

# BD FACSMicroCount



Helping all people  
live healthy lives

A comprehensive solution for  
rapid enumeration of microbes



# BD FACSMicroCount Solution

The BD FACSMicroCount™ system offers a comprehensive solution for rapid enumeration of microbes. The integrated system consists of an instrument and reagents to support quality manufacturing processes.

The BD FACSMicroCount system can provide results in minutes instead of days for most samples. It detects a wide range of microorganisms including bacteria, yeast, mold, spirochetes, *Mycoplasma*, and parasite cysts. Test results show excellent correlation with traditional methods. Designed for ease of use, the system automatically adds up to three reagents to the sample, mixes, and performs qualitative (pass/fail) and quantitative (counts/mL) tests.

With this degree of automation, the BD FACSMicroCount system provides greater consistency than traditional test methods by eliminating manual steps that can lead to variable results. The system enables organizations to achieve new levels of operational efficiency and continuous manufacturing process improvements, including reduced production cycle times and inventory levels, and improved warehouse utilization. The system also enables a swift, cost-effective response to contamination events.

The BD FACSMicroCount system can address quality testing requirements for manufacturers of pharmaceutical products, personal care products, beverages and probiotics, as well as home cleaning products.

BD training, technical application support, and field service teams are dedicated to helping customers achieve optimal instrument performance, ease of use, and streamlined workflow. Available to advise on a variety of quality testing requirements, the BD support teams are fully committed to the success and satisfaction of their customers.

## How the system works

# Automation Delivers Rapid and Consistent Results

The BD FACSMicroCount flow cytometer uses sophisticated fluidics, laser optics, electronic detectors, analog-to-digital converters, and a dedicated computer to measure microbes in a variety of matrices. An equal level of sophistication and attention to detail is designed into the user interface and software to make the BD FACSMicroCount simple to use.



The instrument can be set up to measure microbes using pre-defined tests or user-defined assays. Four predefined methods in the BD FACSMicroCount software automate testing of performance standards, *E. coli*, product screening, and in-process water tests. The system automates sample processing by programming sample injection, reagent additions, mixing, and data collection parameters. Predefined methods can be optimized to fit unique manufacturer requirements and standard operating procedures.

The BD FACSMicroCount cytometer can load up to 42 samples at a time from four sample trays. Both trays and tray blocks are magnetized so that trays are automatically detected when loaded in the instrument. Samples are prepared for analysis by labeling microbes with a nucleic acid-specific fluorescent dye. The patented BRAG3\* labeling compound is used in conjunction with the nucleic acid dye as a viability stain. It decreases fluorescence intensity of membrane-compromised cells and diminishes background counts due to extraneous debris.

	Product Screening	Water Analysis	Microfermentation
Assay Format	Qualitative	Quantitative	Quantitative
Sample Volume	0.1 mL default	Variable volume range 0.10–0.50 mL	Variable volume range 0.10–0.50 mL
Throughput/Hour	20 samples	12–15 samples	12–15 samples*
Reagents	Up to 2	Up to 3	Up to 3
Reagent Volumes	200 µL Nucleic Acid Dye 100 µL BRAG3	100 µL Nucleic Acid Dye 100 µL BRAG3 Buffer, 100 µL	Variable ranges—method specified*
Reaction Time/Reagent	90 seconds	175 seconds	Method specified*

\*Dependent on selected method parameters

Application	Time to Result BD FACSMicroCount
Product Screening*	24 hours
Water Screening	5 minutes
Direct Swab/Environmental	5 minutes
Direct Enumeration	Under 8 minutes
Bioburden	Under 10 minutes

\*Using the BD FACSMicroCount, most personal care, OTC pharmaceuticals, and household cleansers take 24 hours. Exception: toothpaste (30 hours), fiber capsules (48 hours).

# EASE OF USE

The BD FACSMicroCount system reduces the time to result over traditional methods, yielding reductions in production cycles and cost savings. These time savings can translate directly to additional savings from reductions in inventory and warehouse costs.



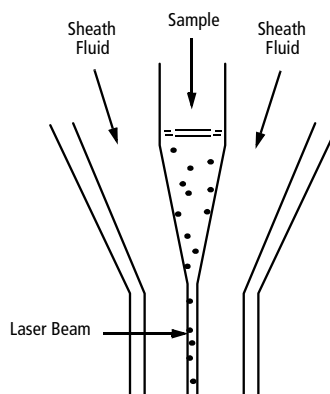
The BD FACSMicroCount system has 3 standard sample trays that load up to 12 samples and fit into positions 2, 3, or 4 of the tray block. The other tray can load up to 6 samples and fits into position 1 of the tray block. This tray can be used for priority samples.



