study.

The pH values of the 10 hospitals studied, shown in Table 4.30, show that efficacy in controlling *Legionella* by copper and silver levels, ranging from 0.151mg/l to 0.539mg/l copper and 0.021mg/l to 0.043mg/l silver, was not affected in water with pH values as high as 8.5. This can be attributed to the design of the copper and silver ionization systems installed in the hospitals, which automatically remove calcium and magnesium ions from the electrodes, as well as the regular manual cleaning of the electrodes.

The BSRIA project of 1996 suggested, however, that a pH value greater than 7.6 could adversely influence the copper and silver ionization *Legionella* inactivation process. It was suggested that the silver concentration dropped dramatically to below 0.01mg/l due to an increase in the pH of the tests carried out with hard water. The pH had increased to 8.6, which resulted in rapid scale formation on the surfaces of the electrodes. The scale coverage on the electrodes obstructed, particularly, the release of silver and reduced the availability of silver in the water. The authors found that by

acidifying the water with citric acid to a pH of 7.2, the electrodes de-scaled and the number of copper and silver ions increased in solution (BSRIA TN6/96). The ACoP (L8) and HTM 04-01 documents, therefore, advise that it is difficult to maintain silver ion concentrations above a pH of 7.6 (UK Approved Code of Practice (L8), 2000, UK Health Technical Memorandum 04-01, 2006).

The chloride values of hospital number 5, 7, 9 and 10, of 60.7mg/l, 58.5mg/l, 54.9mg/l and 57.3mg/l respectively, shown in Table 4.30, indicate that average silver levels of 0.043mg/l, 0.031mg/l, 0.037mg/l, and 0.031mg/l with average copper levels of 0.366mg/l, 0.539mg/l 0.398mg/l, and 0.231mg/l respectively achieved *Legionella* control. Table 4.30 also shows that control was maintained by similar average silver and copper levels in the water systems of the other hospitals studied but at lower chloride levels.

Lin *et al.,* (2002) suggested, however, that it was possible that chloride concentrations in water may decrease the availability of silver cations and reduce its biocidal potential (Lin *et al.,* 2002).

The phosphorus values of hospital number 3, 6, and 10, of 1000µg/l, 1500µg/l, and 1030µg/l respectively, shown in Table 4.30, indicate that average silver levels of 0.033mg/l, 0.021mg/l, and 0.031mg/l with average copper levels of 0.151mg/l, 0.0.328mg/l, and 0.231mg/l respectively achieved *Legionella* control. However, control was also maintained by similar average silver and copper levels in the water systems of the other hospitals studied at lower phosphorus levels.

According to Lin *et al.,* (2005), however, phosphorus may also interfere with the efficacy of copper and silver ionization because of complexation with the copper and silver ions, which could result in precipitation of copper and silver phosphates and potential loss of biocidal action (Lin *et al.,* 2005).

Although the data from the study with the 10 hospitals suggests that in reality chloride and phosphorus may not have a great diminishing effect on the biocidal action of

copper and silver ionization against *Legionella*, the chloride and phosphorus levels in the water that is to be treated by copper and silver ionization need to be monitored. Should there be a reduction of the copper and silver biocidal action against *Legionella* then increasing the copper and silver levels in the water may need to be considered to counteract the potentially adverse effect of higher chloride and phosphorus in the water.

Long term treatment with copper and silver ions could theoretically result in the development of resistance to these ions. Development of resistance was, however, not noticed during the 4 years study with the 10 hospitals. *Legionella* were controlled by consistently dosing the water systems with copper and silver, average copper levels ranged from 0.151mg/l to 0.539mg/l and average silver levels ranged from 0.021mg/l to 0.043mg/l. It is possible that because two biocides are utilized, the acquisition of bacterial resistance to both copper and silver would be more difficult.

Studies, under laboratory conditions, have demonstrated the development of effective defence mechanisms against metal ions by *Salmonella, Pseudomonas, Enterococcus, Klebsiella,* and *E. coli* (McHugh *et al*., 1975, Bridges *et al.*, 1979, Haefeli *et al*., 1984, Kaur and Vadehra 1986, Solioz and Odermatt 1995, Silver, 1996, Gaillard and Webb, 1997, Gupta *et al*., 1999, Gupta, *et al.*, 2001). However, according to Silver, (1996), in Gram-negative bacteria, such as *Legionella,* plasmid-mediated resistance to silver involved energy-dependent efflux of silver from the cell. For instance, plasmid-mediated silver resistance in *Salmonella* involved a total of nine genes and was considered unusual in that it included three separate types of resistance mechanisms (Silver, 1996).

