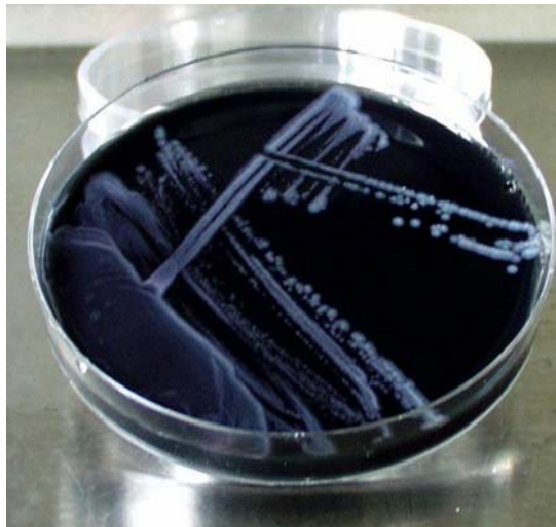


# TECHNICAL CUSTOMER INFORMATION

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## TI-K 0-5 E

### LEGIONELLA PNEUMOPHILA LEGIONAIRES PATHOGENS (LEGIONELLOSIS, LEGIONNAIRES' DISEASE)



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## 1. General Information

*Legionella pneumophila* was first identified in 1976 as the cause of an atypical pneumonia, so-called **Legionnaires' disease**, at a American Legion meeting of US war veterans in Philadelphia. In addition to this severe type of lung infection, with a fatality rate of approximately 15 %, legionellae can also cause influenza-like Pontiac Fever.

**Classical Legionellosis** develops 2 - 10 days after infection, with symptoms of a general feeling of being unwell, limb, muscle and headache, confusion, respiratory distress, a dry cough, shivering and increased body temperature (39 - 40.5 °C). As a rule the disease, which lasts several weeks, is characterised by a particularly aggressive type of pneumonia, lacking evidence of the normal pneumonia pathogens. Treatment with various antibiotics is possible, although controlled studies of their effectiveness have yet to be published [1].

**Pontiac Fever** is characterised by a short incubation time (1 - 2 days) and a milder progression of the illness, which begins with head and limb ache, coughing, fever with shivering, and a general state of confusion. Despite feeling unwell, patients generally fully recover after 5 days. To date, no fatalities have been recorded for this disease.

As a rule, more men contract the disease than women. Ill, elderly people with diminished bodily resistance (immuno-suppressed) and heavy smokers are particularly susceptible. Patients with existing lung damage and diabetes are at especially high risk, and therefore legionellae infections frequently occur in hospitals (**hospital-acquired**). The fatality rate for untreated immuno-deficient patients can reach up to 80 %. In Germany, it is estimated that between 6,000 and 10,000 cases of legionellae pneumonia occur each year, and some 1 - 5 % of hospital-treated pneumonia are diagnosed as legionellosis [1].

## 2. Pathogen

Legionellae belong to the family Legionellaceae, genus *Legionella*. They are found in water as live motile Gram-negative non-sporogenic rod-like bacteria (0.3 - 0.9 µm x 2 - 20 µm or longer), with one or more polar or sub-polar flagella. All legionellae are potentially human pathogens. *Legionella pneumophila* (approx. 90 %) is the most important medically, and includes 14 sero-groups, of which groups 1, 4, 6 are the most significant. In total there are more than 40 species of *Legionella*, including more than 60 sero-groups [1]. In obligate aerobic metabolism, amino acids are used as carbon and energy sources. Carbohydrate can not be utilised.

