

The background of the entire cover is a deep blue color with a faint, repeating pattern of various bacterial shapes, including rods and spheres, rendered in a lighter blue tone. This pattern is most visible in the lower half of the cover.

FOSS

ebook

Reducing bacteria count in raw milk

A guide to opportunities with flow cytometry

June 2017

ANALYTICS BEYOND MEASURE

Preface

For decades, an analytical technology called flow cytometry has played a vital role in improving the hygiene quality of raw milk and today, its importance is only growing. In particular, new developments with the technology are opening up the possibility of more on-the-spot hygiene tests as raw milk is received at the dairy or even on-the-farm.

With the prospect of more rapid tests throughout the supply chain, the days of poor hygiene quality samples with many hundreds of thousands of bacteria per ml could quite literally be numbered.

This e-book aims to bring you up-to-date with developments in flow cytometry with articles, easy to follow technical background and video interviews.

We hope that you will find it useful in the ongoing cross-industry effort to bring down bacteria levels in raw milk.

FOSS

Contents

Article: Beating bacteria in milk	4
The flow cytometry advantage	6
Inside a flow cytometry instrument	7
High-volume bacteria count tests in the laboratory	8
Push button bacteria count at the dairy	9
What to look for in a flow cytometry solution	11
Flow cytometry solutions from FOSS	13

Article:

Beating bacteria in milk

A new official approval for testing bacteria count in raw milk with flow cytometry is just the latest gain in an ongoing battle to reduce bacteria count in raw milk.

Even though a sample of raw milk today looks exactly as it did 50 years ago, the bacteria count is likely to be far lower.

This is good news for consumers and dairy producers alike because bacteriological milk quality is an important parameter for heat treatment procedures, shelf-life prediction and determining the suitability of raw milk for processing different products.

For decades, average levels of bacteria in raw milk have been gradually falling around the world, from hundreds of thousands of Colony Forming Units (CFU) per millilitre in the seventies to less than 10,000 CFU per millilitre today. In many countries, producers consistently attain 5,000 cfu/ml in their raw milk deliveries.



Flow cytometry instruments started out in the 1980's and the method is now approved by both the EU-RL in Europe and NCIMS/FDA in the USA.

Nonetheless, the battle to reduce bacteria levels still goes on with rapid bacteria count analysis of raw milk samples using the flow cytometry technology ever important in the front line.

The importance of flow cytometry tests

Flow cytometry methods were first applied to raw milk testing in the early eighties as a way of automating and speeding up the traditional analysis process.

The traditional method gives an indication of quality based on Colony Forming Units while flow cytometry gives the same indication, but is based on the number of individual bacteria cells delivered in less than ten minutes compared to the two to three days it takes with plate counting.

The rapid results have proved important because catching hygiene breaches in raw milk early is essential for minimizing the impact on raw milk quality. Although pasteurisation will kill the majority of bacteria, their metabolites may cause off flavours and enzymes continue their activities resulting in product defects and reduction of shelf life. With faster information, farmers can be advised about possible problems allowing timely correction for leaks, insufficient cleaning or cooling in the milking system, or health problems in the herd.

Growing acceptance

Not surprisingly, flow cytometry attracted interest amongst milk testing laboratories as a rapid method for bacteria count, particularly as more and more countries tightened up on milk quality and increasingly stringent national regulations for hygiene quality came into force.

Official approvals, however, have been slower. One would think that something that could speed up analysis of bacteria would be quickly adopted. But acceptance and approval is a complicated process and has been going on for about 35 years.

With various industry and government bodies involved, many people need to be convinced of the complex nature of the parameter total bacterial count. In particular, the need to correlate the flow cytometry method, based on counting individual bacteria, with the traditional method, based on counting Colony Forming Units, has caused much discussion which is still going on to this day.

Despite the ongoing discussion, flow cytometry has become the industrial standard for counting raw milk bacteria in many countries worldwide, for instance, the FOSS BactoScan™ FC+ instrument has gained approval from both the EU Reference Labs (EU-RL) and NCIMS/ FDA in the USA and today, more than 90 per cent of all milk within the EU is paid for according to BactoScan results.

A further development is the use of flow cytometry in compact instruments for use at the dairy to check the hygienic quality of deliveries opening up a new chapter in the battle against bacteria in raw milk. [Read more on page 9.](#)

The flow cytometry advantage

Taking just a few minutes per test, flow cytometry is much faster than the traditional plate count method which requires two to three days to generate a result.

This availability of test data is important for dairy farm productivity and subsequent use of milk downstream in the supply chain. For instance, rapid bacteria cell count allows hygienic problems on the farm to be spotted quickly and action to be taken to maintain the quality of milk delivered to the dairy.

