Aquavalens Project

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation"

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Report of suitability of a prototype for multiplex detection of pathogens

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Executive summary

This report explains the recommendations formulated to describe the commercial and technical feasibility of manufacturing an automated system for the multiplex detection of pathogens. The deliverable had to wait for the outcomes of WP6 (filtration and sampling systems) and WP7 (detection technologies) in terms of what is feasible and the types of kingdoms (viruses, bacteria and parasites) that could be detected using molecular techniques. It is concluded that an overall system capable of automatically detecting the three kingdoms would be neither technically feasible nor economically viable. It is recommended that future automated systems concentrate on a given kingdom and clearly specify the range of waters that can be accommodated with the system. It is also indicated that automated molecular detection techniques are not yet technically ready to be deployed in the field.

Aims and objectives

The reading of this report should be carried out in conjunction with the reading of the final reports of WP6 (filtration and sampling systems) and WP7 (molecular detection techniques). Such reports provide indication of the technical suitability of filtration, sampling and detection techniques with respect to the types of waters (raw, surface, ground, etc.) and of pathogens (viruses, bacteria and parasites). The most relevant compilation tables are replicated here for completeness.

The goal of this deliverable and accompanying milestone is to conclude whether technically, and more importantly economically, the manufacturing of an automated system for sampling and detection of multiple pathogens is a viable proposition. The report will discuss first the review of the filtration techniques, followed by the molecular detection techniques and their suitability in being installed into an automated system.

1. Introduction

Automated technological platforms for the detection of pathogens in water can be described as technologies enabling the translation of lab-bench protocols to automated protocols. Such systems could either be installed in laboratories of water companies or in the field. In all cases, such automated systems should have the following characteristics: 1) accelerated diagnostics procedures leading to higher throughput of samples beyond what is currently achieved using highly qualified manpower in laboratories, 2) improved repeatability within and between laboratories and 3) increased safety, with less probability of cross-contamination between samples. Technological platforms should encompass sample preparation, detection and analysis in order to be considered fully automated.

To achieve these aims, the automated systems must possess the following criteria: (1) limit of detection obeying existing regulatory guidelines or compatible with current practice in laboratories, (2) potential for speciation, (3) robustness of detection, (4) low manufacturing and operational costs, (5) potential for simultaneous analysis of multiple pathogens, (6) market demand, (7) fit with existing water management systems, (8) system reliability, (9) size and/or portability of the system.

This report is looking at the potential in an automated system to accommodate simultaneous analysis of multiple pathogens. This can only happen if the right sampling, filtration and enrichment mechanisms are in place, as the system is expect to be fully automated. The next section discusses therefore whether automation of the filtration is achievable in the first instance.

2. Water sampling, filtration and enrichment

To enable detection of low amounts of microbial pathogens in water, large volumes (40 L up to 1,000L) need to be analysed. This can be achieved by various filtration techniques that reduce the water volume while retaining the micro-organisms. An ideal fully automated system would require filtration systems compatible with the three kingdoms (viruses, parasites and bacteria). Moreover, for all three techniques, the microorganisms will need to be eluted from the filters. Both the filtration and the elution methods will need to be optimised to give a sufficient recovery of a control panel of model organisms representing bacteria, viruses and parasites, from treated as well as untreated ground and surface waters. Ideally, the secondary concentration procedures should also be identical amongst the kingdoms.

WP6 concentrated on three candidates filtration systems as shown in Table 1 on the next page. This table represents the final recommendations of the group responsible for the filtration, enrichment and elution methods, a key step towards the diagnostics and monitoring of pathogens using molecular techniques. As evidenced by the table, it is not possible to deliver a universal protocol for the filtration step, which enables the optimum recovery rate of the pathogens. For example, bacteria and parasites are concentrated by e.g. pelleting by centrifugation and viruses are PEG-precipitated, flocculated or (ultra)filtrated by various techniques from the supernatant.

Moreover, the second concentration technique requires centrifugation, which makes the automation of the overall system incorporating molecular detection, either hardly portable let alone transportable for routine field applications, or very expensive, which would be prohibitive for small groups of users of drinking waters. The use of megasonic agitation during elution (see previous deliverables of WP8) might alleviate the use of centrifugation techniques.

