AMSTERDAM INTERNATIONAL WATER WEEK

Abstract Title	ALTERNATIVE CONCENTRATION METHODS FOR IMPROVING DRINKING WATER QUALITY CONTROL
Topic	**O Improving water quality** O Resilient water systems O Circular solutions: Reuse, Recover and Recycle O Transitions in water, agro/food and energy
Challenges and Solutions	Water quality, alternative concentration methods
Author(s), highlight corresponding author	1. Ane López, Aigües de Barcelona, Spain 2. Gemma Saucedo, Aigües de Barcelona, Spain 3. Elena Arbó, Aigües de Barcelona, Spain 4. Belen Galofré, Aigües de Barcelona, Spain
Abstract	Aigües de Barcelona is a water company that supplies drinking water for 3 million inhabitants in the metropolitan area of Barcelona city and manages the water cycle, treating wastewater and using reclaimed water to reduce drought impact. For water quality assessment, microbiological analyses are essential to determine the absence of fecal contamination of water samples and ensure the safety of public health. Although the European Directive already requires mandatory analysis, new methods need to be studied to improve sensitivity and detection levels in order to improve the knowledge of drinking water quality networks. In the same direction, potential risk of biothreat is one of the main issues to manage. In both cases, the use of large water sample volumes increases the sensitivity of the pathogen detection and recovery, since a larger sample size increases the probability of detecting the biological target agent, even at low concentrations. The proportion of pathogens in water is usually low and this may be a limitation for their detection, hence implementing more sensitive

	concentration methods is a very important issue. This has led to the development of different methodologies for the concentration of microorganisms, being the ultrafiltration the most promising methodology. This physical process allows particles above a certain size or molecular weight to be retained in the membrane filter, while smaller molecules or liquids can pass through the pores of the fibers. Within ultrafiltration processes, tangential flow hollow fiber ultrafiltration is considered one of the most efficient techniques for concentrating large-volume water samples, such as dead end ultrafiltration systems InnovaPrep Concentrating Pipette Select (CP Select) (InnovaPrep LLC., US). So, the main goal of this study is to implement the automated and rapìd micro-particle concentrating pipette CP Select, which has been described as a quick and efficient alternative to concentrate biological particles of interest from large volumes of water up to 5 L, obtaining final volumes of concentrate of 150-1000 µL., to improve procedure sensitivity as well as reduce methodology times. Therefore, the aim of this project was to verify the effectiveness and quickness of the pipette method with respect to other techniques of water concentration such as membrane filtration, dead-end ultrafiltration or centrifugation. Methodologies were focused on two targets: Legionella spp. and waterborne viruses. In case of Legionella, the methods used to date of membrane filtration and sonication were replaced by the CP Select concentration, and the following quantification analysis was performed by qPCR. The validation of this study was carried out according to ISO/TS 12869:2019, where it was verified that the protocol ensures correct efficiency and robustness of the method for the detection and quantification of Legionella spp. and Legionella pneumophila. By using this method, we have verified how all validation results were improved: recovery, accuracy, uncertainty, etc. Moreover, time consumption

	is also a remarkable point, as it has been greatly reduced with the new method (Table 1). In general, it was found that this protocol constitutes an optimization of the Legionella detection procedure in water samples that ensures reliable and much faster results. Similarly, the implementation of subsequent analyses of the eluate on culture plate method is currently underway. In the case of large volume concentration, what regards to bacteria and virus concentration procedures, Rexeed ultrafilter (Asahi Kasei Medical Co., Japan) is used to concentrate from 5 up to 1000 liters, depending on matrix sample. Elution methodology currently implemented in our laboratory for viruses control in drinking water samples, includes manual elution of this Rexeed filter and subsequent several centrifugation runs by Centricon Plus-70 centrifugal filters (Merck KGaA, Germany). Obtaining final volumes from 2 to 3 mL. The new method using the CP Select to concentrate the Rexeed filter's elute without using centrifugation steps has been tested in order to obtain a final concentrate of 1 to 2 mL. It has also the advantage of reducing the processing time and avoid some costs, such as, the centrifuge or even the water pump instruments that are used to elute the Rexeed, since we have also tested to elute the Rexeed with the new EasyElute Elution Buffer canister (Innovaprep LLC., US), a cartridge device to ensure an easy elution, safer vacuum method and leak-proof connections (Figure 1). Posterior methodologies used to analyze samples were PCR, qualitative by using multiplex PCR FilmArrayTM system (bioMérieux, France), and quantitative by Lightcycler 96 real-time thermocycler. As mentioned before, the main advantage is the time reduction and less equipment needed for this procedure. Results will be presented in AIWW2023, both virus and the remaining Legionella results.

