



Transforming Open Responsible Research  
and Innovation through CHARM



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# TORCH Project

Anicet R. Blanch

Barcelona, March 2<sup>nd</sup>, 2022



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Spin-off



# Bluephage, a new approach for analysing coliphages as viral indicators of water quality

# Summary

1. Why a new indicator?
2. Coliphages. Who are they?
3. Coliphages in Guidelines, Regulations and Directives
4. How to analyse coliphages? – Standard Methods
5. Bluephage approach. How it works?
6. Bluephage S.L., a spin-off from University of Barcelona

# 1. Why a new indicator?



People without access to safe  
**Drinking water**

**2100** MILLION



**Waterborne diseases** worldwide  
caused mainly by virus, bacteria and parasites

**>400** ORGANISMS



**Annual cases worldwide**  
from waterborne pathogens

**4** BILLION



**Hospitalisations/year** in  
developed countries due to  
waterborne disease

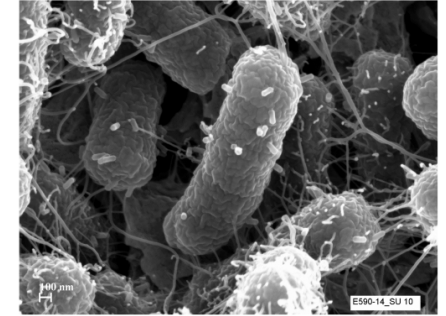
**2** MILLION



# 1. Why a new indicator?

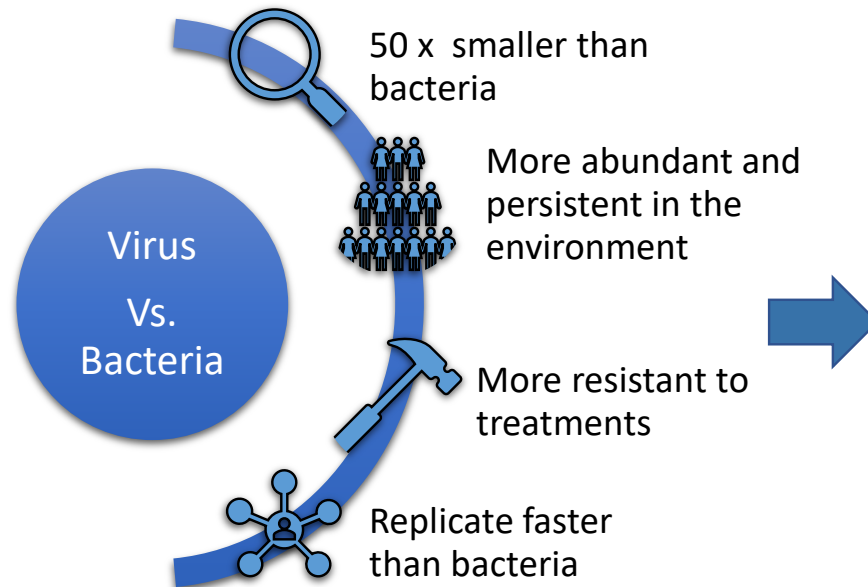
**Microbial water quality assessment and monitoring** is critical for water safety plans and sanitation safety plans

- Established parameters: Bacterial indicators
- New parameters: **Viral indicators (coliphages)**



Comparison: Bacteria vs. Virus size

## Why viral indicators?

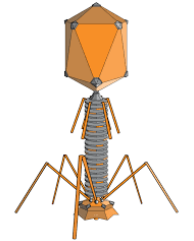


Bacterial indicators are limited and cannot detect the presence of viral pathogens...

...coliphages can

## 2. Coliphages. How are they?

### Bacteriophages: virus of enteric bacteria



- **Different groups of bacteriophages** have been proposed as indicators of faecal and / or viral contamination in water, biosolids and food.
- Bacteriophage groups are **defined with respect to the host bacterial strain** that is used for their detection.
- Bacteriophages are **bacterial viruses**, and in aspects such as their composition, structure, morphology and size of the capsid, they share many properties with animal and human viruses.

