

# A new method for monitoring hygiene in beverage plants

**FLOW CYTOMETRY** | The risk of primary and secondary infection in beverages can be reduced if a beverage production facility is clean at all times and in all areas. Ensuring this is one of the central and cost-intensive tasks of beverage production. But how can you be sure that all pipes and equipment are always clean? How can this be monitored almost in real time?

**VARIOUS METHODS** have been available for this purpose for many years. However, all these methods have in common that it either takes a relatively long time to obtain results or that these are not particularly accurate.

What causes infection? If dirt accumulates on system components or in pipes and

containers, for example as a result of insufficient cleaning, this provides a breeding ground for a large number of microorganisms. In other words, the number of microorganisms increases continuously over a certain period of time. Ultimately, germs harmful to beverages also settle and cause related problems in the product. Thus, it always starts with an initial accumulation of organic dirt and microorganisms.

Due to the fact that dirt and germs are the beginning of an infection, the company Sigrist has tested the newly developed portable flow cytometer BactoSense in various beverage plants.

Extensive tests were carried out in the brewery and beverage industries. Their water network was examined at various points from water inlet to bottling, swab samples were taken from system components, drip samples from bottles were taken from the bottle washing machine and measurements were even made directly in the finished product (see figures 1 and 2).

## So, how is this carried out in practice?

First, a so-called microbiological fingerprint is taken with the flow cytometer and the plant-specific water. For this, a water sample is measured, and after approx. 25 minutes a fingerprint is obtained. This includes determining how high the cell count is, how many living cells are present, how many dead cells are present and also how high the organic proportion of this sample is. The organic proportion is important because it is directly related to the cleanli-

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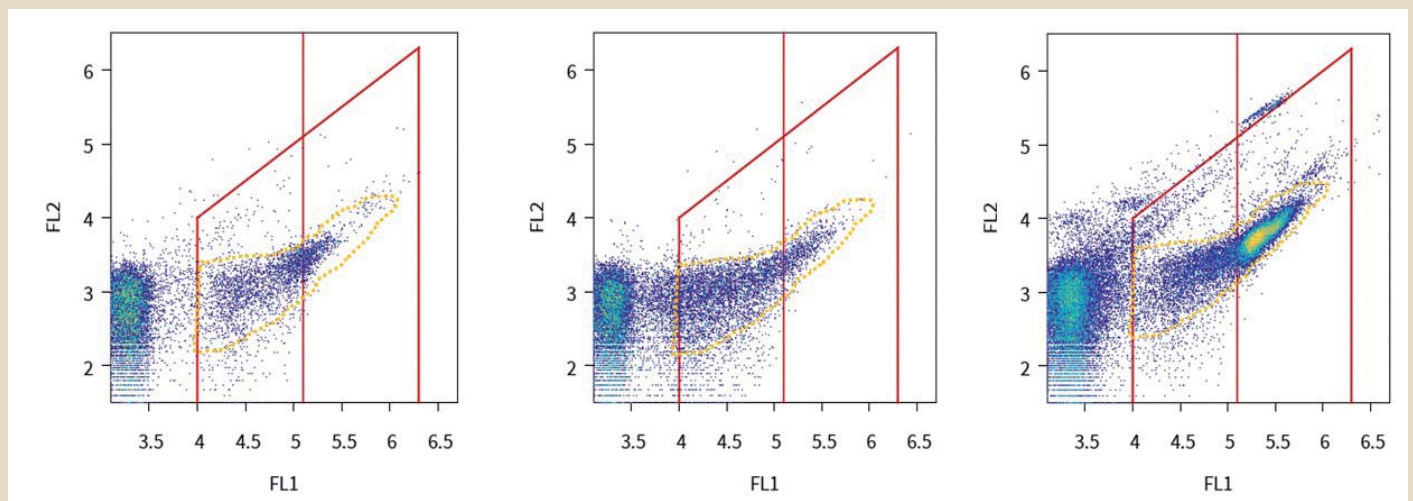


Fig. 1 Dot plots ("finger prints") of various samples. Left: good brewing water; middle: city water; right: contaminated water

