Sevenfold Higher Recovery of Microorganisms Retained from Liquids Using a Novel Dissolvable Filter Technique

Application Note

Detection of low concentrations of Microorganisms present in liquids can be difficult if a rapid detection method like PCR is used for analysis. This study evaluates the DNA isolation efficiency from water samples spiked with *Legionella pneumophila* using a novel technique from the Microsart® Geneprep kit compared to the commonly used method. Commonly, the liquids are filtered and retained microorganisms are washed off from the filter for further DNA Extraction and PCR analysis. The recovery rate of the retained microorganisms is highly depending on the efficiency of the washing step and is most often unsatisfying, leading to questionable results of the subsequent analysis. Using the novel Membrane-dissolving technique of the Microsart® Geneprep kit, a 100% recovery rate leading to a sevenfold higher sensitivity in detection of spiked *Legionella pneumophila* in water samples can be demonstrated in this study.

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I. Introduction

Legionella are commonly occurring pathogenic bacteria, found in both natural and artificial water systems causing serious lung diseases. Especially in man-made water systems like warm water distribution tubings or cooling towers *Legionella* can proliferate massively leading to sporadic diseases or even outbreaks. Cooling towers are common equipment used in refrigeration devices on industrial sites or large public buildings like hospitals. A favorable environment for *Legionella* growth is tempered water between 20 and 45°C containing nutritional components like sediments, scales, sludges or biofilms, which often occur in man-made water systems.

There are two most commonly used methods to detect *Legionella* contamination in water: (1) the classical membrane filtration method with subsequent colony counting and (2) Nucleic acid amplification technique (NAT) with DNA based testing by Polymerase Chain Reaction (PCR). The membrane filtration method with subsequent colony counting requires an incubation time of at least five days (see Fig. 1). Cultivation time is the bottleneck using classical cultivation as detection method. Furthermore single cell fluorescence based assays (for example fluorescence microscopy) demonstrated that plate count method underestimates the real cell number by several dimensions because of stressed or agglomerated cells [1] [2] [3]. NATs are cultivation independent without any need of long incubation times and cumbersome colony counting. However, lack in sensitivity and the small insertable sample volume in NAT test systems have limited its use in the detection of microorganisms.

Different strategies to overcome these limitations after filtration of the liquid have been developed. Some providers recommend to recover the retained bacteria by washing them off the filter after the filtration step. The recovery rate of the retained microorganisms is highly depending on the efficiency of the washing step and is most often unsatisfying, leading to questionable results of the subsequent analysis. A novel technique dissolves the microorganism-containing membrane. By dissolving the membrane, a maximum recovery rate is guaranteed for subsequent DNA extraction, increasing the subsequent PCR sensitivity.

In this study, two kits for NAT analysis are compared using *Legionella*-spiked water samples to evaluate DNA extraction efficiency for liquids ≥ 100 ml and the sensitivity of subsequent *Legionella* detection.

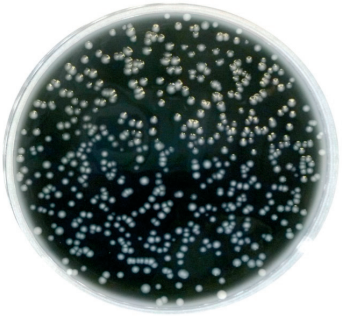

Fig. 1: *Legionella* colonies on BCYE agar after five days of incubation representing the traditional method

II. Material and Methods

Sample Preparation
Distilled water has been spiked with *Legionella pneumophila* at a concentration of 10^5 cells per 100 ml. Sample filtration of 100 ml of spiked water and DNA isolation has been done according to the instructions for use of the kit supplier P and Sartorius´ Microsart® Geneprep kit in parallel [4] [5].

Both sample preparation kits include material for DNA extraction, and deliver ready-to-analyze DNA. Both kits were used according to the respective recommended instructions for use. For subsequent analysis to evaluate the DNA extraction efficiency of both sample preparation methods the AquaScreen® *Legionella* species PCR Real-time kit (Supplier: Minerva Biolabs GmbH) has been used. Ct values have been determined and used for calculation to compare the efficiency both kits. An overview of the different steps of the kit from supplier P and Sartorius´ Microsart® Geneprep is shown in table 1.

Table 1: Sample preparation procedure of the kit from supplier P and Sartorius´ Microsart® Geneprep.