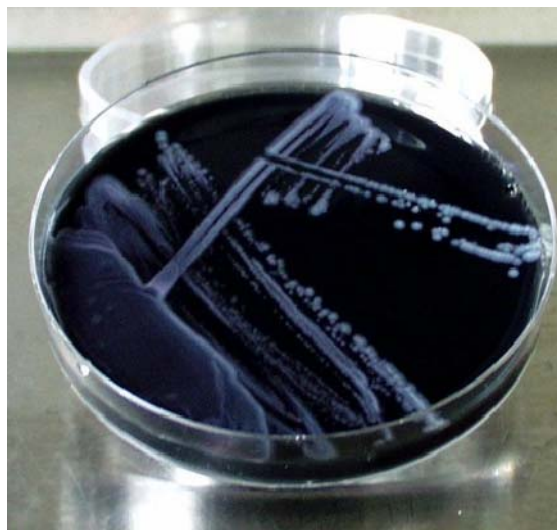


Fig. 1: *L. pneumophila*, sero-group 1

Detection by quick microbiological testing is currently inadequate, requiring instead time-consuming culturing of legionellae on special agar. As legionellae have yet to be isolated from healthy persons, a positive culture always indicates a Legionella infection. Positive RIA or ELISA assays for Legionella antigens in urine also indicate infection [1, 2], only reacting with sero-group 1 antigens, and occasionally cross-reacting with other sero-groups. Direct identification of pathogens from sputum, tracheal secretions and contaminated water is also possible using direct fluorescent serological techniques (DFA) [3], although the sensitivity of this method is relatively low. The value of diagnosis by antibody detection by indirect immunofluorescence tests (IIFT) is only of retrospective value, since the indicative titre increase in serum antibodies is often only reached in the 6th - 8th week of illness. Detection of Legionella DNA by PCR, or other amplification techniques, is also possible [1], although their sensitivity and specificity has yet to be evaluated.

### 2.1 Routes of Infection and Incidence

Based on world-wide studies, it is currently thought that legionellae naturally occur in all bodies of freshwater, but not in sea water, including:

- main water supplies, warm water supplies
- (e.g. blocks of flats, hospitals, hostels, homes, hotels)
- surface waters (especially heated rivers, lakes, ponds)
- cisterns
- damp floors
- swimming pool and whirl pool water, warm water boilers
- cooling water, cooling towers
- water spray of air-conditioning system humidifiers
- (ventilation and air-conditioning systems)
- dental practices (turbo drills, rinse water).

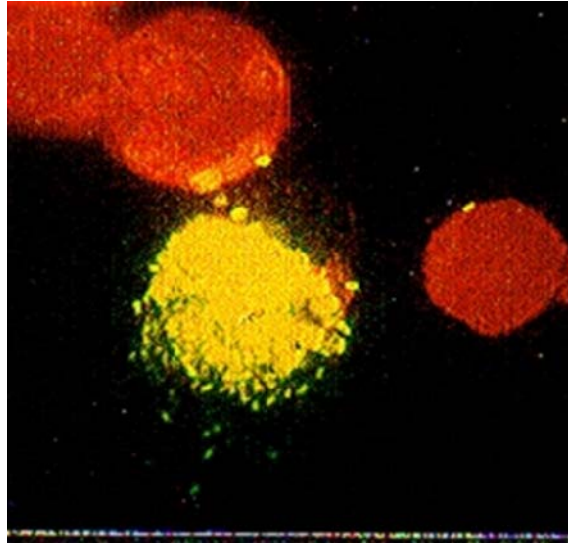
A prerequisite for legionellae is increased water temperature. In nature living legionellae multiply optimally between 25 - 55°C (the so-called risk range), at pH values of 5.5 - 9.2 and dissolved oxygen concentration of 6.0 - 6.7 mg/l [1, 4]. Nevertheless, legionellae have been isolated from water systems ostensibly maintained at 55 - 60°C, where the piping is convoluted thus preventing good water mixing, described in studies on large buildings and hotels in the USA and England [5, 6], drinking water systems in England [7], and domestic warm water supplies in Germany [8]. In Germany, Legionella bacteria have been detected in mains water in West-Berlin and North Rhine-Westfalia [9, 10]. The bacteria can also occur in cold water, although they cannot multiply to any appreciable degree, such that ground water and cold drinking water (below 18°C) support hardly any legionellae. Ideal conditions for the multiplication of legionellae often exist in surface water supply systems, e.g. in pipes and plumbing, air-conditioning. Increased risk of legionellae occurs in old and poorly maintained, or intermittently used water supply systems and storage tanks (multiplication due to long standing times!).

Legionellae in water itself pose no direct risk to health, which is why the pathogen is classed as facultative. The inhalation of a large number of the bacteria as water aerosols (e.g. when showering, in air-conditioned rooms or in whirlpools) can result in illness. The infectious dose depends on the individual's constitution and the virulence (potential for causing illness) of the Legionella strain. To date, direct infection from person to person has not been reported.

Unlike in Spain, compulsory registration of the disease was not required by the German epidemic laws. However, from January 2001 an Infektionsschutzgesetz (protection against infectious diseases law, IfSG, 3. clause, § 7) requires registration (including diagnostic laboratories) of a confirmed Legionella infection.