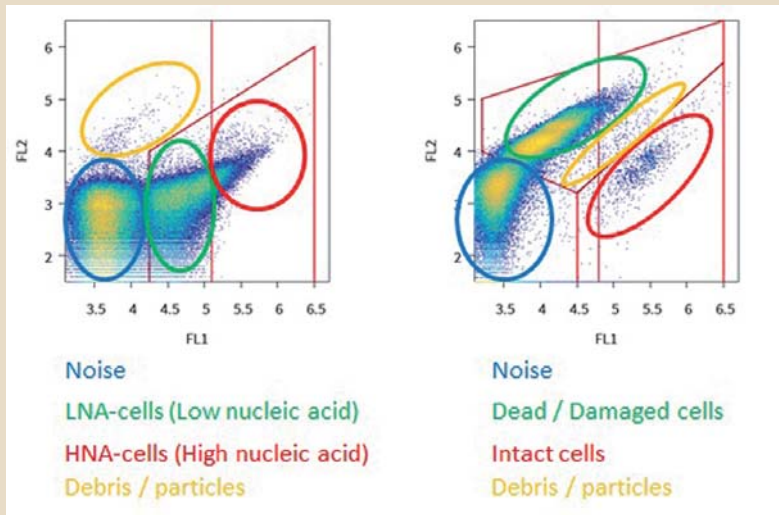


Fig. 2 Comparison of the dot plots of TCC (left) and LDC (right)

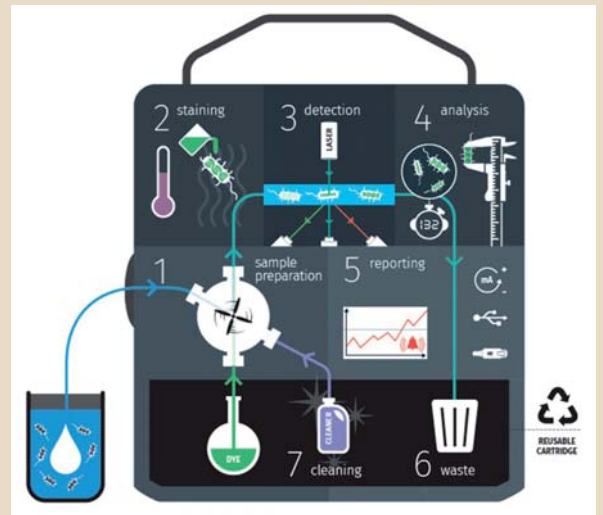


Fig. 3 Process sequence within the BactoSense

ness of the system. High organic content means high food content for recontamination.

**How does the BactoSense work?**

A sample (approx. 5 ml) is fed to the instrument manually or automatically. The sample is automatically mixed with two fluorescent dyes and then measured.

The different dyes penetrate intact (“living”) or damaged (“dead”) cells and cause them to glow green (intact) or red and green (damaged cells). The organic proportion does not absorb the dye and is detected by its own fluorescence and scattering. The entire measurement is fully automatic and finishes each measurement with a cleaning of the device – also automatically (fig. 3).

If water samples are now taken from the pipe system, the fingerprint of the measured water will be close to the original value, as long as the water has not passed through any polluted or contaminated points in the system. If the water has passed through polluted zones, the value of the microorganisms and the organic matter automatically increases significantly from this point. It can thus be determined that there is a problem. By means of a step-by-step control, the problem can now be defined within a very short time.

Since a single measurement only takes about 25 minutes and the device is easily portable, a good overview of the situation can be obtained promptly.

Cleaning of the corresponding components of the plant can be carried out and the success can be checked immediately afterwards.

How can this new technology be used in the case of secondary infections caused by surfaces? By taking defined swab samples from surfaces, such as the infeed starwheel or filling valves, and placing them in a suitable aqueous solution; this solution can then be measured in the BactoSense and, by comparing it with its zero value, the state of the surface can be determined immediately – if there is a lot of organic dirt, or if there are many living or dead cells on the surface. In this way, the cleanliness of surfaces or the effectiveness of a cleaning measure can be determined (figures. 4 and 5).

The advantage of the flow cytometry technology used is that the basic principle has been an established and mature technology for decades but was previously coupled to bulky and complex laboratory equipment. The Sigrist BactoSense now represents a fully automated mobile version of the technology, which can even be installed online directly at a filling line in a beverage plant. In an online installation, for exam-

ple, it was possible to determine the effect of various plant preparation measures (e.g. sterilisation) on the microbiology.

Figure 6 shows the course of the online measurements over one week with the same scaling. The type of sterilization of the plant was varied, and/or new filters had been installed in the fourth diagram.

After applying the optimal method, the possible filling time for mineral water (in this case still mineral water) could be significantly extended from 48 hours to one week. The cleanliness of the filling line was monitored online with the BactoSense almost in real time. The values remained constant over periods of one week. Longer filling times for non-carbonated products with higher product safety are a clear advantage. If the finished product is measured, releases can be issued more rapidly (fig. 7).

