

Sensitive analyses in a minute cycle

Product release of beverages
with largely automated flow cytometers

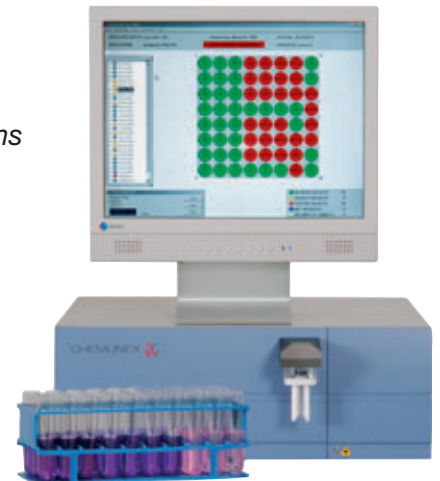
In times of more and more increasing effectiveness, safe and quick product releases are becoming an increasingly important factor. As classical microbiology is limited by the growth rate of microorganisms on culture media, rapid microbiology systems have become more and more interesting to quality assurance managers during the last few years. GETRÄNKEINDUSTRIE interviewed Elke Karches, leader of the microbiological department at Eckes-granini, about the application of fully automated flow cytometers for release testings of finished products. (sw)

GETRÄNKEINDUSTRIE: What were the reasons for Eckes-granini to purchase a rapid microbiology system for quality assurance?

Elke Karches: Because of the new orientation of our marketing strategy and the shifting demands of the consumer, the packaging for Eckes-granini products was changed from the traditional

glass bottle to the modern PET bottle at the beginning of 2004.

The basis for this change is a completely new filling technology with highest demands for aseptic working. In contrast to traditional hot filling, the aseptic cold filling has no protection against contamination in the form of heat influences during the filling process. This means



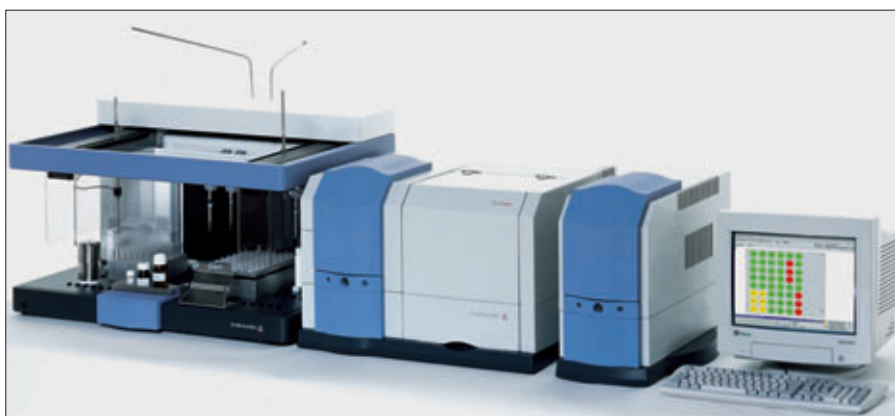
BactiFlow, a semi automatic flow cytometer, is perfect for small and medium batches.

that every microbiological contamination can spoil the product. Besides comprehensive measures that accompany the production, the microbiological assessment of the process and the finished products plays a key role for the assessment of the plant hygiene. In order to be able to make a statement concerning aseptic as fast as possible, we decided to introduce a rapid microbiology system in addition to the installation of the PET plant.

GI: Why did you decide to introduce the D-Count method of Chemunex?

Karches: We wanted the rapid method to meet the following requirements:

- Largely automatic sample treatment,
- A detection level for contaminations as low as possible,



D-Count is a fully automated flow cytometer for medium and large workload laboratories.

- Detection of all microorganisms that can spoil the product,
- Reliability and reproducibility of the results.

A comparison of different methods that are available on the market showed that D-Count is the best system to meet our requirements.

GI: *How did the introduction of the rapid microbiology system take course? Which aspects ought to be considered before and during the installation?*

Karches: In any case, the method should to be validated against the analysis that was used before. For this purpose, a diploma thesis was carried out at Eckes-granini. Therefore, the products that were to be examined were provided with different spoiling germs and the detection of the germ growth was carried out in parallel, with the classical and the cytometric method. As there was a good conformance of the results, the method could be taken as a standard examination method.

A big advantage of this approach was that one person who is not involved in the daily life of laboratories was able to deal extensively with the new apparatus and to pass the experiences on to the subsequent users. The testing method had been validated already before the initial operation of the PET plant, initial problems could be solved already in the beginning, and there was already a certain routine in the handling of the cytometer.



Elke Karches is leader of the microbiological department at Eckes-granini in Nieder-Olm.

GI: *In which production sections does D-Count have the greatest advantages?*

Karches: *The current application of the analysis mentioned is limited to the release examinations of finished products. Within the scope of internal*

Fully automated flow cytometer

The D-Count® method by Chemunex for the microbiological release of fruit preparations and beverages is a fully automated flow cytometer with an autosampler which provides user friendly, very sensitive and quantitative analyses in a minute cycle. Usually, the time for product releases is reduced by between two and five days.

The flow cytometers are able to examine the whole product range of carbonated beverages – this comprises beverages with fruit pulp as well as alcoholic beverages – largely without any interfering influences caused by the product matrix. Apart from that, this method also detects viable and possibly product affecting germs which don't grow on the respective cultural medium (viable but not culturable).

The system automatically labels microorganisms in the products with the patented viability marker Fluorassure, which provides a reliable differentiation between viable and non-viable cells.

After the microorganisms have been labeled, the sample is automatically injected into the quartz flow cell of the fully automated flow cytometer. Here, the fluorescently labelled microorganisms separately pass a laser beam and they are detected by sensitive photo detectors. Afterwards, the result of the analysis is stated in cells per millilitre or grammes.

Today, important D-Count® applications in beverage companies are:

	Release after production
Detection of yeast and bacteria in filterable beverages	48 hours
Detection of yeast in filterable beverages	22 hours
Detection of the total number of germs in non-filterable beverages	72 hours
Detection of the total number of germs in concentrates	48 hours
Detection of the sterility in UHT products	48 hours
Detection of the total number of germs in water	30 minutes
Specific detection of enterobacteria in water	10 hours
Specific detection of alicyclobacillus in beverages and concentrates	48 hours

The sample performance of a fully automated flow cytometer allows to perform 250 up to 1000 analyses per working shift, depending on the kind of application. The system is operated by one person.

future development, an enlargement of the application spectrum is being examined.

GI: *How is this method applied and how does it influence the product release?*

Karches: Due to the testing plan which was developed within the scope of the above mentioned diploma thesis, even slowly growing spoiling germs can be detected reliably. The samples are pre-incubated under aerobic conditions for 72 hours, they are examined with D-Count, and in the case of negative results they can be released immediately. Our company thus saves a period of time of about five days compared to traditional plate-casting process.

GI: *Is a classical culture medium method also applied in parallel?*

Karches: At the beginning, cultural comparison analyses were carried out

in parallel to the flow cytometry. Due to continuously matching results and an increasing reliability in the result assessment of the apparatus, we were able to do without the classical examination.

GI: *Do you see advantages of the new method only for the quality assurance, or is there a general advantage for your company?*

Karches: Due to the rapid microbiological results, the quarantine time could be reduced and storage capacity could thus be saved with a constant delivery reliability. At the same time, the microbiological results, which can be considered as a sensitive and realtime sensor, give information about the whole filling hygiene.

GI: *Mrs. Karches, thank you very much for this interview.* □