Reagent ports dispense up to three reagents as specified in predefined or custom-designed software setups.

## Hydrodynamic Focusing of the Sample Core through the Flow Cell

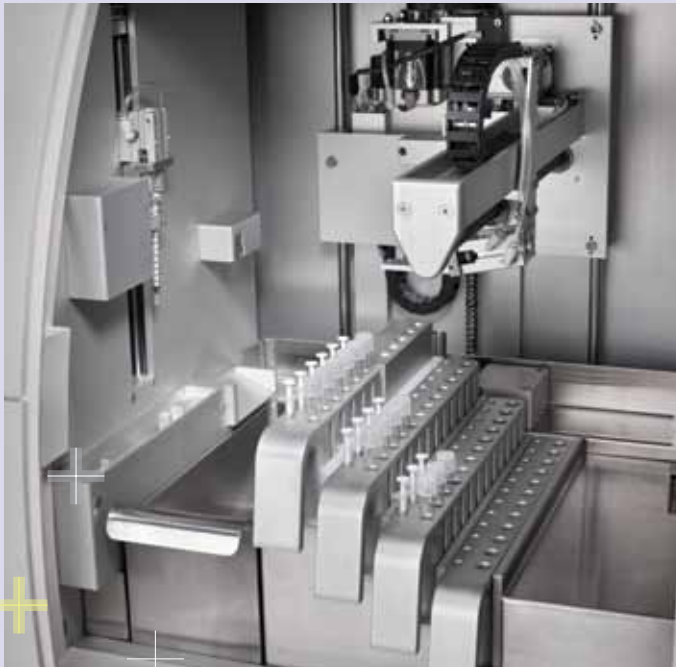
Hydrodynamic focusing guides the microbes in a single-file stream to intercept the laser beam. Once the microbes pass through the laser beam, the optics system collects fluorescence and side scatter information and the data is displayed in real time as an intensity plot or counts/mL.



## How the system works

# Rapid Sample Analysis for Qualitative and Quantitative Results

The BD FACSMicroCount system handles up to 20 samples per hour for qualitative analysis (presence/absence) and up to 15 samples per hour for quantitative analysis (enumeration). Unlike other systems, the BD FACSMicroCount can count live or dead cells, which can be valuable in fermentation applications.



The BD FACSMicroCount software creates an electronic record of each sample, identifying it by date and end time. Data can be evaluated for individual samples or compared to other data sets. Results can be viewed in graphical or tabular data displays to speed analysis. Graphical displays include intensity, fluorescence, side scatter, and counts vs. time. Data analysis can be performed while the instrument is running new samples.

### Types of Microorganisms

- Gram-positive bacteria
- Gram-negative bacteria
- Mycoplasmas
- Spirochetes
- Anaerobes
- Spores – bacterial and mold
- Parasite cysts
- Filamentous bacteria, yeasts, and mold

### Microorganisms Enumerated

- *Aeromonas caviae*
- *Aeromonas hydrophila*
- *Aspergillus niger* spores
- *Bacillus atrophaeus*
- *Bacillus atrophaeus* spores
- *Bacillus pumilus*
- *Bacillus pumilus* spores
- *Bacillus subtilis*
- *Bacillus subtilis* spores
- *Bordetella bronchiseptica*
- *Brachyspira hyodysenteriae*
- *Burkholderia cepacia*
- *Campylobacter jejuni*
- *Candida albicans*
- *Candida glabrata*
- *Citrobacter freundii*
- *Clostridium perfringens*
- *Cryptococcus* spp.
- *Cryptosporidium parvum* oocysts
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Enterococcus casseliflavus*
- *Enterococcus durans*
- *Enterococcus faecium*
- *Enterococcus faecalis*
- *Enterococcus gallinarum*
- *Enterococcus hirae*
- *Enterococcus mundtii*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli*
- *Escherichia coli* O157:H7
- *Escherichia coli* O25:HN
- *Escherichia coli* O15:NM
- *Escherichia coli* O1:NM
- *Escherichia coli* O7:NM
- *Escherichia coli* O78:NM
- *Escherichia coli* ON:H8
- *Escherichia coli* ON:NM
- *Escherichia coli* O8:HN



A robotic arm moves samples and syringes inside the instrument. A pneumatic gripper on the arm, controlled by a pneumatic air line, holds samples and syringes. A vortexer, located to the back of the instrument, mixes samples.

