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Concept for a portable measuring device for the quantitative detection of legionella

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Abstract. According to the drinking water regulation, drinking water systems must be tested for harmful contamination at specified intervals. To meet the legal requirements and save costs and time, a portable analysis method is to be developed. For this purpose, a concept study was conducted. It describes a portable measuring device for the quantitative detection of legionella using real-time PCR. The study includes the construction elements and conditions that are necessary for the collection, processing, and evaluation of the sample. It was found that commercially available PCR systems do not meet the size requirements and can only be represented by an in-house development. In addition, a microfluidic system must be developed that is designed for one-time use and low production cost.

1. Introduction

Legionella pose a persistent threat to health. The infections they cause can lead to severe courses and even death. Therefore, regular testing of drinking water supply systems is essential. Currently, testing for Legionella in drinking water systems is performed by a lengthy and costly microbiological laboratory test. For this purpose, trained personnel must take drinking water samples at the corresponding plant, which are then tested for microbiological contamination in the laboratory test. The legal threshold values for microbiological, chemical and indicator parameters must not be exceeded. If more than 100 legionellae are measured per 100 ml of drinking water, the threshold value is considered exceeded [1] (DVGW Website: Anlage 3 2022). In addition, test strips can be used for self-testing, which indicate discoloration above a certain threshold value of legionella. This test must be performed annually for large public facilities such as hotels and schools, and every 3 years for apartment buildings and housing associations [2].

In this paper a portable method for the quantitative detection of Legionella is presented, which is to evaluate the water sample by real-time PCR. The aim is to enable rapid on-site testing of the Legionella content, which can be carried out in a time- and cost-efficient manner.

2. Methods

In order to perform the sample analysis by real-time PCR, a defined test cycle must be followed. First, the corresponding samples of 100 – 1000 ml each must be taken at the sample valve. Then the sample must be concentrated to a defined volume with a high recovery rate. For the preparation of the PCR it is necessary to perform a live-dead differentiation to exclude the harmless dead Legionella. In addition, a defined volume must be taken from the concentrated sample and mixed with the master mix in the correct mixing ratio. Finally, real-time PCR can be performed, and an evaluation is made by the ratio of sample volume and cycle number.

3. Results and Discussion

According to the Drinking Water Ordinance, water samples must be taken at three important points. Before and after the water heating system and at the last sampling point in the building. It is assumed that a defined sample volume of 100 – 1000 ml is taken and fed into the system. Figure 1 shows the further process. First, the defined sample volume is pumped through a filter with a pore size of 0.22 or 0.44 µm and disposed of in a waste container. Then the filter cake is rinsed with sterile water in the opposite direction. The procedure is controlled by changing the flow of two valves and incorporating check valves. The concentrated sample then ends up in a buffer tank, from which another pump draws a defined quantity for the microfluidics. Here, a decision can be made between sample and air by means of two controlled valves. In addition, a defined amount of mastermix is added to the microfluidics at the ratio of the sample by another pump. The second pump can then move the sample through the microfluidic by pushing air. The pumps should not be in direct engagement with the medium so as not to affect the sample (peristaltic pumps). The materials that are in direct engagement with the sample should be at least bioinert and must be replaced or cleaned after each procedure.

