About Advanced Microfluidics SA : *Advanced Microfluidics SA is an innovative microfluidic device company, specialized in high performance and customized automated liquid handling systems and OEM microfluidic components with a focus on fluidic performance, reduced cross contamination thanks to low internal volume instrumentation and no dead volumes fluidic solution for laboratories and Industries. AMF is based in Switzerland next to the prestigious EPFL research center."* ***Visit us at amf.ch***

MICROFLUIDIC APPLICATION NOTE

Microfluidic flow cytometry application

for bacteria online monitoring in drinking water using BactoSense technology

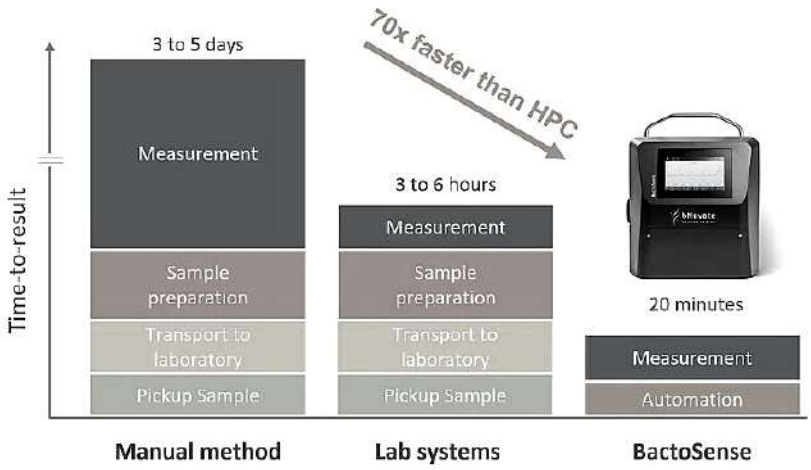

Figure 1. *Time-to-result comparison between HCP manual method, FCM Lab systems method and the BactoSence automated flow cytometer.*

Maintaining drinking water safety and measuring its quality is a permanent challenge. Currently, water evaluation uses the traditional HPC (Heterotrophic Plate Counts) manual method and FCM (flow cytometry) method for ***bacterial enumeration*** *to monitor microbiological quality of drinking water. The HPC method has poor sensitivity and accuracy (~99% bias)* and requires long analysis times with results coming after many days of incubation compared to* ***flow cytometry*** *(~ 99% accurate; ± 15-20 min analysis time)*. But the remaining issue with traditional FCM method, is the time-consuming part of pickup sample, transport to laboratory and sample preparation, which anyway lengthen time of the overall process*.*

BactoSense, the first online fully automated flow cytometer for microbial monitoring

Our partner ***bNovate Technologies*** *develops an automated flow cytometer for online monitoring of bacteria in drinking water.*

BactoSense is a **fully automated flow cytometer at microfluidic scale**. It gives the possibility to monitor directly on-site because each process from sample preparation to reporting is fully automated inside the device which is extremely **compact and precise** because using only **small volumes of sample, thanks to microfluidics.**

Figure 2. *Picture of the BactoSense compact technology*

**Manickum, T. (2020). Total Colony Counts (TCC) By Flow Cytometry (FCM) Should Replace the Heterotrophic Plate Count (HPC) Test for Bacteriological Enumeration of Water-Some Recent Developments in Flow Cytometry: A Review.*

How does it work ?