- *Geobacillus stearothermophilus*
- *Geobacillus stearothermophilus* spores
- *Giardia lamblia* cysts
- *Haemophilus parasuis*
- *Haemophilus somnus*
- *Halobacterium salinarum*
- *Klebsiella pneumoniae*
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Lactobacillus delbrueckii*
- *Lactobacillus lindneri*
- *Lactobacillus plantarum*
- *Lactococcus lactis*
- *Lawsonia intracellularis*
- *Leptospira pomona*
- *Listeria grayi*
- *Listeria innocua*
- *Listeria ivanovii*
- *Listeria monocytogenes*
- *Listeria seeligeri*
- *Listeria welshimeri*
- *Micrococcus candidans*
- *Micrococcus luteus*
- *Moraxella bovis*
- *Mycoplasma bovis*
- *Mycoplasma hyopneumoniae*
- *Nannocystis exedens*
- *Oxalobacter formigenes*
- *Pantoea agglomerans*
- *Pasteurella multocida*
- *Pediococcus acidilactici*
- *Pediococcus damnosus*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Pseudomonas fluorescens*
- *Pseudomonas putida*
- *Ralstonia pickettii*
- *Raoutella terrigena*
- *Saccharomyces cerevisiae*
- *Salmonella adelaide*
- *Salmonella anatum*
- *Salmonella choleraesuis*
- *Salmonella dublin*
- *Salmonella enteritidis*
- *Salmonella hadar*
- *Salmonella heidelberg*
- *Salmonella iverness*
- *Salmonella schalwijk*
- *Salmonella typhimurium*
- *Salmonella worthington*
- *Serratia marcescens*
- *Shigella boydii*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus saprophyticus*
- *Stenotrophomonas maltophilia*
- *Streptococcus bovis*
- *Streptococcus equinus*
- *Streptococcus pyogenes*
- *Tsukamurella paurometabola*

Personal and home care products

# Screening Finished Products and Raw Materials Streamlines the Process and Reduces Risk

The BD FACSMicroCount reliably screens raw material and finished products for microbial contamination to help manufacturers monitor product quality. By delivering pass/fail results for the presence or absence of microbial contamination within 24 to 48 hours, the system offers significant benefits over traditional methods that typically require holding raw materials in quarantine for several days.



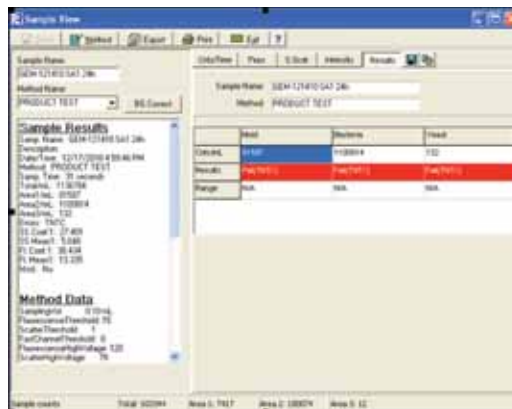
The BD FACSMicroCount system can screen bacteria, yeast, and mold in a single test with results that correlate with traditional plating methods. This application uses the BD FACSMicroCount Product Screening Media Kit to enrich the sample with bacteria, yeast, and mold for rapid growth. In traditional plating methods, bacteria screening is separate from the screening for mold and yeast. Thus, the extra speed of the BD FACSMicroCount method offers additional time savings.

The qualitative product screening application uses a defined method to increase output to 20 samples per hour, the fastest operating mode of the system. This application employs a default sample volume of 0.1 mL and uses only two reagents for reaction times of approximately 90 seconds per reagent, allowing preparation of multiple samples at once.

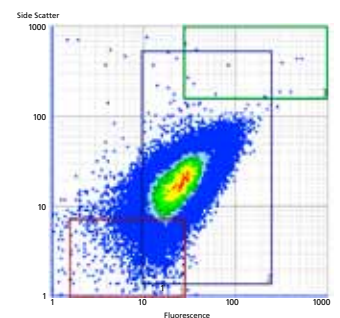
Faster screening cuts the time needed to release products, which directly translates to savings from reduced manufacturing cycle times and inventory levels, and improved warehouse utilization.

## Data Analysis

Data can be presented in graphical or tabular format and data review can be conducted while other samples are being processed. Specific pass/fail criteria can be saved in the methods page for each product sample, creating visual highlighted (red-fail result and green-pass result) indicators for the user to see.



Tabular results



Intensity view results



# PRODUCT SCREENING



## Procedure to Prepare Sample for Processing

### Sample Preparation

Suspend the sample in phosphate buffer or growth enhancement media (GEM) that contains soy lecithin and Tween@20 as neutralizers, and thoroughly mix to achieve a homogeneous product suspension.