Figures/diagrams/illustrations

Table 1. Comparison of recovery, accuracy, uncertainty and time parameters of the two methods implemented: filtration and sonication *versus InnovaPrep Concentrating Pipette Select* (*CP Select*) for *Legionella* spp. concentration and quantification of water samples.

	Filtration & sonication		InnovaPrep Concentrating Pipette (*CP Select*)	
	Leg spp.	*Leg pn*	*Leg* spp.	*Leg pn*
RECOVERY (%)	33,30%	15,30%	81,10%	85,80%
ACCURACY (%)	16%	19,30%	14,90%	16,40%
UNCERTAINTY (LOG)	1,32	1,7	0,52	0,47
CONCENTRATION TIME (min)	60 min		5 min	

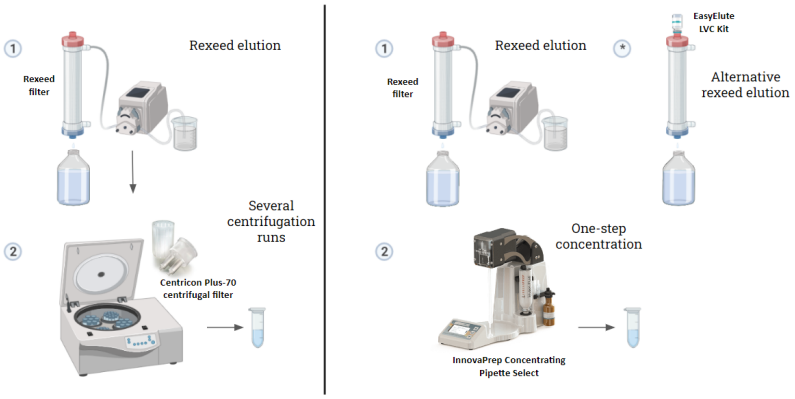

Figure 1. The two concentration methods used for large volume concentration. On the left, manual elution Rexeed filter and subsequent centrifugation runs by Centricon Plus-70 centrifugal filters. On the right, the Rexeed filter manual elution and its alternative Rexeed elution by using the EasyElute Elution Buffer canister,by and the new method using *InnovaPrep Concentrating Pipette Select* (*CP Select*).

ALTERNATIVE CONCENTRATION METHODS FOR IMPROVING DRINKING WATER QUALITY CONTROL

A. López*, G. Saucedo*, E. Arbó* and B. Galofré*

*Aigües de Barcelona, empresa Metropolitana de Gestió del Cicle Integral de l'Aigua, SA. General Batet 5-7 08028 Barcelona

Aigües de Barcelona is a water company that supplies drinking water for 3 million inhabitants in the metropolitan area of Barcelona city and manages the water cycle, treating wastewater and using reclaimed water to reduce drought impact. For water quality assessment, microbiological analyses are essential to determine the absence of fecal contamination of water samples and ensure the safety of public health.

Although the European Directive already requires mandatory analysis, new methods need to be studied to improve sensitivity and detection levels in order to improve the knowledge of drinking water quality networks. In the same direction, potential risk of biothreat is one of the main issues to manage. In both cases, the use of large water sample volumes increases the sensitivity of the pathogen detection and recovery, since a larger sample size increases the probability of detecting the biological target agent, even at low concentrations. The proportion of pathogens in water is usually low and this may be a limitation for their detection, hence implementing more sensitive concentration methods is a very important issue.