Dollenmeier, (2002), also suggested that development of resistance to metal ions was metabolically expensive for bacteria, and that, therefore, the formation of resistance was not likely. The author suggested as well that resistance to biocides occurred most readily with substances that relied on specific protein binding or were susceptible to chemical breakdown or metabolism, such as antibiotics, but that metal ions had such a broad non-specific mode of action, and were also elements that could not be degraded, which made resistance not likely (Dollenmeier, 2002).

Chopra, (2007) suggested that because resistance to metal ions is that metabolically expensive for bacteria, it is also difficult to compete against non-resistant bacteria and

that, therefore, metal resistant strains do not persist through subsequent generations (Chopra, 2007).

According to Edward-Jones, (2009), the low silver levels used in hygiene, personal care and healthcare, the lesser contact time, and the multifaceted mechanisms of silver action on bacteria meant that the development of resistance against silver was unlikely (Edward-Jones, 2009).

The study with the 10 hospitals highlights that the temperature regime, based in this instance on the measurements of temperatures at outlets, described in the ACoP (L8) and the HTM 04-01 documents, was ineffective but that copper and silver ionization was effective in controlling *Legionella* with copper and silver levels ranging from 0.151mg/l to 0.539mg/l copper and 0.021mg/l to 0.043mg/l silver, at temperatures below 50ºC, at chloride values ranging from 8.4mg/l to 60.7mg/l, phosphorus values ranging from 191µg/l to 2100µg/l, and at pH values ranging from 6.9 to 8.5.

The study highlighted that routine monitoring for *Legionella*, performed simultaneously with monitoring for copper and silver levels, identified areas of increased risk of contamination as well as risk of exposure, which allowed for effective prevention of hospital-acquired legionellosis.

During the studies none of the 10 hospitals experienced any endemic hospital-acquired *Legionella* pneumonia. One elderly, male, oncology patient of study hospital 1 contracted Legionnaires' disease from an outlet contaminated with *Legionella*. A rubber lined flexible hose was attached to this outlet. The hose was removed, brushed through 5 times in 500ml distilled water, which was then analysed for *Legionella* by the culture method. The analysis was conducted by the UK Health Protection Agency and the same *L. pneumophila* serogroup 1 bacteria that had infected the stricken patient were isolated. The patient survived, the contaminated hose was replaced with copper pipe, the outlet was run regularly to encourage the copper and silver levels, which were previously below 0.2mg/l copper and below 0.02mg/l silver, and the *Legionella* contamination cleared.

Flexible hoses lined with synthetic rubber are commonly attached to outlets in hospitals because of ease of use. The UK Water Regulations Advisory Scheme

(WRAS) observed that the conditions within flexible hoses may favour the growth of *Legionella*, and that evidence from scientific investigations of the occurrence of *Legionella* in hospitals has shown that some flexible hoses were heavily infected with biofilms which included *Legionella.* Microscopic examination showed that the inner surface of these hoses were rough and pitted. The rough inner surfaces and the pits provided an ideal environment for bacteria to attach and form biofilms that harboured the *Legionella*. The hoses examined were made from the elastomer 'ethylene propylene diene monomer' (EPDM), which is a synthetic rubber (Water Regulations Advisory Scheme, January 2006, EPDM Flexible Hoses). Therefore, flexible hoses lined with synthetic rubber should not be used in water systems where the risk of legionellosis is high, such as in hospitals.

The study also highlighted that both healthcare facility and infection control personnel play an important part in preventing hospital-acquired legionellosis. Remedial work, such as replacing old rubber lined flexible hoses with new hoses at outlets at which *Legionella* were found, flushing of outlets at which *Legionella* and little copper and silver was found, inspecting and cleaning of mixing valves of outlets at which *Legionella* were found, and removing deadlegs, deadends, should be followed up and completed, whilst the presence of *L. pneumophila* in samples should prompt clinical surveillance. Facility and infection control departments of hospitals as well as the producers of control modalities should all work together to successfully prevent hospital-acquired legionellosis. This was also emphasized by Lin *et al.,* (2011).

Lin *et al.,* (2011), not only demonstrated the importance of this synergy between hospital facility and infection control departments and the significance of routine monitoring for *Legionella* but also that recommended control modalities need to be supported by evidence of their efficacy against *Legionella*.