In water, legionellae multiply intracellularly in amoebae and other protozoa [11, 12]. Infected amoebae are especially significant in the transfer to man, since legionellae activate their virulence gene in the host. Infection via infected amoeba explains the well-known dose effect associated with outbreaks of legionellosis (no infection despite contaminated water system, or infection despite low Legionella contamination). Some 13 species of amoebae and two species of ciliates have been identified as hosts for Legionella pneumophila, mostly commonly Hartmannella

vermiformis and Acanthamoeba castellanii: both have been shown to support increased virulence in *L. pneumophila* [13]. Interestingly, *L. pneumophila* shows a very wide range of hosts, including human macrophages. Recently, in addition to legionellae able to multiply on artificial media, numerous LLAP organisms (legionella-like amoeban pathogen) have been identified, many of which are non-cultivable and possibly can survive as true endosymbionts in amoebae. The ability of pathogenic bacteria to multiply within amoebae is a wide-spread and currently underestimated phenomenon. In evolutionary terms, the association of bacteria and amoebae is credible, as the host's intra-cellular compartment protects against growth-inhibiting environmental influences such as bactericides or antibiotics.



**Fig. 2: *L. pneumophila* multiplication in amoebae**

An additional feature of Legionella ecology is that bacteria (together with amoebae) occur in biofilms, where they can form up to 10% of the total microbe population [14]. Biofilms are microbial slime layers that coat the inner or outer surface of a water system. They are a potential nutrient media for legionellae and present difficult problems for the disinfection of such systems in that they absorb the biocide, de-activating it, or inhibit its penetration. There are numerous chemical, physical and enzymatic methods for tackling the problem, unfortunately often yielding poor results. In biofilms, legionellae can reach a life-phase in which they can survive, but are nonculturable and therefore undetectable. In amoebae, the bacteria switch to a normal growth phase and multiply on artificial nutrient media [15].

In addition to amoebae and biofilms, other factors influence the colonisation and survival of legionellae in water systems: other species of bacteria (antagonists or synergists) temperature, pH value, oxygen partial pressure, calcium deposits (calcium carbonate), algal growth and the material properties of the water system. Corrosion is suspected of encouraging legionellae growth, whereby iron ions released may be crucial for the growth of legionellae [4]. Given the variety of such factors, it is perhaps not surprising only a fraction Legionella ecology is known to date.

### **3. Source of Infection**

#### **How can infection be prevented?**

The prevention of legionellosis has to be based on two core strategies: first is prevention of a massive build-up of microbes in warm water, aerosol-forming systems; second limiting or preventing contact with aerosols. In the following section, various technical systems are described (2.1) that have been shown to be involved in the transmission of legionellosis and Pontiac fever.

### **Warm water supplies**

According to studies by the State Medical Investigation Authority in Braunschweig [16] and other sources [8], the most dangerous source of infection is warm water supplies: Legionella was detected in all the drinking water system biofilms investigated [17]. The epidemiological significance of legionellae in (warm) water supplies is unequivocally related to the prevailing water temperature. The risk range is between 25°C and 55°C: with temperatures less than 20°C and greater than 60°C this significance does not exist.

The incidence of legionellae has increased dramatically in recent years due to energy-saving measures stipulating a reduction in water temperatures below 60°C, as in the USA. According to the "Accreditation Manual for Hospitals" [18], water temperatures in hospitals were set at 110-113°F (= 43-45°C). After numerous cases of Legionnaires' disease, these regulations were rescinded [18, 19].

### **Ventilation and air-conditioning systems**

In the 1976 Philadelphia outbreak, legionellae was transmitted via the air-conditioning system humidifying water and thus into air in the hotel where the meeting took place. The risk of infection via air-conditioning humidifying systems is particularly high if the water is lukewarm or warm and atomised in circulation, and not conditioned. For this reason the VDI directive 6022 [20] for humidifying water in AC systems restricts the total permissible microbe content to 1000 CFU (Colony Forming Units)/ml. The limit for total legionellae is only 1 CFU/ml, and such systems must be subject to a microbiological hygiene inspection every two years.

### **Whirlpools, showers**

A further source of infection are warm water jet systems with aeration heads, and particularly showers and whirlpools. Infection occurs by inhalation of Legionella-infested aerosols. In recent years, the increased popularity of whirlpools has been accompanied by some spectacular outbreaks of Legionella infections, such as one in 1999 in the Netherlands, where *L. pneumophila* infections were detected in 226 of the some 8000 visitors to a whirlpool exhibition, 18 of whom died as a result of the infection.

### **Dental units, humidifiers in the home**

In the case of dental equipment, the problem of microbial contamination with other types of bacteria, as with whirlpools, has been known for some time. Here equipment and materials should be replaced as recommended and, if applicable, microbicides of proven effectiveness deployed. For domestic appliances producing aqueous aerosols (air humidifiers, mouth washers, inhalers etc.) it is essential that they are regularly and thoroughly cleaned. At critical temperatures, the water should not be allowed to stand, but be regularly changed and appliances not in use maintained dry.