This has led to the development of different methodologies for the concentration of microorganisms, being the ultrafiltration the most promising methodology. This physical process allows particles above a certain size or molecular weight to be retained in the membrane filter, while smaller molecules or liquids can pass through the pores of the fibers. Within ultrafiltration processes, tangential flow hollow fiber ultrafiltration is considered one of the most efficient techniques for concentrating large-volume water samples, such as dead end ultrafiltration systems InnovaPrep Concentrating Pipette Select (CP Select) (InnovaPrep LLC., US).

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Methodologies were focused on two targets: Legionella spp. and waterborne viruses.

In case of *Legionella*, the methods used to date of membrane filtration and sonication were replaced by the *CP Select* concentration, and the following quantification analysis was performed by qPCR. The validation of this study was carried out according to ISO/TS 12869:2019, where it was verified that the protocol ensures correct efficiency and robustness of the method for the detection and quantification of *Legionella* spp. and *Legionella pneumophila.* By using this method, we have verified how all validation results were improved: recovery, accuracy, uncertainty, etc. Moreover, time consumption is also a remarkable point, as it has been greatly reduced with the new method **(Table 1)**.

In general, it was found that this protocol constitutes an optimization of the *Legionella* detection procedure in water samples that ensures reliable and much faster results. Similarly, the implementation of subsequent analyses of the eluate on culture plate method is currently underway.

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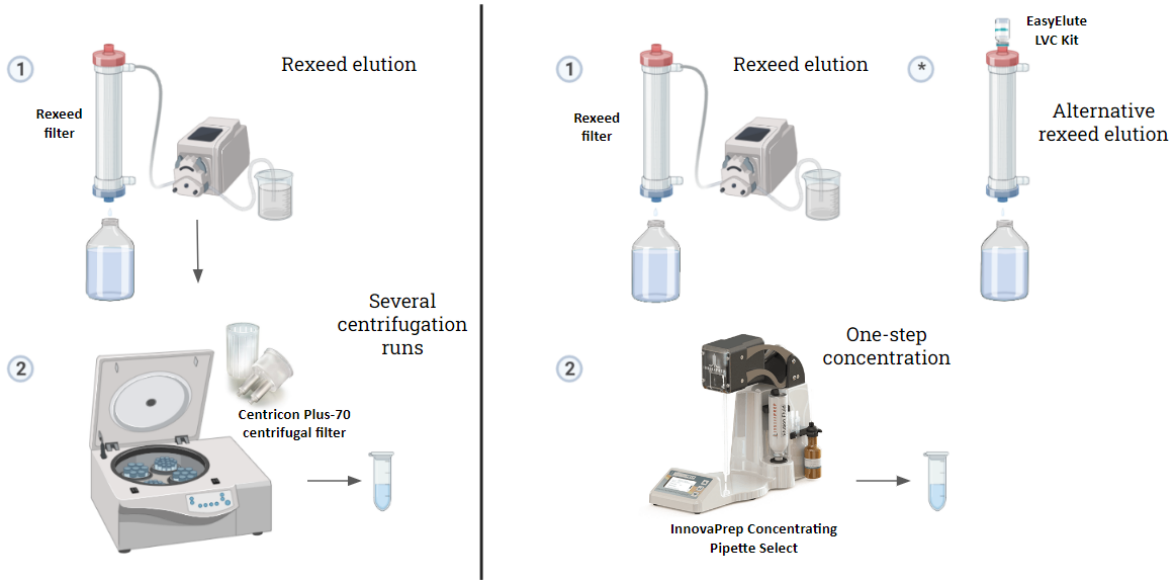

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Posterior methodologies used to analyze samples were PCR, qualitative by using multiplex PCR FilmArray™ system (bioMérieux, France), and quantitative by Lightcycler 96 real-time thermocycler. As mentioned before, the main advantage is the time reduction and less equipment needed for this procedure. Results will be presented in AIWW2023, both virus and the remaining *Legionella* results.