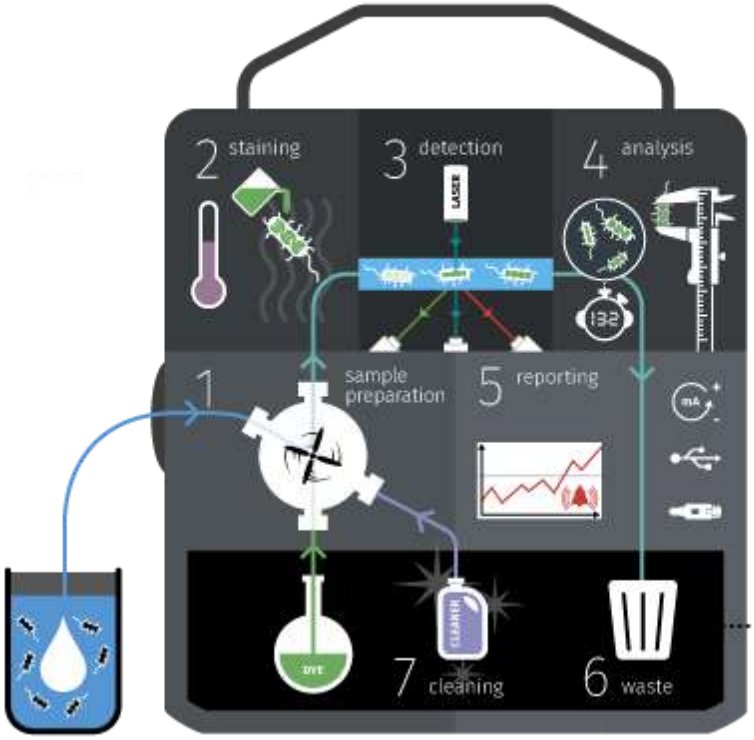

Figure 3. Inside functionalities of the BactoSense fully automated sample preparator and microfluidic flow cytometer.

After an **automated sample preparation (1)** and **staining (2)**, it is precisely counting microbial cells in water in only 20 minutes using the **microfluidic flow cytometry detection method (3)**. With automated online monitoring prior distribution, the BactoSense is designed for continuous surveillance, and immediate warning in case of contamination.

All chemicals including waste are stored in an **integrated cartridge (6)**. It is so easy to operate that it can be simply plugged in and used by anyone to monitor water installations, water distribution networks, or water quality control.

1 Automated microfluidic sample preparation

This process is fully automated thanks to the **AMF Sample preparation module** mounted in the system, ***shown on figure 4***. A 2mL sample volume of water is required. The water sample is taken and **SYBR Green I (SG)** dye solution is distributed evenly throughout the sample, and automatically mixed.

2 Sample staining

Once mixed, the sample is then automated heated at 37°C for 10 minutes, allowing the incubation phase where the dye attaches to the DNA of microbial cells.

3 Microfluidic flow cytometry used for bacteria cells detection

Optical detection technique:

Fluorescence: 535/43 (FL1), 715 LP (FL2), Side scatter 488/10 (SSC).

The resulting DNA-dye-complex fluoresces, giving off green light when illuminated by blue light: it absorbs at 497 nanometers blue light (λmax = 497 nm) and emits green light (λmax = 520 nm) detected by FL1 sensor. The detection limit is 100 cells/mL- 5000000 cells/mL.

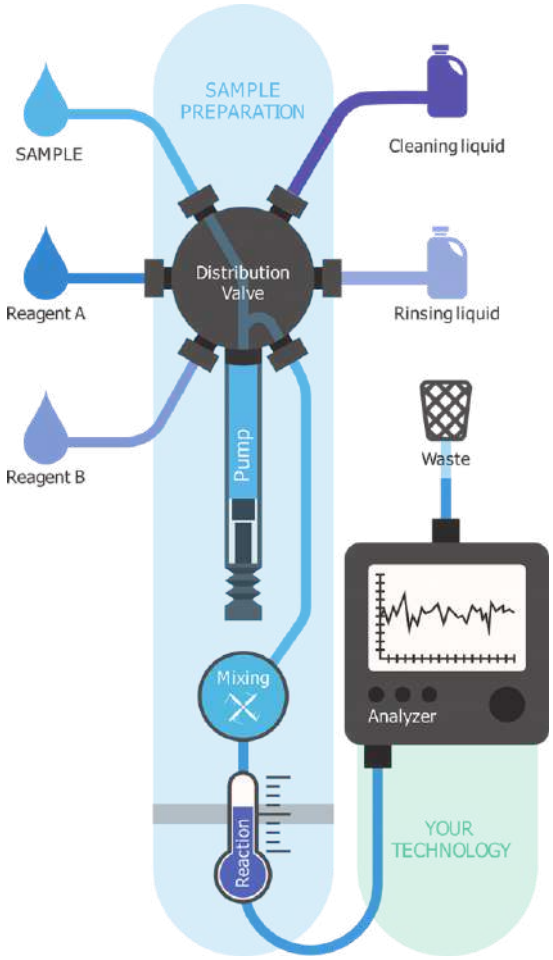

Figure 4. AMF Automated sample preparation principle.