### **Cooling water, cooling towers**

Under certain conditions, cooling tower steam and condensate are a potential source of danger. Comprehensive emissions investigation programmes detected legionellae in virtually all cases [4]: including both force-ventilated and naturally-ventilated cooling towers. A particularly large accumulation of legionellae was found in the diffusion zone in force-ventilated cooling towers: steam condenses on the ventilator blades, drips back down the diffuser and is carried back up by the air stream, circulation which results in an increase in legionellae concentration after each cycle due to the evaporation of water.

Few reports exist that link illness with the operation of wet cooling towers. Two publications report Legionella infections in power stations. In one case [21], it concerned a small reverse cooling plant in a compressor house in the grounds of a power station -thus not linked with the naturally-ventilated wet cooling tower. In the other case [22], the cause of the infection was the highly suspect works practice of cleaning a steam turbine's piping with high-pressure hoses, without adequate respiratory protection. Infection by legionellae in this area has to be reckoned with and for cleaning teams, a face mask is strongly recommended.

Current knowledge indicates that the spread of infection over great distances, as aerosols, cannot be ruled out, even if to date no concrete evidence has been produced. The low risk of infection (for the surrounding area and population) from wet cooling towers is, however, only the result of taking into account the specific criteria which comprise basic legionellae ecology [4, 23]. Only low numbers of Legionella present a low infection risk.

#### 4. Fighting Legionellae

##### 4.1 Increased water temperature

German investigations [16] and other sources [24] have shown that water systems maintained at a constant temperature of 60°C do not support Legionella colonies. However, 60°C alone is not sufficient to eradicate the microbe if it has already established itself in the system. Observations on the ability of legionellae to multiply at nominal water temperatures of 70°C indicate it is unlikely all parts of the water system are heated to the intended temperature. Several factors, such as sediment, wall incrustation or dead-end pipes may constitute so-called 'blind spots' in which the lethal temperature is not reached, and which, in the event of a temperature drop may lead to recontamination of the entire system [24]. Multiple flushing with water above 70°C, often recommended, does not eliminate legionellae from the system.

##### 4.2 Microbicides

Tackling Legionella bacteria in water from the circulatory spray humidifiers in air-conditioning and cooling systems would appear to be urgently necessary. This is possible using microbicides developed by modern chemistry over the past few years. A significant problem is the reduced efficacy of many biocides against biofilms, which by dissipating the biocide, protecting the target micro-organisms, hinder optimal biocidal effectiveness [25].

In tests on cooling tower water, N-heterocycles has been shown to be very effective in very low doses against Legionella pneumophila and Legionella gormanii.

The BKG Water Solutions products Ferrocid 8583 and Ferrocid 8599, which contain the active agent mentioned above can be used to combat legionnaires' pathogens in cooling towers, cooling water and the humidifying water of air-conditioning systems, such that a germ reduction of several orders of magnitude can be expected. The use of Ferrocid 8599 for air-conditioning system water reservoirs at the concentration recommended poses no threat to human health [26].

In a modified quantitative suspension test (following DIN prEN 13623), different concentrations of Ferrocid 8580 were checked for their bactericidal efficacy against Legionella pneumophila sero-group 1. All test concentrations and tested pH values showed complete elimination of the germ after one hour. Growth was only observed after 5 minutes at the lowest concentration of Ferrocid 8580 used (10 g / m<sup>3</sup>). Thus, according to the modified DIN prEN 13623, Ferrocid 8580 can be considered as an effective bactericide against Legionella pneumophila sero-group 1 [27].

The effect of Ferrocid 8580 on legionellae in biofilms was investigated in different real-life simulation tests [28]. With pure culture biofilms of Legionella pneumophila on membrane filters and cultured mixed biofilms (Legionella pneumophila and other organisms), with a experimental flow velocity of 0.5 m/sec, significant lethality was observed. Legionella pneumophila was partly quantitatively killed. In the latter investigations, after the addition of Ferrocid 8580, over 90 % of bacteria were eliminated from the biofilm, a result of considerable technical significance, indicating that under these conditions biofilm cells can be effectively eliminated from water systems. Statements of efficacy of solid HOBr-splitting, organic halogen compound regarding legionellae is still open to question, as shown by practical experiments by a US working group [29]. Other studies [30] show varying effectiveness of HOBr-splitting, organic compounds against Legionella, but not against cooling water amoebae.