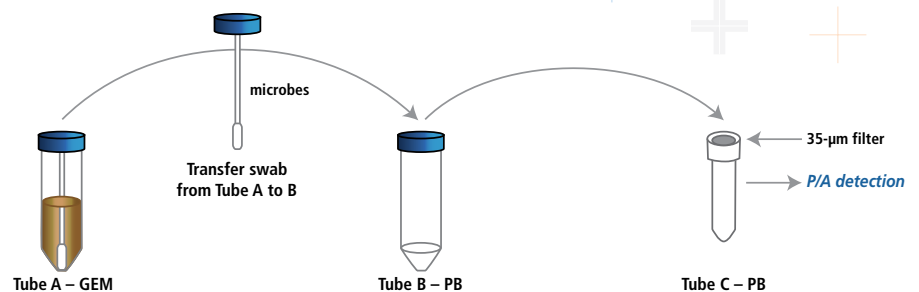
Transfer 1 mL of the suspension to Tube A.

### Enrichment

Incubate the samples for 24 to 48 hours at  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with gentle agitation.

### Post-Enrichment Processing

Transfer the swab from Tube A and place it in Tube B, and vortex for 15 seconds. Leave the tube undisturbed for 10 minutes.



Place a filter cap on Tube C and filter 100  $\mu\text{L}$  of the sample from Tube B into Tube C. Load samples onto the BD FACSMicroCount cytometer and analyze.

## Water quality testing

# Screening Purified and Process Water for Microorganisms

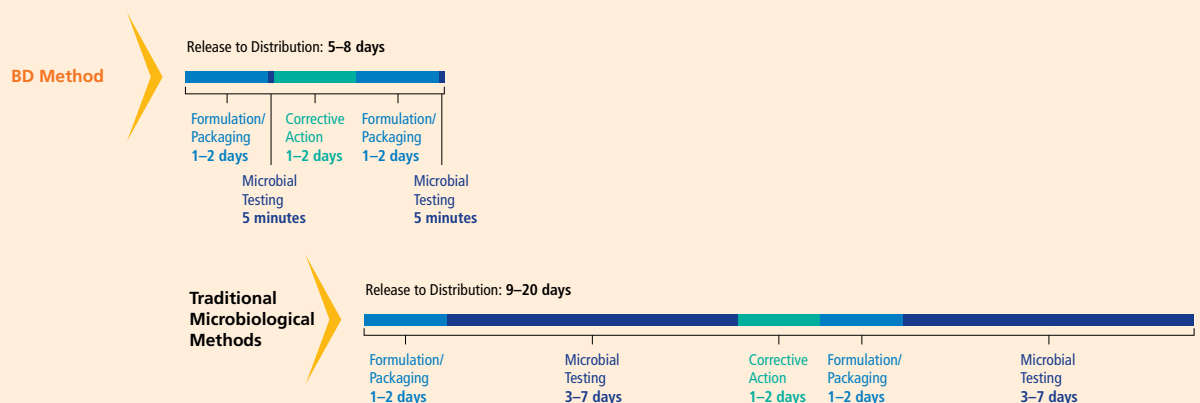
Purified water is a primary ingredient in the manufacturing of pharmaceuticals, personal care products, and many other industrial products. Monitoring the microbial content of water ensures product quality and helps manufacturers realize reductions in maintenance and operating costs by identifying sources of clogging and microbial corrosion.