Supplier P Filtration and DNA isolation for liquids	**Sartorius' Microsart® Geneprep Filtration and DNA isolation for liquids**
1. Filtration of 100 ml sample	1. Filtration of 100 ml sample
2. Filter (incl. retained *Legionella* cells) is folded and transferred with the help of forceps into a 2 ml reaction tube containing lysis buffer	2. Filter (incl. retained *Legionella* cells) is picked up with the help of a new designed single-use device (Microsart®@solve) (Figure 1 – 4) and dissolved within the device (Figure 5). The microorganism-containing liquid is transferred into a 2 ml reaction tube by shaking or centrifugation in seconds
3. Sonication in an ultrasonic bath	3. Chemical cell lysis
4. Heat lysis	4. Phase separation via centrifugation with the help of a phase separation paste
5. Lysate is bound to silica matrix in spin columns	5. Alcoholic DNA precipitation after transferring the upper phase in a new reaction tube
6. Washing step 1	6. Washing of the DNA pellet
7. Washing step 2	7. Drying of the DNA pellet
8. Washing step 3	8. Rehydration of the DNA pellet
9. Drying of the silica matrix	
10. DNA elution	
PCR setup and analysis with AquaScreen® L. specc. (according to the instructions for use [6])	

Figure 1 – 5: Show handling steps according to instructions for use of the Microsart® Geneprep kit.

PCR setup
For detection of *Legionella* species by PCR, AquaScreen® L. specc. (Minerva Biolabs) was used according to kit instructions. All tests were run at least as four-fold replicates, means n ≥ 4.

Calculation of Ct values
Ct values of samples processed by the product of supplier P have been compared with Ct values of samples processed with the Microsart® Geneprep.

Delta-Ct (ΔCt) has been calculated using the mean values of each method:
$\Delta Ct = (\text{mean-}Ct_{\text{supplier P}}) - (\text{mean-}Ct_{\text{Microsart® Geneprep}})$

The difference in sensitivity was calculated as follows:
$\Delta Sensitivity = 2^{(\Delta Ct)}$
In general it can be considered: The lower the Ct value, the earlier DNA is detected. The higher the difference of Ct values of two methods, the higher is the difference in recovery rate and sensitivity. A ΔCt of ~ 3.3 corresponds to about one log level improved sensitivity.

III. Results

For the detection of *Legionella pneumophila* using Microsart® Geneprep and the product from supplier P in parallel for sample preparation a mean delta-Ct value of $\Delta Ct \sim 2.8$ was determined. This results in a sevenfold ($2^{(2.8)} \approx 7$) increased recovery rate when using the Microsart® Geneprep method compared to the method of supplier P.

Test results of the benchmarking can be found in table 2. All spiked samples have been tested positive for *Legionella* with both methods used.

Table 2: Ct values of detected *Legionella* after DNA extraction by kits of Supplier P and Sartorius´ Microsart® Geneprep, the ΔCt of the mean Ct values and the calculation of the difference in sensitivity based on ΔCt show approximately sevenfold higher sensitivity of *Legionella* detection using the Microsart® Geneprep method

Sample	Ct value	Ct mean value	Delta-Ct	Calculation of ΔSensitivity = $2^{(\Delta Ct)}$
PCR Negative Control	No Ct	No Ct	N.A.	
	No Ct			
Sartorius´ Microsart® Geneprep	27.23	26.7	2.8	$2^{(2.8)} = 6.964$
	26.98			
	25.87			
	26.81			
Product Supplier P	30.10	29.5		
	29.00			
	29.53			
	30.51			
	29.05			
	28.84			
PCR Negative Control	No Ct	No Ct	N.A.	
	No Ct			

IV. Discussion

This study compared two different methods to extract DNA from retained microorganisms from liquids, using *Legionella* in water samples for investigation. Up to a sevenfold increase in sensitivity can be reached when using the Microsart® Geneprep sample preparation kit compared to a competitive product from supplier P.

As all detection assays are only as sensitive as the prior sample preparation method, it is crucial to recover microorganisms as effective as possible. As it is almost an impossible task to wash off all retained organisms from a filter without loss, the recovery rate from common methods and subsequent detection quality remains uncertain. In contrast, the Microsart® Geneprep kit includes a dissolvable filter which guarantees that 100% of retained microorganism can be recovered in solution for further DNA isolation and detection. This ensures a highly effective sample preparation and supports the quality of subsequent PCR analysis for contaminated liquids in the most effective way.

Rapid and sensitive determination of *Legionella* bacteria in an outbreak scenario is crucial for helping to protect public health. This study shows that the Microsart® Geneprep sample preparation kit provides a highly effective solution to recover 100% of retained microorganisms from liquids and strongly increases the subsequent detection sensitivity for harmful organisms.

V. References

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