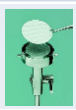
RAPID QUANTIFICATION OF BACTERIOLOGICAL CONTAMINATION OF SEA, LAKE AND RIVER WATER

Blue DNA Companion, Clermont-Ferrand, France

/ CONTEXT

Bathing water, such as sea, lake and river water, may be subject to microbiological pollution. *Enterococcus spp and E.coli* are used as water-quality indicators in the European Union (Directive 2006/7/EC) and the United-States (2012 Recreational Water Quality Criteria – RWQC published by U.S. Environmental Protection Agency (EPA)). In this work, we propose a multiparametric detection of these 2 microorganisms in less than 3 hours after bathing water collection in accordance with EU and EPA regulations. This rapid and quantitative analysis is based on RT-PCR specifically dedicated to viable bacteria.

Water sample	Filtration	Mechanical lysis	Nucleic acid extraction	MIC RT-PCR
	10 min	5 min	30 min	90 min
				

/ MATERIALS

- GenoSPYD filtration kit (Blue DNA Companion, Clermont-Ferrand, France)
- Precellys Evolution homogenizer for mechanical lysis (Bertin Technologies, Montigny-le Bretonneux, France)
- Automated magnetic extraction platform such as MagSPYD (Blue DNA Companion, Clermont-Ferrand, France) King Fisher ml, 96, Flex, Duo or Ideal robot
- MIC PCR machine
- GenoSPYD q-PCR quantitative preloaded kits for *E.Coli* and *Enterococcus spp.* (Blue DNA Companion, Clermont-Ferrand, France)

/ PROTOCOL

- Micro-organisms collection: each sample was filtered on a 0.45µm membrane. Filters are then loaded into a GenosSPYD lysing tube with 1.5 GenoSPYD Bacterial Lysis Buffer.
- Mechanical lysing: the lysing tube containing the filter is then loaded in an homogenizer either Precellys Evolution or Minilys, with the following program:

> 3 cycles of 45 sec at 6500 rpm with a 30 sec pause between cycle on Precellys Evolution.

> 3 cycles of 45 sec at maximal speed with a 30 sec pause between cycle on Minilys.

- Extraction step: the RNA contained into the lysate were purified and concentrated using GenoSPYD RNA Mag extraction kits and an automated magnetic extraction platform.
- One step qRT-PCR was realized using GenoSPYD q-PCR quantitative preloaded kits which include an internal control, and an MIC-PCR machine.

/ RESULTS

4 samples of contaminated water were analyzed respectively: 10000 CFU, 1000 CFU, 100 CFU and 10 CFU.

Figure 1 shows results and efficiency for *Enteroccocus spp.* analysis.

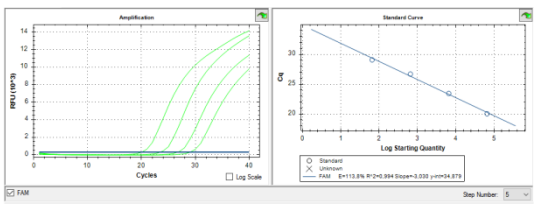


Figure 1: qRT-PCR amplification curves for Enterococcus spp.

	LOQ	LOD without enrichment
E.coli	100 CFU	1 CFU
Enteroccocus spp	100 CFU	50 CFU

Table 1 : LOQ (limit of quantification) and LOD (limit of detection) for E. Coli and Enteroccoccus spp. analyzed in water

/ CUSTOMER

/ CONCLUSION

The GenoSPYD automated product solutions associated with Precellys homogenizers allows to detect and quantify specific RNAs from viable micro-organisms such as *E.coli, Enterococcus spp., Staphylococcus aureus, Pseudomonas aeruginosa*. in less than 3 hours. Regulatory pollution indicators in water can be detected with a limit of quantification of 100 CFU.

This water testing method has been validated by key players in the industry such as Suez Environment.

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