In the US the following evaluation steps of modalities preventing hospital-acquired legionellosis have been suggested (Stout and Yu, 2003, Stout, 2007):

- Demonstrated efficacy *in vitro*;
- Anecdotal experience in individual sites;
- Controlled studies of sufficient duration (years) in single sites; and
- Confirmatory reports from multiple sites

The study with the 10 hospitals reports on the efficacy of copper and silver ionization to control *Legionella* in multiple UK hospital water systems over a period of 1½ to 4 years as well as on the efficacy of copper and silver ionization in these water systems in preventing legionellosis. This represents the last 2 of the above evaluation steps.

Previously, in laboratory assays, copper and silver ions have already been shown to effectively kill *Legionella in-vitro* (Landeen *et al.,* 1989, Lin *et al.,* 1996, Rohr *et al,* 1996), anecdotal reports of the efficacy of copper and silver ionization systems have also been presented (Thompson *et al.,* 1990, Lee *et al.,* 1994, Liu *et al.,* 1994, Mietzner *et al.,* 1997, Mathys *et al.,* 2002, Oesterholt, 2006), and studies have also been conducted in the US into the efficacy of copper and silver ionization to prevent hospital-acquired legionellosis over a prolonged period (Selenka *et al.,* 1995, Rohr *et al.,* 1999, Reynaga *et al.,* 2001, Stout and Yu, 2003). Copper and silver ionization is, therefore, a *Legionella* control modality to have fulfilled all of the 4 evaluation criteria.

This US evaluation methodology has, however, not been adopted in the UK. The recommendations in the ACoP (L8) and HTM 04-01 documents are currently not supported by evidence of their efficacy in controlling *Legionella* in water systems. At the time of writing this thesis, however, a review of the ACoP (L8) is proposed but it is unclear whether or not the efficacies of the recommended control modalities are going to be evidence-based, and whether or not routine monitoring for *Legionella* as well as for the biocidal agents applied and interaction between facility and infection control departments is going to be recommended.

5.2 Initial experiment with rigs

According to the ACoP (L8) and HTM 04-01 documents, the plumbing materials commonly used for hot water and cold water are copper, polyethylene, stainless steel and iron. Unplasticized polyvinyl chloride (uPVC) is also often used.

However, in spite of research showing that *L. pneumophila* can colonize stainless steel, that biofilm formation and *L. pneumophila* colonization is heavier on polyethylene in comparison to copper, and that iron tubercles of eroded galvanized pipes provide nutrients and a habitat for microorganisms including *Legionella* (Haas *et al.*,1983, Schofield and Locci, 1985, Keevil *et al.*, 1993, BSRIA Application Guide Ag 2/93, Camper, 1996, BSRIA, 1996, Geldreich, 1996, Geldreich and Le Chevalier, 1999, van der Kooij *et al.*, 2005, www.corrosion-doctors.org), no reference to this is given in the ACoP (L8) and HTM 04-01 documents.

Instead, the ACoP (L8) and the HTM 04-01 documents specify that a hot water and cold water installation must comply with the requirements of the Water Supply (Water Fittings) Regulations 1999, which provides a list of products and materials that have been assessed for compliance. This assessment is carried out in the UK according to the British Standard BS6920, which ensures that the material does not contribute to poor water quality by producing unacceptable taste and odours, by releasing chemicals, or by encouraging microbial growth. The procedure uses a natural river water inoculum and a water sample of known chemistry. Bacterial growth is determined indirectly by measuring oxygen consumption. The procedure, therefore, does not provide information on the rate of biofilm formation on different plumbing materials or indicates which organisms are present within the biofilm (BS6920-01, 2000).

Experiments with model systems have indicated that polyethylene can promote microbial growth more than copper (Keevil *et al.,* 1993, van der Kooij *et al.,* 2005). Although the model systems constructed for these experiments endeavoured to replicate small hot water systems, they did not simulate a copper and a polyethylene piped hot water system of a typical small UK hospital, in which water is stored, heated, blended and circulated. Biofilm formation and *Legionella* growth were also

only examined in these model systems and *Legionella* control measures were not tested.

For this project, copper and polyethylene piped model rigs were, therefore, constructed to not only examine the differences in biofilm formation and *Legionella* growth in copper and polyethylene piped water systems but also to examine *Legionella* growth at a hot water temperature of below 46ºC, simulating a blended water system, and to examine the efficacy of hot water temperatures of 50ºC, as well as the efficacy of copper and silver ionization at temperatures below 46ºC, in controlling *Legionella* in these rigs. The rigs were uniquely designed and operated in that their pipes were divided into removable sections to examine biofilm formation, and *Legionella* growth, on the inner surfaces of the different materials. Water was also removed from and added to the rigs daily, therefore, consistent conditions were effectively maintained within the rigs.