## 2. Coliphages. How are they?

**Bacteriophages behave like animal and human viruses attending to:**

- Circulation through filters (membranes, ultrafiltration, "soil", etc.)
- Adsorption to surfaces (particles, membranes, etc.)
- Resistance to physical and chemical disinfection
- Environmental persistence

## 2. Coliphages. How are they?

The relationships observed between bacteriophages and human infectious viruses are essential for risk models

$10^4 - 10^5$  Somatic Coliphages for 1 Infectious Enterovirus

**Ratio in secondary and tertiary treatments**



# 2. Coliphages. How are they?

## Groups of Bacteriophages proposed as indicators

Defined according to the host bacteria that they infect

COLIPHAGES

### Somatic coliphages

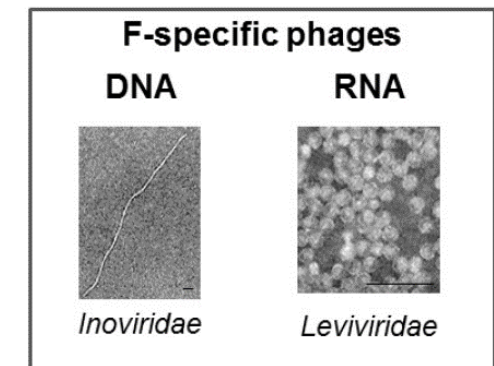
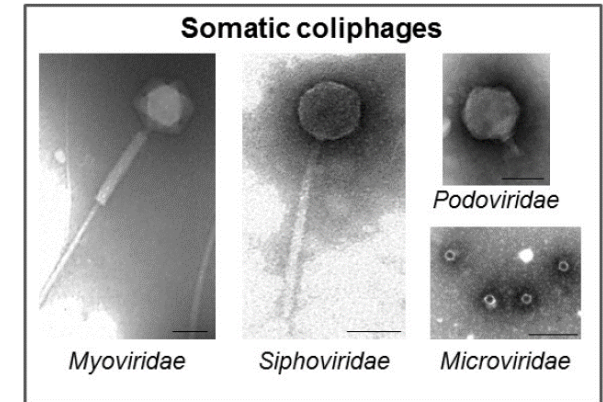
- They infect *E. coli* through the cell wall
- Host (non written agreement) strains CNI3, WG5 derived from *E. coli* C (ATCC13706)

### F-specific coliphages










- They infect *E. coli* and other enterobacteria through the sex pili encoded by the plasmid
- Host strain *Salmonella typhimurium* WG49 and *E. coli* HS

### *Bacteroides fragilis* bacteriophages

They infect *Bacteroides fragilis* through the cell wall (HSP40, RYC2056)



# 3. Coliphages in Guidelines, Regulations and Directives

	Biosolids	Groundwater	Recreational water	Drinking water	Reclaimed water	Integrity membranes & UV	Direct Potable reuse
Australia 	2012 (WA)		2008 (emerging interest)	2011 *	Queensland 2010 WA 2011		
Canada 			2008	2001 (Quebec)			
Colombia 	2014						
EEUU 		2006	2018		2011 (North Caroline)	2015	2015
EU 				2020	2020		
Italy 	Draft 2018						
France 	2021				2014		
South Africa 				1996 *			
WHO 				2017 *	2017		2017 *