Steps in procedure	Method characteristics		Filtration procedure		
			Hollow fiber	**Monolithic affinity**	**Glass wool**
			Task 6.1	Task 6.2	Task 6.3
Water type			Drinking water Surface source water	Drinking water (untreated)	Surface water Drinking water (untreated)
Volume (L)			50-200 L	60-100 L	5 – 50 L
Kingdoms	**Virus**		MC_0, MS2, NoV	MNV, NoV GII, HadV, MC_0	HAV, TBGV, MNV, NoV GII, HAdV, MC_0
	Bacteria		C. jejuni, L. mono S. enteritidis, E. coli O157	C. jejuni, L. mono, S. typhimurium	C. jejuni, L. mono, S. typhimurium
	Protozoa		Cryptosporidium, Giardia	Cryptosporidium	Cryptosporidium
1st Concen-tration	Mechanism		Size exclusion	Adsorption by ionic interaction	Adsorption by ionic interaction
	Requirement of preconditioning of filter		Yes	No	Yes
	On-site filtration		Yes (tap or pump)	Yes (pump or pressure)	Yes (pump or pressure)
	On-line filtration		No	No	No
	Elution from filter		Back flush (pump, tubing)	Rinse (pump, tubing)	Rinse (pump, tubing)
	Water type sensitive	Turbidity	Yes (turbid eluate)	Yes but limitations	No
		pH	No	No	No
2nd Concen-tration	Virus /bacteria		PEG-NaCl Centrifugal filter	PEG-NaCl Centrifugal filter	PEG-NaCl Centrifugal filter
	Protozoa		IMS PEG-NaCl Centrifugal filter	PEG-NaCl	

Table 1: Summary of the recommended filtration procedures according to kingdoms (protozoa, viruses, bacteria). Courtesy of WP6.

Costs of the filtration methods will also have an effect on the automation of the systems. Ideally, re-usable filters or commercial availability of the filters should be preferred. Table 2 provides a breakdown on the filtration techniques in terms of costs. Hollow fiber ultra-filters normally have a cut-off of ~30KDa and will retain parasites, bacteria and viruses. They are disposable (cost ~€20), thus avoiding the risk of cross contamination and filtration can either

be achieved by coupling the filter directly to a tap or pumping an environmental water sample through the filter. They have mainly been used for filtration of tap water but have also been used for surface waters. Other types of filters are not commercially available and a supply chain would need to be created to benefit form them in a multiplex detection system.

Steps in procedure	**Specificities**	**Cost (€) of concentrate**		
		Hollow fiber	Monolithic affinity	Glass wool
		Task 6.1	Task 6.2	Task 6.3
1st Concen-tration	Filters / devices	26 Not reusable	Homemade Not reusable nor commercially available	Homemade Not commercially available
	Chemicals	10	5	5
2nd concen-tration	Virus /bacteria	PEG-NaCl 15 Centrifugal filter 30	PEG-NaCl 15 Centrifugal filter 30	PEG-NaCl 15 Centrifugal filter 30
	Protozoa	IMS 50 PEG-NaCl 15 Centrifugal filter 30	IMS 50 PEG-NaCl 15 Centrifugal filter 30	
NA-Extraction	DNA/RNA	15	15	15
Detection	qPCR (per rxn)	4	4	4
	qRT-PCR (per rxn)	8	8	8
Total (min/max)		**85/139**	**54/108** price of filter not included	**39/58** price of filter not included

Table 2: Breakdown of costs of the filtration techniques. Courtesy of WP6

3. Detection techniques

The various detection techniques proposed are presentable in table 3 and were provided by WP7, the work-package responsible for the detections techniques to be tested. Without repeating the work achieved and presented in the various deliverables of WP7, it transpires that the automated platforms so far (microLAN) or capable to be automated are indicators of microbial pollution through determination of ATP or other agents indicative of microbial activity.

An attempt was made in the past through Shaw Waters, whose system was purchased by Parker Hannifin. Market studies carried out earlier in the project have indicated that the system was not commercially viable with preference indicated for an automated filtration system only with molecular detection carried out by skilled personal in the case of large water companies.