The initial experiment with the first rigs built, the A rigs (copper and polyethylene), was carried out with the view to investigate whether this rig design was capable of supporting growth of *Legionella* and, if so, to compare microbial growth between polyethylene and copper pipes.

Samples were taken once every week over 582 days and were analysed for viable bacteria by the standard culture method (BS EN ISO 6222:1999). This standard is the industry standard used to establish microbial activity in water systems, and specifies incubation of samples at 37ºC and at 22ºC to differentiate between bacteria that proliferate at body temperature, ~37ºC, and can potentially cause disease in humans, and bacteria that are ubiquitous in the environment and proliferate at 22ºC.

Both rigs were inoculated with viable bacteria. More viable bacteria were introduced to the copper rig than to the polyethylene rig. In total, viable bacteria counts (TVCs) of 8052CFU/ml, incubated at 37ºC, and 12238CFU/ml, incubated at 22ºC, were introduced to the copper rig, whereby 6567CFU/ml, incubated at 37ºC, and 8218CFU/ml, incubated at 22ºC, were introduced to the polyethylene rig.

The TVC analysis results of the 67 samples taken individually over the 582 days from the rigs demonstrated that a population of viable bacteria was successfully maintained

in both rigs. The results were, however, variable, fluctuating wildly. No replication was found in the results and an overall trend could not be established. This could have been due to 3 litres of water with viable bacteria being drawn from the rigs and 3 litres of fresh water, with possibly less viable bacteria, being added to the rigs daily. This would explain fluctuating viable bacteria levels found in real situations.

What was seen was that there were apparently fewer bacteria isolated at 22ºC than at 37ºC were found in both rigs. Although many bacteria thrive at 22ºC as well as at 37ºC, and the 2 temperatures are close enough to be relatively unselective, this possibly suggested that the population had adapted to 37ºC, which could perhaps be explained by the rig water temperatures being kept between 40ºC and 45ºC.

More bacteria that responded to incubation at 22ºC were found in the copper rig than in the polyethylene rig, which was surprising because of the superior biocidal property of copper cited previously in studies conducted by Schofield and Locci, (1985), Keevil *et al.,* (1993), BSRIA, (1996), and van der Kooij *et al*., (2005).

A new method to analyze the pattern of biofilm development was developed for the study with the rigs, whereby pipe sections of each rig were periodically removed and brushed through 5 times aseptically in 500ml of distilled water, after which the sample was dried and weighed. The viable biological material within the brushed samples was also analyzed by the standard culture method (BS EN ISO 6222:1999) in total viable counts (TVCs).

In spite of a considerable population of planktonic organisms, substantial biomass, in weight and in TVC results, did not develop over 478 days, indicating that the conditions within the rigs were not conducive to biofilm growth. This could be explained by the biocidal action of copper in the copper rig cited in other studies, the smooth surface characteristics of polyethylene, the fluid mechanics within both rigs (high flow rates), and insufficient bacterial adhesive force. Also, a dynamic system may have developed in the rigs whereby biofilms formed and dispersed rapidly, due to the high flow rates, preferentially developing in parts of the rig away from the tested sections, such as around the pushfit connections, which was difficult to follow experimentally.

The planktonic *Legionella* results of the samples taken from each rig after *Legionella* was introduced to the rigs showed that substantial *Legionella* populations had developed in the rigs, indicating that the conditions in the rigs were favourable for *Legionella* growth and that biofilms may have, therefore, been present.

2700CFU/l of *Legionella* was introduced to each rig. The bacteria developed quickly to counts of 22000CFU/l in the sample taken from the polyethylene rig and 48000CFU/l in the sample taken from the copper rig 39 days after introducing the bacteria to the rigs. After the 39 days the *Legionella* results were, however, variable and an overall trend in *Legionella* growth in the rigs could also not be determined. This was not unexpected as a chemostat was effectively operated by drawing water from and adding water to the rigs daily.

The average results of the analysis for planktonic *Legionella* showed that the *Legionella* colony forming unit counts in the copper rig samples were higher than in the polyethylene rig samples

A new method to analyze the presence of *Legionella* in biofilms (sessile *Legionella*) was also developed for this study. Pipe sections of each rig were removed, brushed through aseptically 5 times in 500ml of distilled water, after which the sample was analyzed by the standard culture method (ISO 11731:1998). This method has since been adopted by the UK Health Protection Agency in Birmingham.

The results of the *Legionella* analysis of these samples also showed that the *Legionella* colony forming unit counts in the copper rig samples were higher than in the polyethylene rig samples.