\* Guidelines

# 4. How to analyse coliphages? – Standard Methods

## International Standardization Office. ISO



**10705-1: 2002.** Water quality. Detection and enumeration of bacteriophages.

Part 1: Enumeration of F-specific RNA bacteriophages

**10705-2: 2002.** Water quality. Detection and enumeration of bacteriophages.

Part 2. **Enumeration of somatic coliphages**

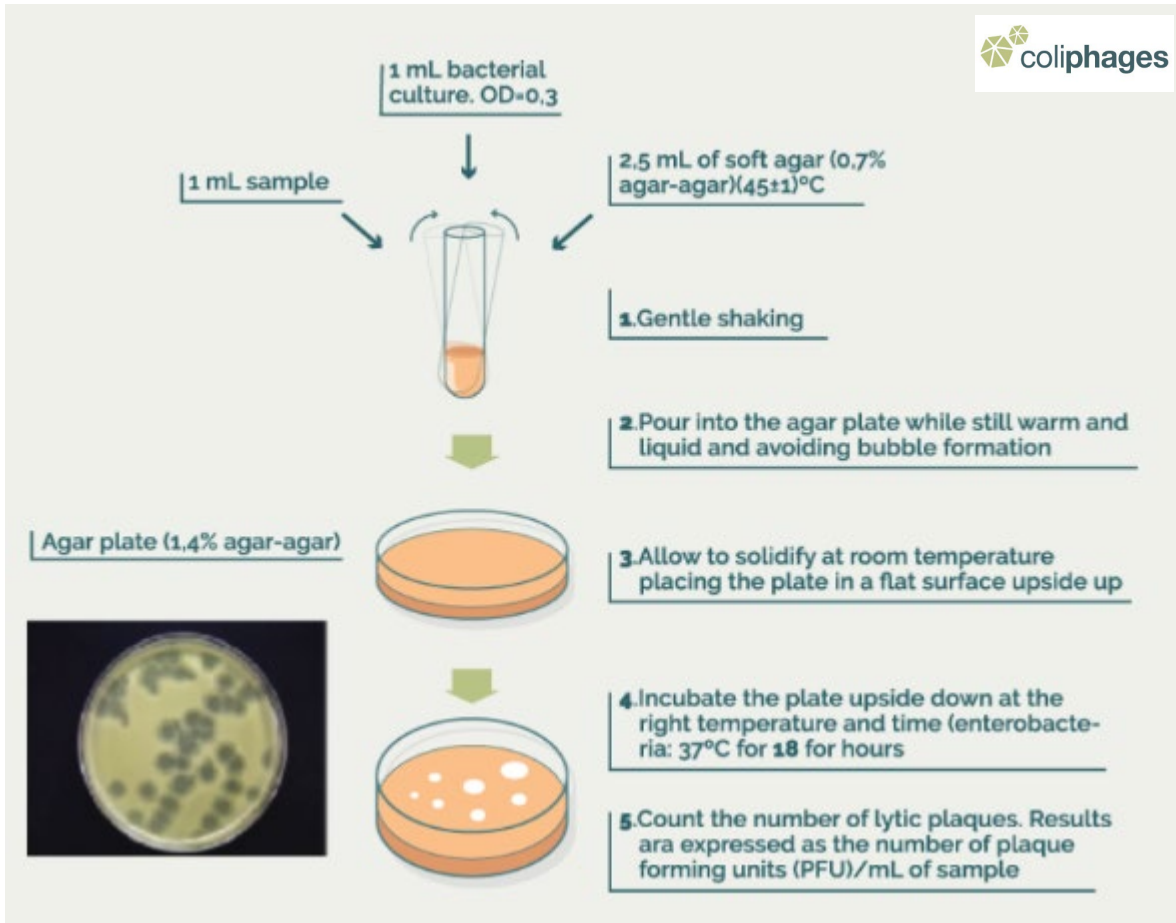
**10705-3: 2003.** Water quality. Detection and enumeration of bacteriophages.

Part 3: Validation of methods for **concentration** of bacteriophages **from water**

**10705-4: 2001.** Water quality. Detection and enumeration of bacteriophages.

Part 4. Enumeration of **bacteriophages infecting *Bacteroides fragilis***

# 4. How to analyse coliphages? – Standard Methods

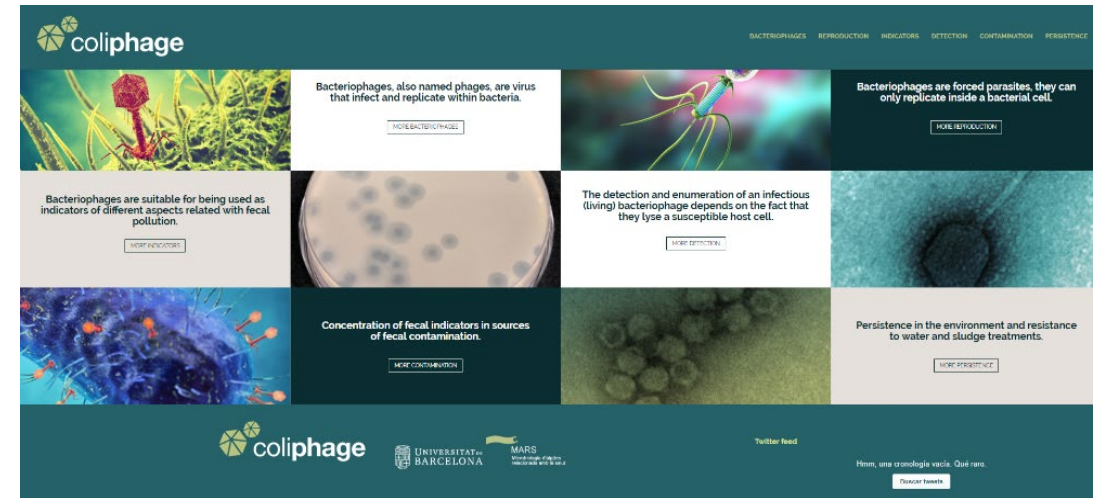


still **multi-step processes** that require several media and operations, pre-preparation of calibrated control and reference materials and...