Water	**Conc.**	**Platform**	**Analytical category**			**Temporal resolution**			**Targets**	**Analytical detection limit (assuming 100% recovery)**	**Main customer(s)**
			sa	im	sv	M	H	D			
DW	none	Biosensor (inline)		X	X	X			Indication of microbial pollution/ bacterial activity	10^{-4} dilution of wastewater	LWC, FFC
DW SW	MF [100 mL]	microLAN (online)	X	X	X		X		Indication of faecal pollution		LWC, SWO, FFC
SW	UF [50 L]	qPCR (offline)	X				X	X	MST	> 5 target copies/L	LWC, SWO, FFC, LAB
SW	UF [50 L]	qPCR (offline)	X				X	X	Pathogens	> 5 target copies/L	LWC, SWO, FFC, LAB
DW	UF [1000	qPCR (offline)			X			X	Pathogens	1 (0.25) target	LWC, SWO, FFC, LAB

	L]									copy/L	
SW	MF [100 mL]	SPC (offline)	X				X	X	Vibrio, Campylobacter, Cryptosporidium		LWC, SWO, FFC, LAB
DW	MF [1-3 L]	SPC (offline)			X		X	X	Vibrio, Campylobacter, Cryptosporidium		LWC, SWO, FFC, LAB
SW	MF [100 mL]	Vermicon (offline)	X				X	X	Bacteria		LWC, SWO, FFC, LAB
DW	MF [1-3 L]	Vermicon (offline)			X		X	X	Bacteria		LWC, SWO, FFC, LAB
SW		AquaTAS (onsite)	X				X		Viruses		LWC
DW		Parker (online)		X	X		X		Parasites		LWC
SW	UF [50 L]	VOCMA	X				X	X			LWC

Table 3: Symbols in the table are as follows. DW: treated drinking water, SW: surface or source water (intended to be treated). UF: ultrafiltration, MF: membrane filtration (elution). LWC: large water companies, SWO: small water organisations, LAB: laboratories, FFC: food and fruit companies. M,H,D: minute, hour, day. Courtesy of WP7.

Therefore, it appears again, that, as in the filtration method, the case for automated detection systems that encompass all kingdoms is not economically viable and do not answer market needs at this stage. The same conclusion applies in the stronger case of multiplex pathogen detection. Existing regulatory framework in Europe does not place either demands for systematic monitoring control of the pathogens, and therefore do not create the market push, slowing down technological solution that could satisfy these needs.

4. Conclusions and future prospects

It is clear that fully encompassing automated filtration and detection systems that process all types of waters and pathogens are currently either not technological feasible, nor economically desirable. Attempts were made in the past even for single pathogen detection systems either did not succeed economically (Shaw Waters system, bought by Parker Hannifin) or were left at the demonstration stage (CryptoDetect CARD by Rheonix). Fully automated commercialised online detection systems deal at the moment with faecal indicators and show promise of commercial success (MicroLAN).

These pessimistic conclusions do not however indicate that future systems cannot be

manufactured and trialled by companies. In that respect, the following Table 4 attempts to provide some pointers as the different monitoring scenarios that could be conceived and their potential of automation. It is hoped that these tables will be of use for future technologists in order to answer the demand of end-users.

Different monitoring scenarios and potential for automation

System type	Monitoring scenario #	Water type	Type of detection	Comment	Type of sampling	Potential for automated filtration (and elution)	Potential for fully integrated solution	Comments
Large systems	Operational	Finished	ATP or enzyme	Early-warning type system *	Systems seem to work with small volumes – is any concentration needed?	Possibly to add concentration – needs to be continuous, rapid method	ATP system will sample directly from the mains and perform automated detection	
	Operational	Finished	Molecular methods (PCR)	Direct detection of index pathogens Online? Unlikely due to cost and reagent storage	Concentration of large volumes (50-1000L); viruses, bacteria and protozoa; collect over time for temporal averaging ^	Concentration system, located at treatment works – could either provide concentrated sample (tangential filtration) OR eluted samples or the filter for transportation (dead end filtration) If filter transported automated elution system at lab could be useful Further concentration	Most likely too expensive and issues of reagent storage therefore unlikely to have fully integrated solution online at treatment works	Cryptosporidium monitoring already generates filters at the lab – improve to be for all kingdoms, if elute is there a storage consideration before sample collection? How about for concentrated sample storage?