The higher planktonic and sessile *Legionella* counts found in the copper rig were surprising considering the biocidal property of copper against *Legionella* cited in studies conducted previously (Schofield and Locci, 1985, Keevil *et al.,* 1993, BSRIA, 1996, van der Kooij *et al.,* 2005).

Schofield and Locci, (1985), found that colonization by *L. pneumophila* was the least on copper materials, the most on rubber, and that the bacteria also appeared to be able to colonize silicone tubing and adhere to stainless steel (Schofield and Locci, 1985).

Keevil *et al.,* (1993) and Rogers *et al.,* (1994), found that *Legionella* comprised a very low proportion of the microbial population on the copper coupons tested whilst, of the plastic materials tested, polyethylene appeared to be most heavily colonized. The authors suggested that copper ions could either inhibit *Legionella* selectively or the organisms that support *Legionella* growth (Keevil *et al.,* 1993, Rogers *et al.,* 1994).

The BSRIA (1996) study also observed that the number of *Legionella* recovered from the biofilms that had formed on the copper and glass reinforced plastic coupons was only significant on the glass reinforced plastic coupons and not on the copper pipework because this appeared to be naturally biocidal (BSRIA TN 9/96), and van der Kooij *et al.,* (2005), found that *Legionella* concentrations in water from the polyethylene pipes were more than 10 times higher than those from the copper pipes (van der Kooji *et al.,* 2005).

The conditions in the copper rig were possibly more favourable for *Legionella* growth than the conditions in the polyethylene rig. The presence of more viable bacteria, isolated at 22ºC, may have provided more nutrients and the rougher surfaces of the copper pipes may have given better microbial attachment for biofilm formation. Enhanced biofilm formation and rougher surfaces of copper piping was demonstrated by Kandlikar *et al.,* (2001) and Renner and Weibel (2011).

The *Legionella* analysis results of the samples from the test carried out simultaneously with brushed through sections 1 to 16 of the copper rig showed that the rig represented a dynamic situation in which biofilms developed and declined rapidly over time. Parts of these biofilms may have also sheared off and may have colonized other parts of the rigs, which emphasizes the potential complexity of biofilms and microbial activity in real water systems.

The highest *Legionella* count was found in brushed through section 10 that had been in the copper rig for 353 days. This may have been because biolayers had developed upstream this section, which had sheared off and re-formed in section 10, therefore, providing better conditions for *Legionella* to thrive, and suggested local interaction between biofilms. The drop in *Legionella* counts in section 3, 2 and 1 that had been in the copper rig for the longest (496, 539 and 547 days) may have been because

biofilms had sheared off and settled elsewhere in the rig, therefore, providing less favourable conditions for *Legionella* to grow in these sections.

Because water was circulated through the rigs continuously at a relatively high rate, and because 3 litres of water were drawn off and 3 litres of fresh water were added to the rigs daily, dispersion of biofilms most likely occurred frequently, which is supported by the variability in the TVC and *Legionella* results.

Less *Legionella* were found in the samples taken from the copper rig A than in the polyethylene rig A after rigs A were used to inoculate rigs B and C. This could be explained by the removal of sections and water from rigs A for rigs B and C and by doing so a substantial amount of the established biofilms that had formed beforehand in the copper rig A was removed from the rig. The conditions within the copper rig A may have, therefore, become less favourable for *Legionella* to grow.

Although a superior biocidal action of copper compared to polyethylene was not demonstrated in the initial experiment with rigs A, less *Legionella* were found in the copper rigs B and C than in the polyethylene rigs B and C. The *Legionella* counts found in polyethylene rigs B and C were ~473% higher than those found in copper rigs B and C.

Interestingly, the *Legionella* found in all rigs during this study, *L. pneumophila* serogroup 2-14, were different from the species introduced to the rigs, *L. pneumophila* serogroup 1 and *Legionella* non-*pneumophila*. This suggested that the local environment in both rigs promoted the growth of *L. pneumophila* serogroup 2-14, and that the inoculum introduced could have contained a mixture, including serotypes not readily detectable in the inoculum at the initial stages.

5.3 Comparison experiment with rigs

The *Legionella* colony forming unit counts also reduced considerably whilst copper and silver ionized water was added daily to the rigs A for 83 days, ~99% in the polyethylene rig, and ~98% in the copper rig. This was achieved at reduced temperatures, average 44ºC in the polyethylene rig and 42ºC in the copper rig, with an average of 0.042mg/l silver and 0.866mg/l copper in the polyethylene rig, and of 0.031mg/l silver and 0.874mg/l copper in the copper rig.