**Applying Standard Methods  
still > 16h! (> overnight)**

# To know more .....



Extraction of bacteriophages from sludges, soils and treated biowastes

<https://www.ub.edu/ubtv/video/extraction-of-bacteriophages-from-sludges-soils-and-treated-biowastes>

[www.coliphages.com](http://www.coliphages.com)

CEN/ISO working document: <http://diposit.ub.edu/dspace/handle/2445/170949>

Detection and enumeration of somatic coliphages

<http://www.ub.edu/ubtv/video/detection-and-enumeration-of-somatic-coliphages>

Preparation of culture reference material

<http://www.ub.edu/ubtv/video/preparation-of-culture-reference-material>

Bacteriophages preparation of a highly concentrated control stock

<http://www.ub.edu/ubtv/video/bacteriophages-preparation-of-a-highly-concentrated-control-stock>

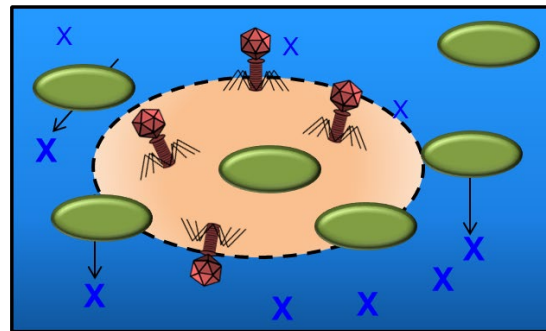
# 5. Bluephage approach. How it works?

## Technology:

A patented bacterial host strain for coliphages that turns its growing culture into **blue colour** in presence of infective virus.

**US Patent Granted US 9.932.645 B2** (April 3<sup>rd</sup> 2018)

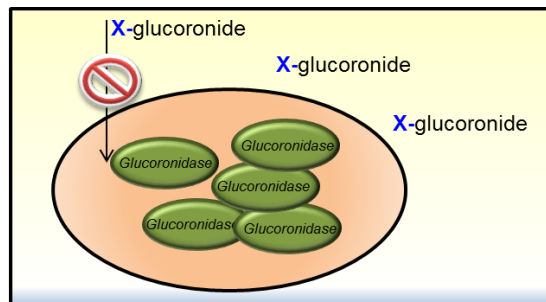
**EPO 3068894** (April 17<sup>th</sup>, 2019)



→ **X** is detected:  
the media turns **BLUE**



**Blue colour**  
(non-safe water)



→ No signal



**Yellow colour**  
(safe water)

Growing media based on ISO (with x-glucuronide)