Further advantages of this new type of flow cytometer are the simplicity of its handling. Sample preparation as well as measurement and evaluation are fully automat-

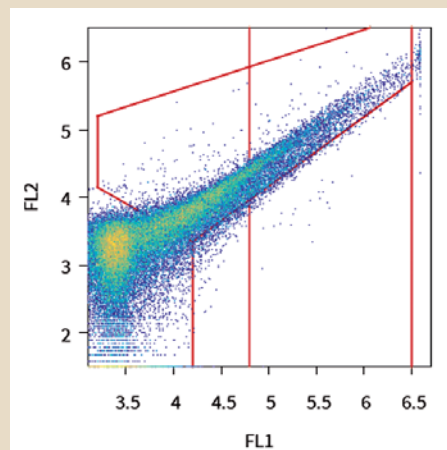


Fig. 4 Swab discharge starwheel

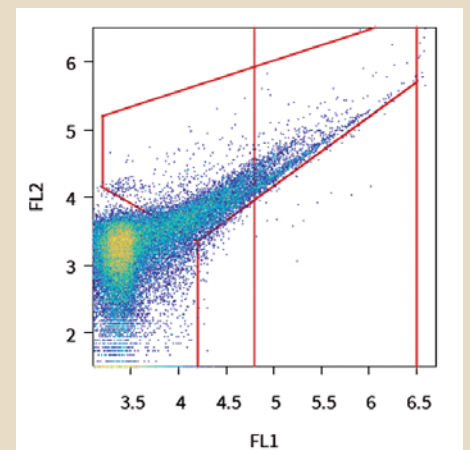
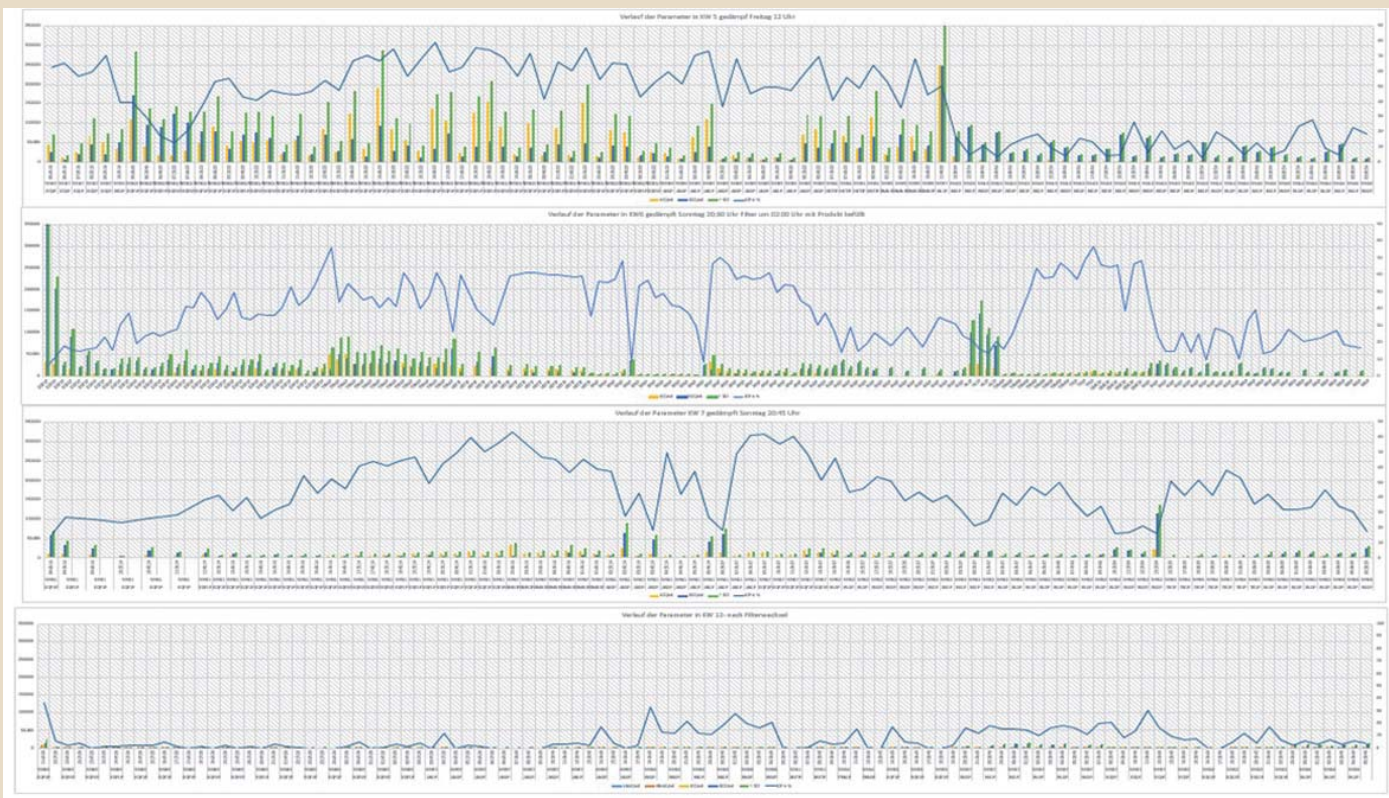


