Risk assessment and monitoring of Legionella by culture and q-PCR in a newly built block of flats associated with a small outbreak of legionnaires´ disease

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Detection of *Legionella*-decontamination in water by real-time-PCR and pre-treatment with ethidium monoazide - Differentiation of viable and dead cells

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The contamination of drinking water supplies in hotels, hospitals and public buildings with *Legionella* is well known. Hospital acquired legionnaires disease is often associated with a high fatality rate. In order to suppress the multiplication of *Legionella* in these buildings technical measures and decontamination by hypoclorination or super heating are applied.

For the exact detection of the contamination the culture method is currently mostly used, but the method with the increasing impact is the real-time-PCR. Since PCR-based assays cannot discriminate between live and dead bacteria further studies are needed.

Ethidium monoazide (EMA) is a photo activated stain, which penetrates only dead bacterial cells with compromised cell walls and cell membranes. It intercalated into DNA of those cells and also in free extracellular DNA. The covalent binding of EMA to DNA is induced by photo activation. The photo induced cross linking result in the insolubility of DNA. Following this DNA can't be extracted.

For the first experiment we prepared different artificial water samples including *Legionella pneumophila* strain Corby. To get an overview of the affectivity of EMA we tested different incubation-times, different concentrations and different exposure times. In the most experiments we use 5 min EMA treatment with 50µmol and an exposure-time of 2 min. For the real-time-PCR we use a 529 bp-fragment, because statistically EMA intercalate into DNA only every 10-80bp.

We treated the water samples with heat, sodium hypochlorite, silver, formaldehyde, Ampicilin and UV-light to get a reduction of the viable bacteria.

With some of these methods a reduction of cultivable bacteria can be verified with the help of EMA, but in some others not. This effect is probably caused by the different antimicrobial mechanisms of the different decontamination-methods.

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Risk assessment and monitoring of *Legionella* by culture and q-PCR in a newly built block of flats associated with a small outbreak of legionnaires' disease

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During the investigation of a small outbreak of legionnaires' disease in a newly built block of flats, several risk factors were uncovered. The outbreak encompassed two men (age 44 and 65) of which the oldest one died. Two interventions (heat treatments of 70ºC for 12 and 24 hours respectively) and permanent changes of the flow and temperature were conducted to overcome the high concentrations of *Legionella* in the water. Water samples (104 in total, both cold and warm water) from taps and shower hoses were collected and cultivated. The samples consisted of: A samples: the first one litre, B samples: one litre collected after flushing until constant temperature and water from shower hoses at a temperature of 38ºC. The survey pointed at important risk factors 1) low temperature of the hot water in some of the most distant taps 2) low flow of the water circulating system because of too small pipe dimensions and too low pump capacity 3) high numbers of *Legionella* in the shower hoses 4) stagnancy of water before residents move in and in unoccupied apartments and 5) lack of or inappropriate control measures.

Following cultivation, DNA from all water samples was extracted by a simple method with Chelex-100. The samples will be investigated by an in-house q-PCR (Quantitative Real Time PCR). The q-PCR is a Taq-Man based assay with 5S primers detecting *Legionella* spp and mip primers detecting *Legionella pneumophila*. The two *Legionella* q-PCR assays have been validated according to the Afnor standard (T90 471) with standards calibrated according to the international standard from Legionelles centre National de Référence (SRM_LEGDNA_01 and CQE_LEGDNA_01). The results from culture (CFU/L) and PCR (GU/L) will be compared, and on this background it will be evaluated if q-PCR could have been used for fast risk assessment in the initial phase of the investigation and to follow the effect of the interventions in the later phase of the investigation.