					Separation into kingdoms (depends upon WP7 multiplexed systems but lysis easier for separated systems)	replacing centrifugation (at lab) – similar system working with smaller volumes? Combination of filters?	Integrated secondary concentrations methods with detection systems is a possibility if secondary concentration methods can be automated (see table of secondary methods)	In Aquavalens not time or resource to build a mini filter system – could suggest a design though Automation of secondary concentration techniques? Challenging. Use of microfluidics?
	Surveillance	Finished	Molecular (PCR) – identify and speciate	Wider range of pathogens Identify pathogen load after treatment – assess effectiveness, evaluate new technologies	Sample large volume from specific site (50-1000L; more likely less) Less time critical Separation into kingdoms at secondary concentration	Portable filtration and elution unit (storage of concentrate/eluate must be considered)	Unlikely to have fully integrated portable system due to cost but could be possible Alternatively have sampling unit as for operational monitoring which is portable and at	Only resources to produce one in Aquavalens but could be useful to integrate several filters with one pumping unit in a van for sampling several sites – suggest a set-up and design for this

					stage		lab integrated further processing and detection	
	Surveillance	Raw	Molecular (PCR) – identify and speciate	Wider range of pathogens Identify pathogen load in source waters for QMRA	Sample large volume from specific site (50-100 L) Less time critical Separation into kingdoms at secondary concentration stage	As above (though probably will utilise different filters for raw water)	As above (though the secondary concentration process might be different for raw waters)	Ideal solution is a flexible system allowing for connection of different filter types
	Investigative	Finished	Molecular (PCR) – identify and speciate	Only when outbreak (might no longer be present in finished water so the raw water sampling critical here)	Sample large volume from specific site (50-100 L) Rapid systems essential to respond to outbreak	Portable filtration and elution unit (storage of concentrate/eluate must be considered)	Unlikely to have fully integrated portable system due to cost but could be possible Alternatively have sampling unit as for operational monitoring which is portable and at lab integrated	Ideal solution is a flexible system allowing for connection of different filter types and as for surveillance monitoring could be useful to process multiple samples simultaneously

							further processing and detection	
	Investigative	Raw	Molecular (PCR) – identify and speciate, find outbreak source	Only when outbreak	Sample large volume from catchment sites (50-100L) Rapid systems essential to respond to outbreak	As above and for the surveillance raw water	As above and for the surveillance raw water	As above
Small systems	Operational Surveillance Investigative	Considerations similar to large systems in terms of automation though the selected pathogens and frequency of monitoring etc might be different, most likely greater pressure on low-cost and easy to operate systems Can we adapt the systems developed above to work with low-cost pumping systems, e.g. bag rolling system?						
Food	Operational	Finished	ATP or enzyme	Early-warning	Systems seem to work with small volumes – is any concentration needed? Especially as food producers volumes less	Utilise one of the systems developed for the large systems depending on if applied on irrigation site or food processing factory	Utilise automated integrated secondary concentration and detection unit	Gather more information on the requirements for testing water for food production to check which systems would be suitable

					than large water plant?			
		Finished	Molecular methods (PCR)					
	Surveillance							
	Investigative							

Table 4: Overview of monitoring situations and the appropriate types of detection and sample processing and where and how automated and/or fully integrated solutions are appropriate

The definitions of the terms surveillance, investigative and operational monitoring are adapted from Allan, I.J., et al., *A toolbox for biological and chemical monitoring requirements for the European Union's Water Framework Directive.* Talanta, 2006. **69**(2): p. 302-322 and *World Health Organisation*. 2011: Guidelines for drinking-water quality:

(i) *surveillance* monitoring aimed at assessing long-term water quality changes and providing baseline data on raw water sources allowing the design and implementation of other types of monitoring; or the monitoring undertaken by water providers, or the regulatory bodies, to ensure water treatment processes are providing the required level of public health protection; or the verification of treatment processes, validating them for future use.

(ii) *operational* monitoring aimed at providing additional and essential data on water bodies at risk or failing environmental objectives of a WSF/WSP; this operational monitoring should provide information for instant action.

(iii) *investigative* monitoring aimed at assessing causes of such failure; or at understanding the pathogen load in source waters; or tracking the source of an outbreak.