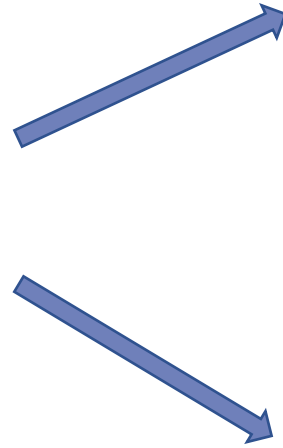


# 5. Bluephage approach. How it works?



Non-safe

Safe



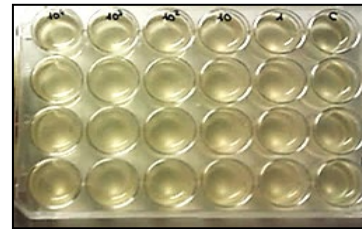
0 h



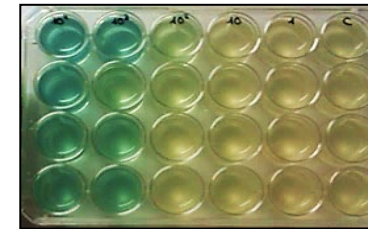
1:30 h



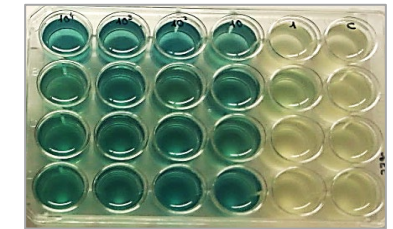
3:30 h



UFP/pou 10<sup>4</sup> 10<sup>3</sup> 10<sup>2</sup> 10 1 C



10<sup>4</sup> 10<sup>3</sup> 10<sup>2</sup> 10 1 C



10<sup>4</sup> 10<sup>3</sup> 10<sup>2</sup> 10 1 C

- Adaptable to miniaturized systems and 100 mL volumes

# References of interest

## Bluephage related scientific publications

1. Muniesa, M., E. Balleste, L. Imamovic, M. Pascual-Benito, D. Toribio-Avedillo, F. Lucena, A. R. Blanch, and J. Jofre. 2018. Bluephage: A rapid method for the detection of somatic coliphages used as indicators of fecal pollution in water. *Water Res.* 128:10-19. <https://doi-org.sire.ub.edu/10.1016/j.watres.2017.10.030>
2. Toribio-Avedillo, D., J. M. Diaz, J. Jofre, A. R. Blanch, and M. Muniesa. 2019. New approach for the simultaneous detection of somatic coliphages and F-specific RNA coliphages as indicators of fecal pollution. *Sci.Total Environ.* 655:263-272. <https://doi-org.sire.ub.edu/10.1016/j.scitotenv.2018.11.198>
3. Toribio-Avedillo, D., J. Martin-Diaz, P. Blanco-Picazo, A. R. Blanch, and M. Muniesa. 2020. F-specific coliphage detection by the Bluephage method. *Water Res.* 184:116215. <https://doi-org.sire.ub.edu/10.1016/j.watres.2020.116215>
4. Méndez, J., Toribio-Avedillo, D., Mangas-Casas, R., Martínez-González, J. 2020. Bluephage, a method for efficient detection of somatic coliphages in one hundred milliliter water samples. *Sci Rep* 10, 2977 (2020). <https://doi-org.sire.ub.edu/10.1038/s41598-020-60071-w>

## Some review on coliphages as indicators

1. Blanch, A. R., F. Lucena, M. Muniesa, and J. Jofre. 2020. Fast and easy methods for the detection of coliphages. *J.Microbiol.Methods* 173:105940. <https://doi-org.sire.ub.edu/10.1016/j.mimet.2020.105940>
2. Jofre, J., F. Lucena, A. R. Blanch, and M. Muniesa. 2016. Coliphages as Model Organisms in the Characterization and Management of Water Resources. *Water* 8:199. <https://doi.org/10.3390/w8050199>



# 6. Bluephage S.L., a spin-off from University of Barcelona



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Safe water for a **better world**



November 2016

# 6. Bluephage S.L., a spin-off from University of Barcelona

Parameter	ISO	Bluephage EASY KIT	Bluephage RAPID KIT
Sensitivity	1 PFU/1mL	1 PFU/1mL 1 PFU/100mL	1 PFU/100mL <sup>&amp;</sup>
Ease to use	*	**	***
Applicability	Water, Food, Biosolids	Water, Food, Biosolids	Water, Food, Biosolids
Availability of strains	Not included	Included	Included
Preparation of material (h) (previous steps)	<b>40-60</b>	<b>0</b>	<b>0</b>
Preparation of inoculum culture (h)	<b>3-4</b>	<b>2</b>	<b>0,16</b>
Results time (h)	18-20h	18-20	<b>6</b>
Hours Technician	<b>4</b>	<b>1</b>	<b>&lt;0.5</b>



<sup>&</sup>Sensitivity 79% and Accuracy 83,8%

\*Not very easy \*\*Easy \*\*\*Very easy



Depending on the Kit (volume analysed)



# 6. Bluephage S.L., a spin-off from University of Barcelona

## 1. Preparing the sample



36 ± 2°C

Prewarm the water sample at 36 ± 2°C. It is very important that the sample reaches this temperature before starting the analysis.

## 2. Preparing the host strain



Room temperature

Prewarm the Host Strain Vial at room temperature. Remove the seal from the cap and gently rotate it clockwise to release the host strain. Shake vigorously to resuspend the host strain and let it stand for 10 minutes upside-down at room temperature.

## 3. Water sample



36 ± 2°C

Measure 100 mL of the water sample. The 100 mL mark is just an orientation reference. Open the BP Testing Bottle and pour in the water sample.

## 4. Mixing the resuspended host strain and the sample



Open the Host Strain Vial and check that all the contents in the cap have been resuspended. Pour the host strain into the BP Testing Bottle. Close the bottle.

## 5. Starting the test. Resuspension of Bluephage Medium.



Press the top of the BP Testing Bottle cap to make the Bluephage Medium fall inside the bottle. Shake gently to completely dissolve the Bluephage Medium in the water sample. Do not turn the bottle up and down.