Milk with a high bacterial load is an undesirable raw material for dairy production especially for products such as butter. Although pasteurization kills the majority of bacteria, their metabolites may cause off flavours, and enzymes continue their activities resulting in product defects and reduction of shelf life. Most milk payment schemes therefore grade milk according to the level of bacteria.

With a rapid bacteria cell count, farmers can be advised about possible hygiene breaches enabling them to correct for leaks, insufficient cleaning or cooling in the milking system or health problems in the herd.

Flow cytometry is also highly consistent. The traditional plate count method is subject to test-to-test variables such as the sample preparation with a petri dish, the timing of the incubation period and a count by the naked eye. In comparison, flow cytometry is an automatic, machine-based test which counts with accuracy, repeatability and reproducibility. It can analyse raw milk samples directly without prior heating or dilution, which could otherwise affect precision.

Superior accuracy through individual bacteria count

Because the flow cytometry method counts all bacteria as single cells and not clusters it gives a uniform high performance with reproducibility of results. Unlike the plate counting method, results cannot be influenced by the operator and raw milk samples can be tested directly without prior heating or dilution, which can affect precision.

Accuracy: Typical $S_{y,x} < 0.25$ log units in the entire measuring range.

Reference method: Standard Plate Count (IDF Standard 100B: 1991).

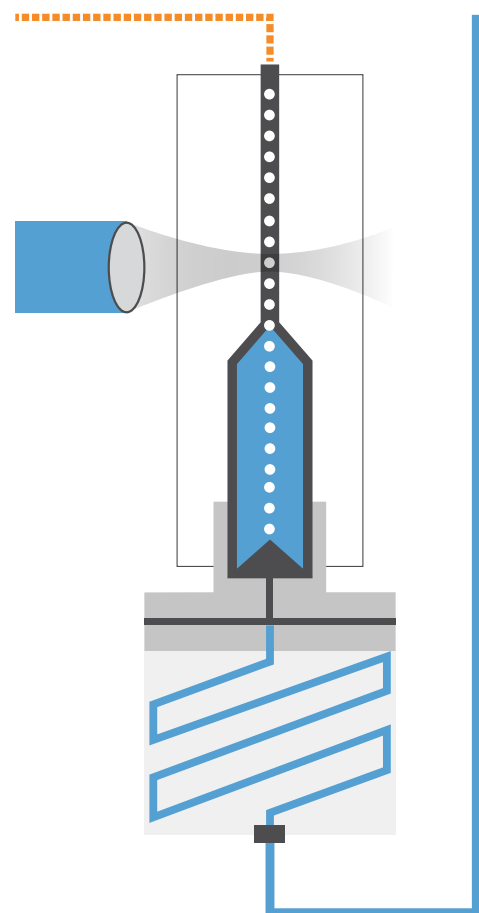
Inside a flow cytometry instrument

Fluorescent flow cytometry is used to analyse the physical and chemical characteristics of particles in a fluid as it passes through laser light. In the context of food and agricultural analysis, it provides rapid analysis of bacteria and somatic cell count in milk samples.

Bacteria count method

Bacteria count demands an initial incubation step to stain the bacteria and to break down all particles, except for cells to be counted.

1. During incubation, the cells are stained with a DNA-specific, staining medium, a fluorescent dye.
2. A precise syringe system is used to pass them through a flow cell one-by-one where they are exposed to a light beam from a laser source.
3. The laser excites fluorescence from the dye and the stained cells emit one light pulse for every cell passing the beam.
4. The fluorescent light is detected by a highly sensitive detector which gives electronic impulses.
5. The pulses are counted and displayed in a pulse height analysis (PHA) diagram on the instrument screen.



A syringe system is used to pass cells through a flow cell one-by-one where they are exposed to a light beam from a laser source

The Bacteria count takes nine and half minutes including the incubation element.

High volume tests in the laboratory

Step inside a modern raw milk-testing facility.

Throughout the laboratory, conveyor belts ferry milk samples to and from analysis instruments with rows of plastic vials lined-up like soldiers on parade, marching neatly towards the waiting analysers. Radio frequency identification (RFID) keeps track of the samples using intelligent sample identification based on programmed chips containing all relevant sample data attached to the bottom of the sample vial.

Today, flow cytometry is used by raw milk testing laboratories around the world to perform thousands of bacteria count tests per day on samples collected from dairy farms. But if a single test takes nine minutes how is this possible?

The key is to handle the time-consuming incubation step in a smart way with multiple incubation facilities to handle several samples at a time. But the demands do not stop there. Handling, testing and tracking thousands of samples also needs to be performed effectively and often the bacteria count test needs to be combined with other tests such as a host of milk quality tests performed by other infrared instruments in the laboratory.

Modern flow cytometry instruments are therefore increasingly integrated with automated sample delivery systems, scanning systems and software systems to keep track of all the data generated. Until recently, flow cytometry for bacteria cell count was largely limited to these sophisticated laboratory environments. Now the advent of new instruments designed specifically for lower volume testing at the dairy has changed that landscape.