Microfluidic flow cytometry principle:

*Flow cytometry is a common technique used to detect and measure the physical and chemical characteristics of suspended cells or particles in a fluid as it passes through at least one laser. It measures close to **99% of all particles** that are within the liquid sample matrix, so it is a **precise detection technique.** Microfluidic flow cytometers use the **principle of hydrodynamic** focusing for presenting cells to the laser. The sample is injected into the center of a sheath flow. The flow chamber is conical in shape, the reduced diameter forces the cell into the center of the stream. This way the cell passes through the laser one cell at a time.*

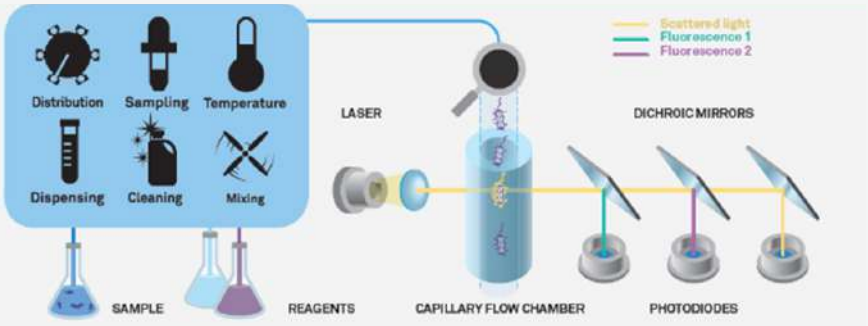

Figure 5. *Flow cytometry principle*

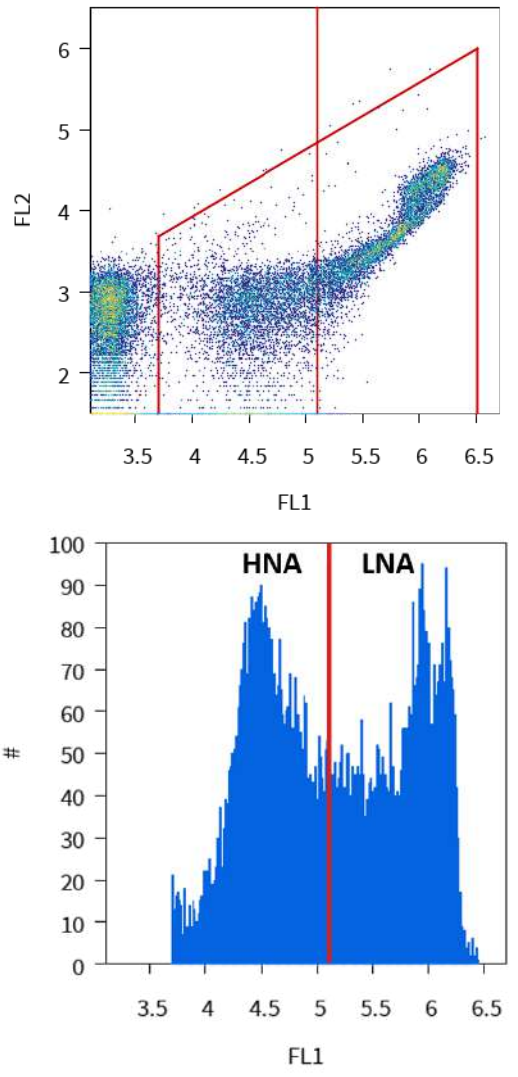

Figure 6. *Cytogram for the analysis of a raw, untreated water sample (Total Cell Count through time) using the BactoSense*

4 Results and analysis

What parameters am I measuring?

Here are several standard parameters to quantify water cleanliness:

- **TCC Total Cell Count /mL** (count of all points within the gate): Current WHO guidelines stipulate a count of zero TTC bacteria for ingested water
- **ICC Intact Cell Count**
- **HNAC High Nucleic Acid Count /mL**
- **LNAC Low Nucleic Acid Count /mL**
- **HNAP High Nucleic Acid Percentage** = The HNAP (%) is the High nucleic acid percentage = HNAC/TCC × 100%.

With the sample analysis, every cell generates signal peaks on the **3 channels: SSc, FL1 and FL2**. The signal peaks of FL1 and FL2 are represented by one dot in the dot plot, ***as shown on figure 6***.

Finally, a cytogram is generated by the data processing: The TCC (total cell count) value is the count of all points within the gate. The LNAC/mL (low nucleic acid count) is the count of all points in the "right" gate compartment. The HNAC/mL (high nucleic acid count) is the count of all points in the "left" gate compartment. The **detection limit is 100 cells/mL- 5000000 cells/mL**.

5 Reporting

The results are available within 20 minutes. A large intuitive touch screen serves as user interface. The measuring interval and further settings can be programmed simply and quickly thanks to the embedded software.

6 & 7 Waste management and cleaning

The generated waste products are **enclosed in the sealed cartridge**, which allows for safe handling and easy disposal. The system is cleaned and rinsed. This involves waste generation and removal. This reduces sample contamination and the effect on subsequent measurements.