## 6. Running the test



36 ± 2°C 1 h  
+  
30 ± 2°C 5 h

Incubate the BP Testing Bottle at 36 ± 2°C during 1 h. Then, transfer the BP Testing Bottle to an incubator at 30 ± 2°C during 5 h more. Total incubation time is 6 h.

## 7. Reading the results



Read the test results after 5h. If the yellow colour is maintained (negative), it indicates absence of somatic coliphages. If colour changes to green-blue (positive), there is presence of somatic coliphages. The colour change always occurs before 6h if there is a presence of bacteriophages, even at very low concentrations (1 PFU / 100 mL). Do not extend the incubation and reading of tests beyond the time when the negative control presents a start of color change due to enter in the natural death phase of the culture. In case of confirmation of negative results, see Spot Test at Section 8.

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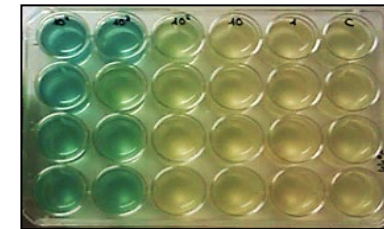
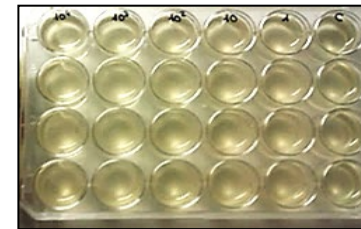


- Somatic coliphage analysis
- All kinds of water matrices
- Sample volume 100 mL
- Results in pfu / 100mL
- Results after 6 hours of incubation

## How is it quantified?

Digitization of colour change through image analysis and mathematical calculation. A confidence interval, automation and traceability of samples are established.