The *Legionella* results of the samples taken from the brushed through sections during treatment with copper and silver ionized water, were, however, inconclusive because no results before treatment were available for the same sections analyzed, and variability of the TVC and *Legionella* results, found previously, had highlighted that biofilms may have been unstable because of frequent dispersion. Biofilms may also have been removed from the rigs by draining 3 litres of water from them daily.

Before copper and silver ionized water was added to the polyethylene rig, *Legionella* counts found in the samples of the brushed through sections were:
Section 19, in place in rig since design stage, 5600CFU/l
Section 18, in place in rig for 79 days, 0CFU/l
Section 17, in place in rig for 114 days, 13600CFU/l.

The *Legionella* counts found in the samples of the polyethylene rig brushed through sections during treatment were:
Section 16, in place in rig for 133 days, day 13 of trial, 2900CFU/l
Section 14, in place in rig for 175 days, day 55 of trial, 30400CFU/l
Section 13, in place in rig for 203 days, day 83 of trial, 0CFU/l.

Before copper and silver ionized water was added to the copper rig, *Legionella* counts found in the samples of the brushed through sections were:
Section 19, in place in rig for 58 days, 6700CFU/l
Section 17, in place in rig for 79 days, 1500CFU/l
Section 16, in place in rig for 114 days, 800CFU/l.

The *Legionella* counts found in the samples of the copper rig brushed through sections during treatment were:
Section 15, in place in rig for 133 days, day 13 of trial, 200CFU/l
Section 14, in place in rig for 175 days, day 55 of trial, 6600CFU/l
Section 13, in place in rig for 203 days, day 83 of trial, 900CFU/l.

A peak *Legionella* colony forming unit count, of 59200CFU/l, found in a sample taken from the copper rig on day 55 of treatment, and the reduction in counts thereafter, suggested that biofilms may have been broken up by the copper and silver, dispersing significant numbers of *Legionella* into the water. Too many *Legionella* may have been dispersed to be controlled by the available silver and copper at that time.

As expected, the *Legionella* populations of rigs B, where temperatures were maintained below 46ºC, without copper and silver addition, increased. More *Legionella* were, however, found in the copper rig than in the polyethylene rig, which was surprising due to the biocidal property of copper against *Legionella* cited in previous work (Schofield and Locci, 1985, Keevil *et al.,* 1993, BSRIA, 1996, van der Kooij *et al.,* 2005). The conditions in the copper rig were possibly more favourable for *Legionella* growth than the conditions in the polyethylene rig. Less *Legionella* were, however, found in a sample of a brushed through section that had been in the copper rig for 203 days but these results were inconclusive because, as suggested previously, biofilms may also have been actively dispersing, and may have been removed from the rigs by draining the rigs' water daily.

The study with the rigs C was conducted to establish the efficacy of controlling *Legionella* by temperatures of 50ºC as this is the control modality adopted by most UK building owners in response to the recommendations in the ACoP (L8) and 04-01 documents. The results of the *Legionella* analyses of rigs C showed, however, that less control was achieved compared to in rigs A in which copper and silver ionized water was added daily.

The results of the 10 hospitals studied already highlighted that the temperature regime, recommended in the ACoP (L8) and HTM 04-01 documents, did not completely control *Legionella* in the water systems of the hospitals. The large number

of contaminated hot outlets that were blended by cold water to avoid scalding, which is a requirement when the temperature regime is applied as a control modality, suggested that *Legionella* proliferation could actually be promoted by applying the temperature regime. This was also supported by the high *Legionella* colony forming unit counts found in the samples taken from rigs B, in which non-scalding water temperatures were maintained.

A greater reduction in the *Legionella* colony forming unit counts, of 76%, was found in the copper rig C than in the polyethylene rig (48%). Less *Legionella* were also found in the samples taken from the 3 brushed through copper sections, although these results were again inconclusive.

It was impossible to maintain the ACoP (L8) and HTM 04-01 documents' recommended temperatures consistently at all outlets throughout the 10 hospitals, and it was also difficult to maintain the temperatures of rigs C above 50ºC. The temperatures of rigs C also fluctuated, similar to the hot water temperatures of the 10 hospitals studied.

The *Legionella* results of the samples taken from rigs C also demonstrated that even when a temperature of 50ºC, and above, is maintained, *Legionella* is not controlled. This was also observed in the study carried out with the 10 hospitals and from data gathered from other sites.

Because the study with the 10 hospitals showed that control by copper and silver ionization of the *Legionella* was maintained, it could be assumed that copper and silver ionization also controlled biofilms as biofilms are the breeding grounds for *Legionella*.