Fig. 5 Swab filling valve



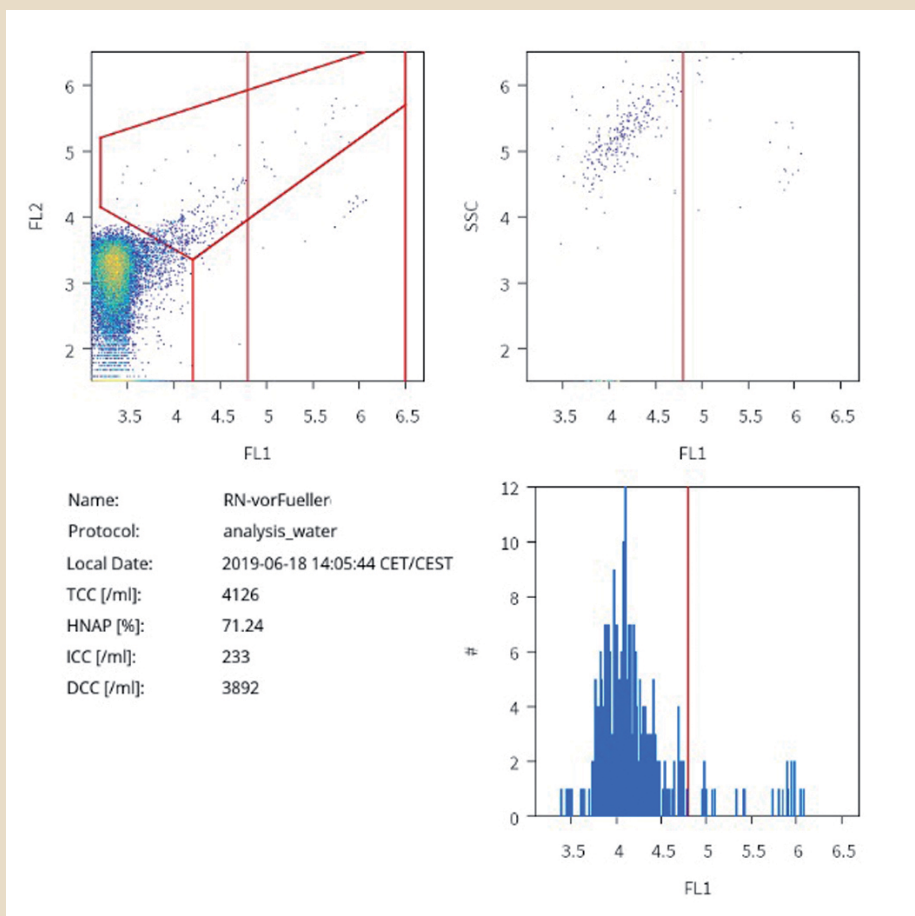
**Fig. 6 Microbiological outcome as a result of differing sterilization of the plant (top and 2x middle) as well as after the installation of a new filter (bottom)**

ic. After each measurement, the instrument is automatically cleaned. No manual work is necessary here. Furthermore, it is not necessary to have special microbiological training. Only the evaluation of the results requires a certain experience and knowledge of the specific fingerprints of the water and the product.

The chemicals used in the Sigrist BactoSense, which have to be handled in classical flow cytometry, are contained in a closed cartridge. With BactoSense, no chemicals, detergents or waste can escape. With this cartridge, approximately 1000 measurements can be made before the cartridge needs to be replaced. This only takes a few minutes and can be done menu-driven with your own personnel and without tools. The cartridges are refilled in an environmentally friendly way so that practically no resources are consumed.

**Conclusion**

The cytometer described here is an easy-to-use microbiological instrument, which provides certainty about the cleanliness and the microbiological condition of the plant almost in real time and in many places. Cost savings can be achieved through optimized cleaning cycles, extended bottling times and faster product releases.



**Fig.7 Result overview, here: sampling prior to bottling**