Payment samples have an individual chip attached to the bottom of the sample vial.



Push button bacteria cell count at the dairy

The increasing use of flow cytometry in the milk laboratory has naturally led to questions about how to share the power and convenience of tests with dairy producers to follow in the footsteps of other analytical technologies such as near and mid-infrared. These have brought rapid and simple chemical-free tests to the dairy for most quality control areas except one – hygiene.

Here too, progress is now being made that can open up a new flank in the push against bacteria in raw milk.



Bacsomatic™ how it works video

Measuring just 40 x 40 x 40cm, a small flow cytometry instrument from FOSS offers a fast alternative to manual assay or semi-automated methods requiring reagent handling. It delivers results for both bacteria count and somatic cell within nine and half minutes and the handy format makes it ideal for use at the dairy, on large farms or in smaller milk-testing laboratories.

The flow cytometry method is the same as used in the large fully automated instruments used by milk-testing laboratories to process hundreds of tests per hour, but with a design aimed at the lower throughput needs of dairies needing to test raw milk deliveries arriving each day by tanker trucks.

The availability of a rapid and easy to perform test allows rapid decisions on how to handle deliveries and how best to process the milk according to the hygienic quality.

With its smart flow system it is also possible to integrate bacteria and somatic cell count in one small, handy instrument as explained in this video interview with the scientists behind the new solution.

Elements of a good flow cytometry instrument

1. Repeatability



Bacteria counting involves a lot of zeros, for example, around 10,000 bacteria per ml is a perfectly normal count in a high quality milk sample and the numbers can go as high as 1,000,000 per ml in a poor quality sample. A tiny amount of sample-carryover can therefore have a dramatic impact on the result of the next test and, to avoid this, any flow cytometry system must include an effective cleaning flush between tests. Some Bacteria also have a tendency to gather in clumps so effective sample preparation as part of the instrument flow system is an important factor in achieving a good repeatability.

2. Throughput



For applications in raw milk testing laboratories, throughput is the lifeblood of effective customer service and profit for the laboratory.

Fed by automated sample trains, modern flow cytometry instruments can test up to 600 samples per hour for somatic cell count and up to 200 per hour for bacteria count.

The bacteria count is slower due to the incubation step as explained in the bacteria count method description above. However, despite the fact that an incubation takes eight minutes, a clever use of multiple incubation chambers allows a number of tests to be performed in parallel to achieve a high throughput of tests.

A design trade-off is made for smaller handy instruments where manual placement of samples reduces throughput compared to the large fully automated laboratory solutions

used in milk testing laboratories. Nonetheless, the time to result of individual tests should be the same, allowing useful applications in the supply chain, for example, with rapid checks on raw milk arriving in tankers at the dairy.

3. Robustness

As implied by the high-throughput demands, a high volume flow cytometry solution must handle a virtually constant flow of samples or cleaning (flush) liquids. Critical points in the flow system such as the pumps and valves must be robust and any servicing must be made as simple as possible.

4. IBC to CFU Conversion table

The Bactoscan™ and BacSomatic™ instruments report results as Individual Bacteria Count (IBC). However, many laboratories are obliged to present their results in Colony Forming Units (CFU), which are obtained by a traditional plate count method. Software supporting the use of a conversion table to convert from IBC to CFU should therefore be part of the flow cytometry solution.

5. Reagent handling



Liquid reagents are involved in the method for example, for the dye and for cleaning. To make the operators working environment as easy and safe as possible, ready-to-use reagents can be provided in enclosed bag systems which effectively avoids skin contact. The dosage can also be controlled automatically to ensure the same reagent mix and dosage every time, reducing the risk of human error for highly consistent results.

6. True network capability for effective instrument management

True networking is much more than an internet connection. It should allow you to look inside the instrument to check performance and stability. This is a must to allow remote management and surveillance of multiple instruments from a single location for example, to check the results from regular pilot check sample tests.

FOSS products using flow cytometry for bacteria cell count

Learn more about
BactoScan™ here



Based on proven FOSS technology, BactoScan™ measures the hygienic quality of milk by analysing bacteria in raw milk. Delivering results in just minutes, BactoScan allows farmers or milk testing laboratories to take action fast to preserve and enhance milk quality. Up to 200 tests per hour can be performed.

Learn more about
BacSomatic™ here



BacSomatic™ is the first ever integrated bacteria and somatic cell counter for testing the hygienic quality of milk.

The small, easy-to-use instrument offers a fast alternative to manual assay or semi-automated methods requiring reagent handling. It delivers accurate results for bacteria count and somatic cell in less than nine and half minutes or in less than one and half minute for somatic cell count alone. The handy format makes it ideal for use at the dairy, on large farms or in smaller milk-testing laboratories.