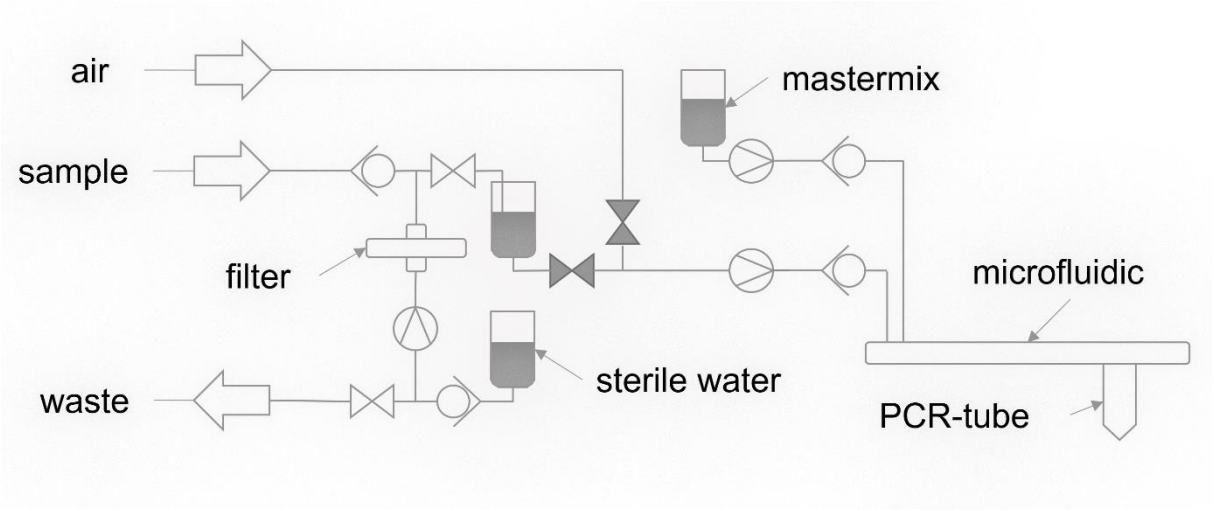

Figure 1: Concept method for sample reduction and mixing with the mastermix using microfluidic.

The microfluidics has the task to mix mastermix and sample and to enable the live-dead differentiation. It must also transfer the sample into the PCR. Figure 2 shows two chips that can perform this function. Both have sample and master mix inlets at the bottom and a prechamber for dispensing both volumes. This is followed by a cavity for mixing the media and a device for inserting a PCR tube. The chips are intended for single use and should therefore be manufactured by suitable processes (injection molding). They should also be made of a biocompatible material such as polycarbonate to avoid contaminating the sample.

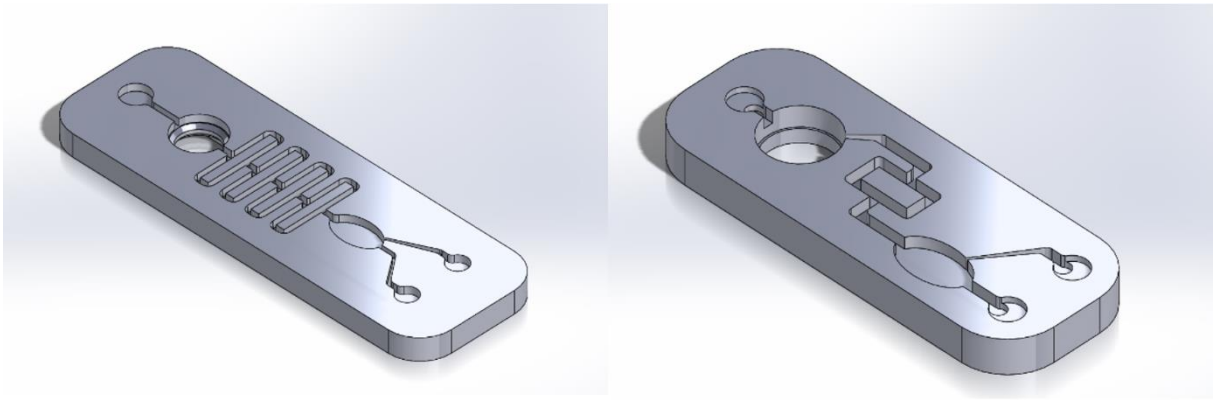

Figure 2: Microfluidic chip for mixing sample with mastermix and PCR-tube attachment.

Once the sample is in the PCR tube, amplification of the Legionella contained in the sample can be performed. This is then evaluated by fluorescence spectroscopy. The behavior of the entire system must be considered when calculating the amount of Legionella. For an accurate determination, the original sample volume, the recovery rate of the concentration and any dead volumes must be known. If PCR is performed with a reference and negative control, the Legionella content of the collected sample can be calculated from this. For the implementation of a portable device, a PCR would have to be developed which fits into a smaller scale and contains only the necessary components for the measurement of Legionella.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that there are no conflicts of interest.

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