Experience with chlorine is also poor. As Best et al. (1983) showed [31], even with the higher chlorine content of US drinking water (up to 0.5 ppm free chloride), legionellae were detectable. In the warm water system of Kingston Hospitals (Surrey, UK), even a chloride concentration of 50 ppm (!) for 24 hours, was not enough to kill off particularly resistant legionellae. Legionella in Acanthamoeba cysts are resistant to 50 ppm chloride [32]. Success with chloride against legionellae not associated with amoebae or in biofilms are, however, generally achieved with chloride concentrations of 2-6 ppm free chloride [33].

As an alternative to chlorine disinfection, copper and silver ions are often used in conjunction with free chloride [33]. Although copper ions in particular reduce Legionella, this method fails in the presence of amoebae [34].

### 4.2.1 Handling Precautions for Microbicides

Use biocides safely. Always read the label and product information before use.

### 4.3 Preventative Measures

Due to the difficulty of fighting legionellae in amoebae and biofilms, the following measures, described in detail in [35, 36], are recommendable:

- Avoid water temperatures ranging from 25°C to 55°C.
- Avoid lengthy standing times for water - biofilms can develop and thus encourage legionellae.
- Avoid 'dead ends' in the distribution systems.
- Routine cleaning and chlorination every six months for vaporisation cooling systems.
- Thermal sterilisation every six months for warm water heaters and hot water distribution systems.
- Maintain the cleanliness of cooling systems by use of a routine water treatment program.
- Routine inspection of cooling systems for microbial contamination.
- Avoid materials that encourage colonisation and growth, and employ easy-to-clean constructions.
- Avoid the introduction of substances that encourage the growth of bacteria.

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6. Glossary

aerobic	oxygen requiring
anaerobic	does not require oxygen
algae	uni- or multicellular aquatic plant group
bactericide	kills bacteria
ciliates	highly differentiated protozoa
DFA	direct fluorescence antibody method
ELISA	<u>E</u> nzyme <u>l</u> inked <u>i</u> mmuno <u>s</u> orbent <u>a</u> ssay
endosymbiont	mikro-organisms that live in eukaryote cells
eukaryont	organism, whose cells have a distinct nucleus (= protozoa, moulds, plants, animals)
facultative	selective, not exclusive
Gram	characterisation for a particular bacteria, based on the cell wall structure
contamination	infestation, e.g. with micro-organisms
makrophages	free-ranging immune system cells, that ingest particulate matter and micro-organisms
µm	micrometer, 10 <sup>-6</sup> m, 0.001 mm
mikrobicide	kills micro-organisms
micro-organisms	life-form, only visible with a microscope
pathogen	agent that causes disease
PCR	<u>P</u> olymerase <u>C</u> hain <u>R</u> eaction
protozoen	eukaryote life-form, mostly animal
RIA	<u>R</u> adio- <u>I</u> mmuno- <u>A</u> ssays
cysts	quiescent phase of protozoa

**Table 1: Ferrocid and its properties**

Product	Ferrocid 8580	Ferrocid 8583	Ferrocid 8599
<b>Area of application</b>	cooling and water processing systems	cooling cycles, cooling towers, water processing systems	circulating water in air- conditioning units (USB)
<b>Legionella effective component</b>	dibromnitripropionamide	subst. N-heterocycles	subst. N-heterocycles
<b>Product data</b>			
Appearance:	clear, colorless to amber liquid	clear, yellowish solution	homogenous, clear to slightly opaque, yellowish fluid
Density (20°C):	1.20 ± 0.02 g/cm <sup>3</sup>	1.05 ± 0.01 g/cm <sup>3</sup>	1.02 ± 0.02 g/cm <sup>3</sup>
pH-of concentrate:	approx. 4	4.8 ± 0.2	9.2 ± 0.2
Viscosity (Brookfield, 100 UPM; 20°C):	approx. 45 mPa s	14 ± 5 mPa s	13 mPa s
Miscibility with water:		approx. 1:1	
Frost sensitivity:	from - 5°C	± 0°C	ab 0°C
Solidification point (DIN 51583):	- 20 ± 1°C	- 2°C	- 4°C
PO4-content: Flash point (DIN 51755):		0.35 ± 0.05 % 45°C	
<b>Test- concentration</b>	10 - 40 ppm (pulse dosing)	10 - 100 ppm (cycle)	400 - 600 g/m <sup>3</sup> (cycle)
<b>Killing rate Legionella pneumophila (Serogruppe 1)</b>	> 105 bacteria are killed after 10 minutes > 90 % bacteria are killed in biofilm	effective against L. pneumophila and L. gormanii	effective against L. pneumophila and L. gormanii

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