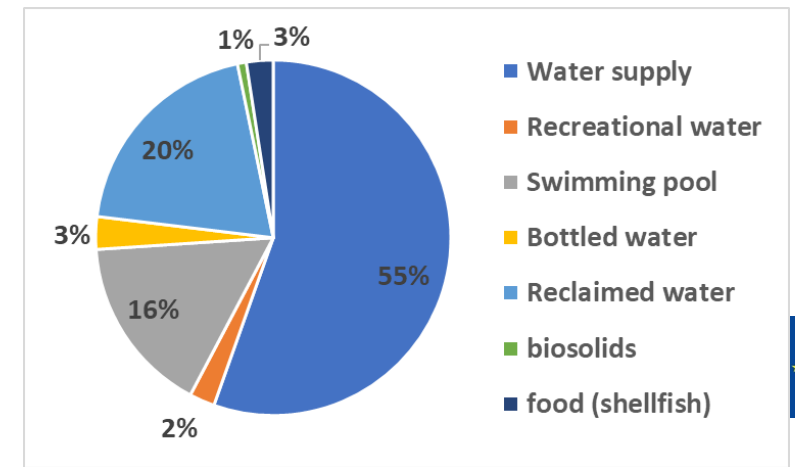
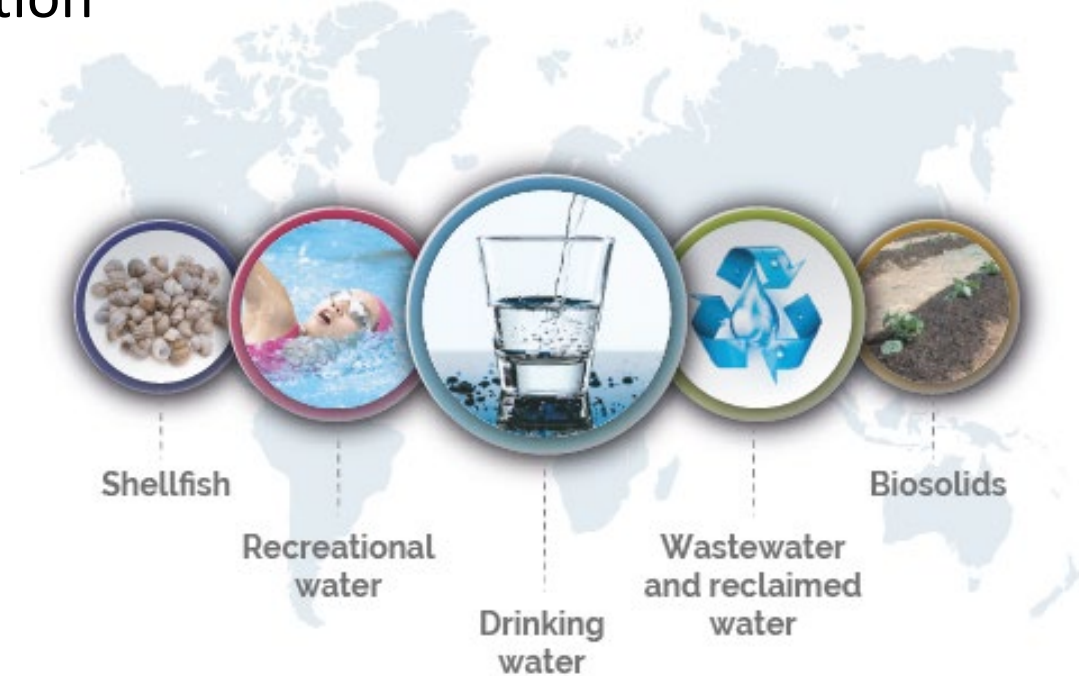
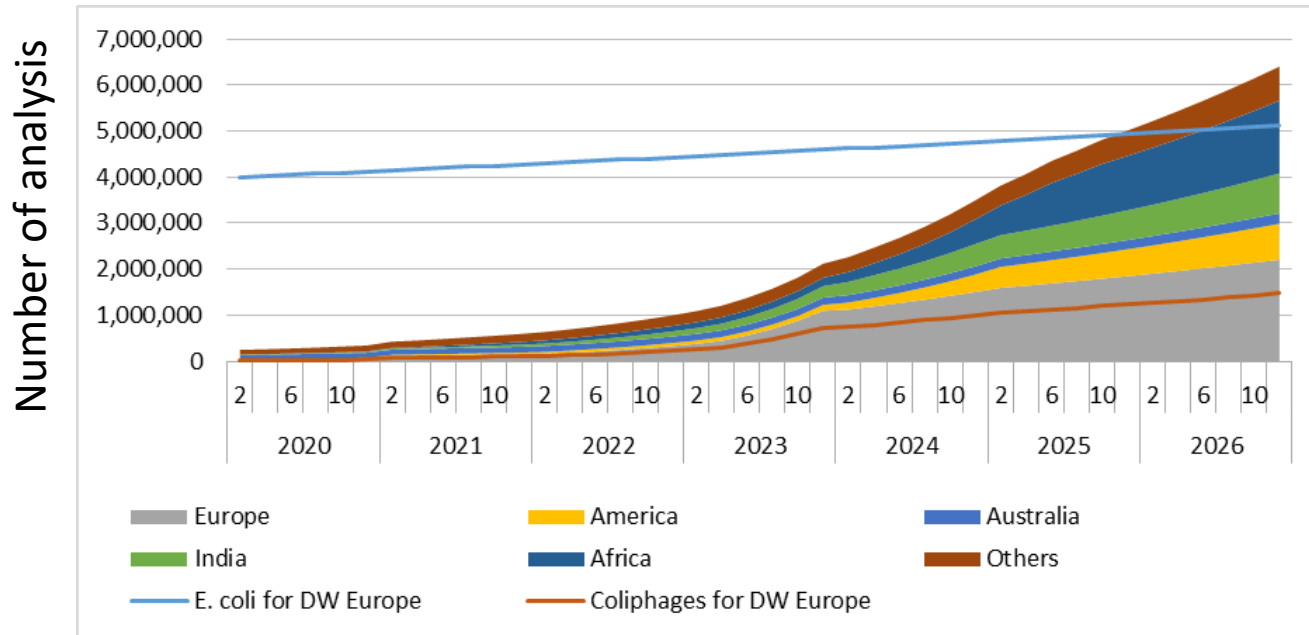
- Camera device (mobile, tablet...)
- APP Download
- Photography
- Reading results