Control of biofilms by copper and silver ionization was also demonstrated by the BSRIA project of 1996. It was demonstrated that, where operated at concentrations of 0.4mg/l copper and 0.04mg/l silver, copper-silver ionization was effective for the control of biofouling. Biofilms were analysed in the copper pipework circuits and the glass reinforced plastic (GRP) tanks by removing small copper coupons from the copper pipework circuits and GRP coupons from the tanks. These coupons were covered with biofilms and disinfected by copper-silver ionization. The cistern GRP

and cold water circuit copper coupon samples of the rig that was supplied with hard water demonstrated a 30% drop in biofilm coverage on commencement of disinfection. After 14 days of disinfection, the percentage coverage on the surface of the hot water circuit copper coupon was reduced to less than 5%. The percentage coverage on the cistern GRP coupon sample began, however, to increase again to 30% after 21 days. The authors suggested that biofouling returned on the cistern GRP coupon because it was difficult to maintain silver concentrations above 0.01mg/l due to scaling of the silver electrodes, which obstructed the release and presence of silver. Copper-silver ionization disinfection resulted in a rapid decrease in biofilm coverage on the cold water copper coupon samples of the rig that was supplied with softened water, from over 50% to less than 5%. A more gradual constant decrease was noted, to less than 10%, on the cistern GRP and the hot water copper coupons (BSRIA TN6/96).

Shih and Lin (2010) also demonstrated that copper-silver ionization was effective in controlling biofilms. A model plumbing system, consisting of four transparent PVC biofilm sampling pipes, was designed. A 14-day inoculation period was followed by 120-hours disinfection. The inoculum solution consisted of a bacterial suspension (3 x 10^6cfu/ml) of environmental isolates of *P. aeruginosa, S. maltophilia,* and *A. baumannii*, which are biofilm-associated sessile pathogens. Copper-silver concentrations were maintained at 0.2mg/l copper and 0.02mg/l silver to 0.8mg/l copper and 0.08mg/l silver and achieved complete inactivation of the biofilm-associated *P. aeruginosa* within the first 24 hours. Biofilm-associated *S. maltophilia* was completely inactivated in 48 hours, and 99.9% of biofilm-associated *A. baumannii* was killed in 12 hours (Shih and Lin, 2010).

Whether or not control of biofilms was achieved by copper and silver ionization was, however, inconclusive in the study carried out with the rigs A because biofilms were potentially unstable and moving through the pipe sections, and because biofilms may have been removed from the rigs A by draining 3 litres of water from them daily this was considered essential to maintain uniform conditions in the rigs.

Although superior biocidal action of copper was observed in studies conducted previously (Schofield and Locci, 1985, Keevil *et al.,* 1993, van der Kooij *et al.,* 2005),

this was not seen in the studies carried out with the rigs as more average *Legionella* colony forming unit counts were found in the copper rigs A and B than in the polyethylene rigs A and B.

Although superior biocidal action of copper was not demonstrated by the studies with rigs A and B, more *Legionella* control was, however, found in the study with copper rig C, which could possibly be due to the superior biocidal action of copper cited in previous work (Schofield and Locci, 1985, Keevil *et al.,* 1993, BSRIA, 1996, van der Kooij *et al.,* 2005).

5.4 The Robbins device experiment

Experiments with the Robbins device were conducted to clarify the results of the studies with rigs A and B, which did not demonstrate the superior biocidal action of copper cited previously in other work (Schofield and Locci, 1985, Keevil *et al.,* 1993, BSRIA, 1996, van der Kooij *et al*, 2005). The Robbins device offered a simplistic means to observe in a short time whether or not bacterial growth was less on copper than on polyethylene.

Tests 1, 2, and 3 with the Robbins device were conducted by releasing copper and polyethylene discs in 10ml distilled water and shaking the samples for 30 seconds. These tests showed more bacterial growth in the copper disc samples than in the polyethylene disc samples after 24 hours, and, therefore, supported the findings of the studies with rigs A and B. The rougher surface of the copper discs may have given a better environment for microbial attachment and for biofilm formation than the smoother surfaces of the polyethylene discs.

The Robbins device was also used to observe bacterial growth on rubber discs that were cut from the Ethylene Propylene Diene Monomer (EPDM) rubber inners of a flexible hose, commonly used in UK water systems.

Tests 1, 2, and 3 with the Robbins device were conducted by releasing also these rubber discs in 10ml distilled water and shaking the sample for 30 seconds. These tests showed greater growth in the rubber disc samples than in the copper and polyethylene disc samples after 24 hours.

Adhesion and biofilm formation on materials used for medical devices, including silastic rubber, has been studied in the past using a Robbins device. Jass *et al.,* (1995) used a chemostat coupled Robbins device to monitor the colonization of *P. fluorescens* and *P. putida* on silastic rubber surfaces (Jass *et al.,* 1995).