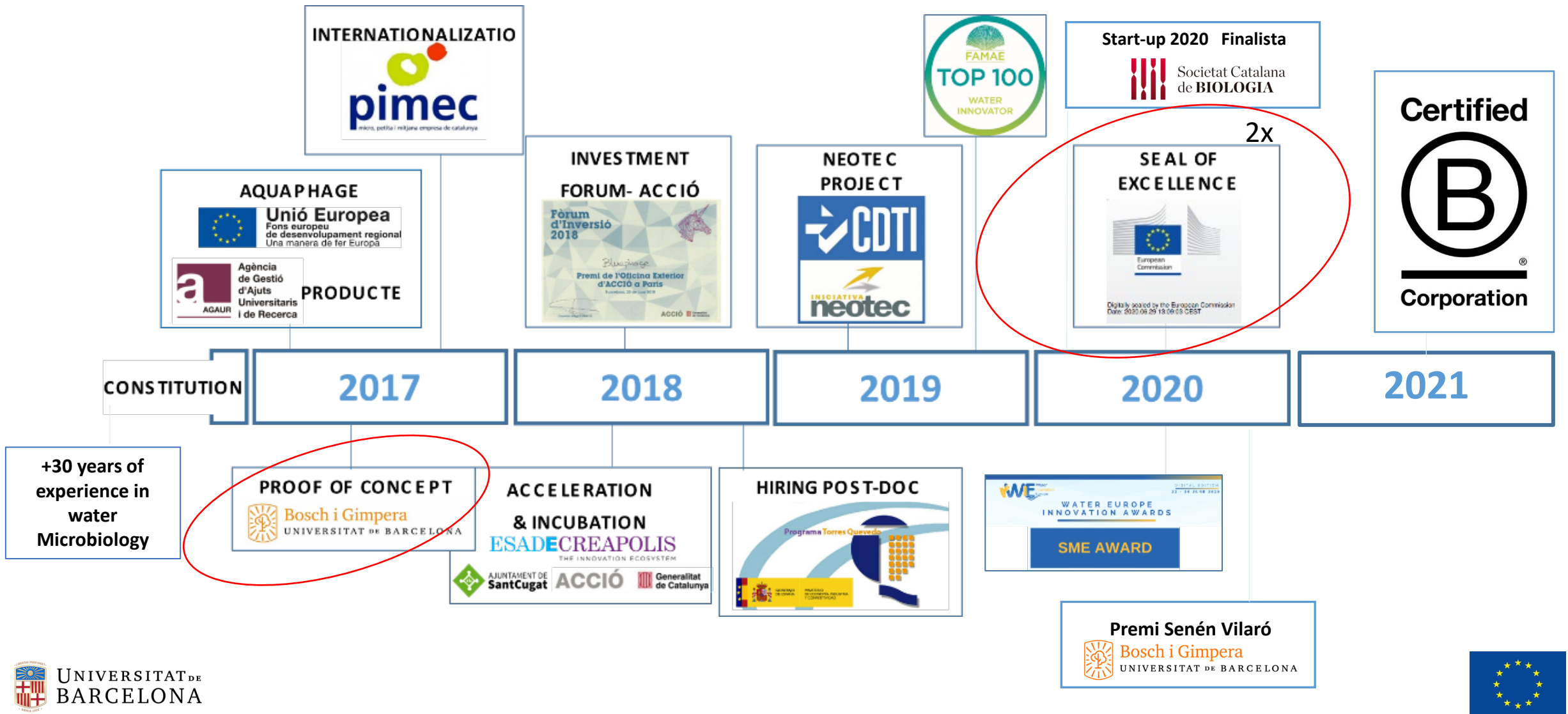


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Water analysis: a growing market driven by regulation



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Commercial Director



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CDO



Miriam Pascual  
CQO



Ariadna Jorba  
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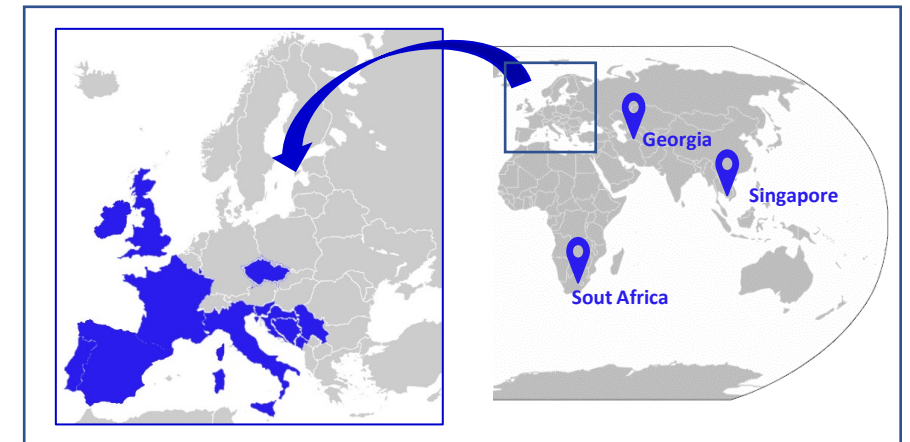


@bluephageWATER



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## DISTRIBUTORS





## Acknowledgements

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**Toribio-Avedillo, Daniel**



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