Greater bacterial growth on rubber, natural and synthetic, materials was has also been demonstrated in other studies (Schofield and Locci, 1985, Keevil *et al.,* 1993, Rogers *et al.,* 1994). These studies did not use a Robbins device and were conducted with coupons of rubber inserted in complex model water systems.

Mats of cells and slime-like debris was heaviest on rubber and least on copper materials of the model hot water system built to examine colonization by *L. pneumophila*. Abundant growth of *L. pneumophila* on rubber was also observed. Polyethylene was not tested in this study (Schofield and Locci, 1985).

Keevil *et al.,* (1993) found that the most prolific biofilms developed on the surface of elastomeric materials. The biofilm covered the entire elastomer surfaces after only 24 hours and contained more than $8.9x10^6$cfu/cm^2 on latex and $2.7x10^6$cfu/cm^2 on ethylene propylene rubber. The highest populations of *L. pneumophila* were found on latex and ethylene propylene rubber as well. Copper and polyethylene were also tested in this study (Keevil *et al.,* 1993).

Rogers *et al*., (1994) also investigated biofilm growth and *L. pneumophila* colonization on latex, ethylene-propylene rubber, and polyethylene, and found that latex and ethylene propylene rubber were also most heavily colonized with *L. pneumophila* (Rogers *et al.,* 1994).

Tests 4 were conducted with the Robbins device to also compare bacterial growth on the copper, polyethylene, and rubber discs but by brushing the discs in 10ml distilled water instead of shaking them in 10ml distilled water for 30 seconds. These tests showed that most viable bacteria were released from the rubber discs but that more viable bacteria were released from the polyethylene discs than from the copper discs, which could be explained by the biocidal action of copper or by that not all viable bacteria were removed from the copper discs through brushing them. The surfaces of copper are rougher than the surfaces of polyethylene, and Donlan, (2002), had reported that surface roughness of materials can allow microbes to reside in a protected area (Donlan, 2002).

As with the studies with rigs A and B, the findings with the Robbins device also did not demonstrate the superior biocidal action of copper but indicate that the surface roughness of copper may provide a better surface for bacterial attachment and growth than the smoother surfaces of polyethylene.

That rubber encourages bacterial growth was demonstrated by the Robbins device experiment. As this could enhance the risk of harbouring *Legionella*, rubber lined flexible hoses and other synthetic rubber materials used in water systems should be identified and assessed for the possibility of contamination by *Legionella.*

To summarize, this thesis set out to investigate microbial control using copper and silver ionization systems.

The results demonstrated that the temperature regime as described in the ACoP (L8) and the HTM 04-01 documents, of obtaining above 50°C after a 1 minute run of hot outlets and below 20°C after a 2 minutes run of cold outlets, which was the *Legionella* sole control modality applied to the water systems of 10 hospitals in the UK, did not achieve complete control of *Legionella*. *Legionella* were not completely controlled at hot outlets at which temperatures were maintained above 50°C and also not at cold outlets at which temperatures were maintained below 20°C. A major issue was seen when the temperature regime was applied in the hospitals studied because temperatures needed to be kept at most outlets below 46°C to avoid scalding. The results of the samples taken from outlets at which hot water was deliberately blended by mixing valves with cold water demonstrated that a considerable number of blended outlets were contaminated with *Legionella.*

Legionella were also not completely controlled in the copper and polyethylene rigs, designed to simulate a typical hot water system of a small UK hospital, in which water is stored, heated, and circulated. Furthermore, the results of the copper and polyethylene rigs, in which temperatures were deliberately maintained below 46°C, to simulate blended water, demonstrated that *Legionella* actually proliferated at these temperatures.

On the other hand, the results of the samples taken from the hospitals demonstrated that substantial *Legionella* control was maintained by copper levels ranging from 0.151mg/l to 0.539mg/l and silver levels ranging from 0.021mg/l to 0.043mg/l, and in the presence of chloride concentrations ranging from 8.4mg/l to 60.7mg/l, phosphorus concentrations ranging from 191µg/l to 2100µg/l, and a pH ranging from 6.9 to 8.5. *Legionella* were also substantially controlled in the copper and polyethylene rigs to which copper and silver ionized water was added daily for approximately 3 months.

Surprisingly, the results of the studies with copper rigs A and B did not demonstrate the superior biocidal action of copper. However, the Robbins device results of the brushed copper, polyethylene and rubber discs did show greater bacterial growth on rubber and polyethylene than on copper.

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