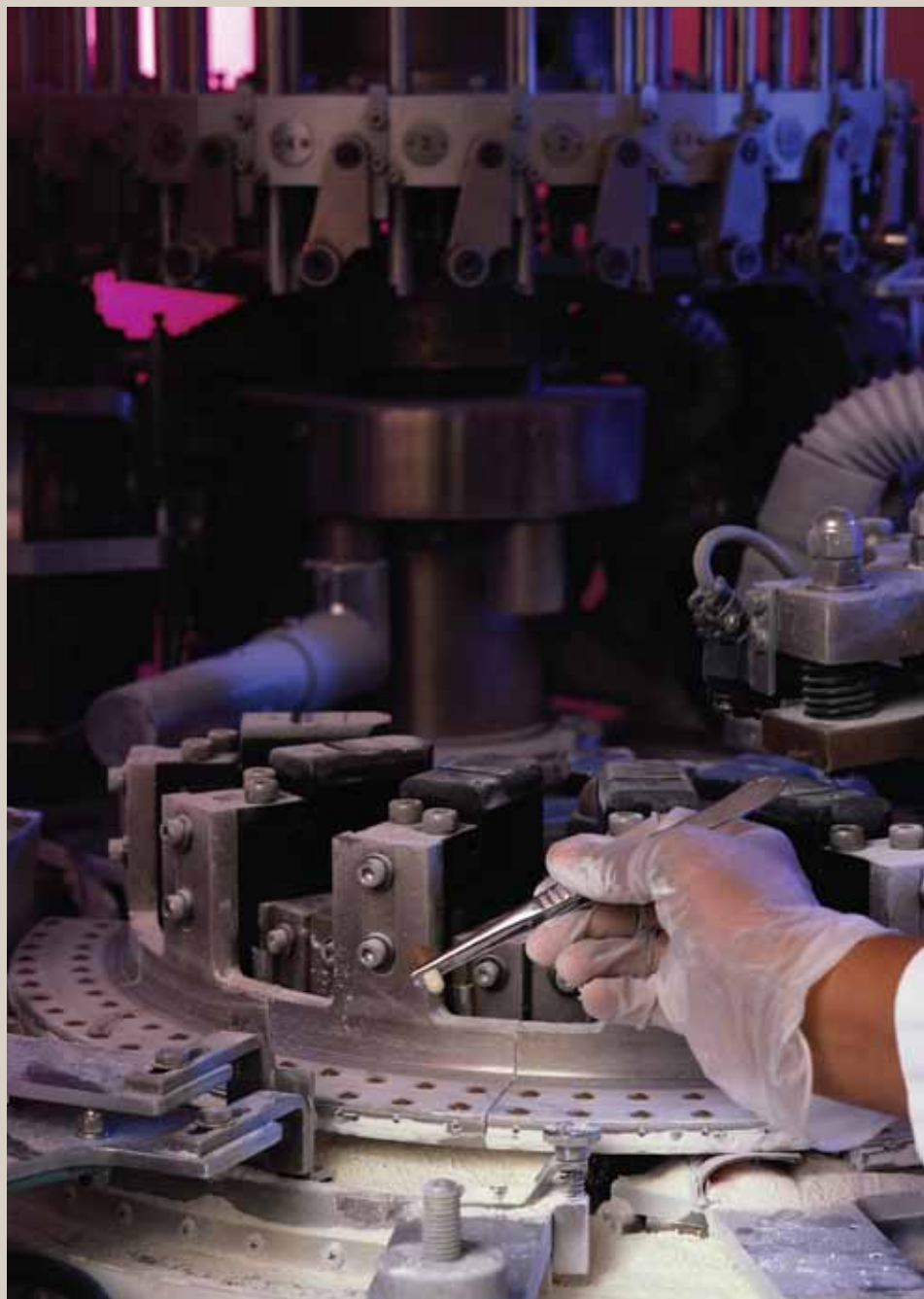
The BD FACSMicroCount system is an easy-to-use and rapid screening solution that provides reliable, quantitative results in less than 5 minutes, so testing is completed with significant time savings over traditional testing methods, which can take several days.

The BD FACSMicroCount automates the process to keep the testing as simple as possible. After the water sample is collected, it is transferred to a sterile tube and loaded into the instrument. When the Water Analysis application is selected, the software activates the high-throughput option and automatically performs reagent additions, mixing, and sample injections. For rapid preparation of multiple samples, the default reaction time for each reagent is 175 seconds. Up to 42 samples can be loaded at a time, and the application can test up to 15 samples per hour.

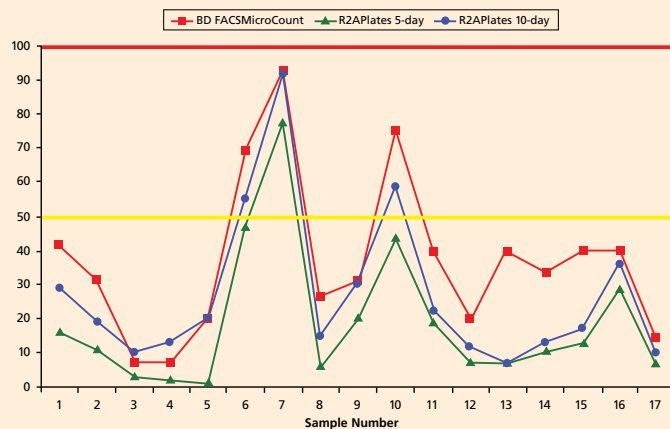
### Comparisons of Estimated Response Time for Contamination Events



The BD FACSMicroCount system enables manufacturers to respond to contamination events faster by giving results in just 5 minutes instead of the 7 days needed in traditional methods. In addition, by eliminating manual counting steps that can lead to variable results, the system provides greater consistency than traditional test methods. This reliability comes with no compromise in accuracy: quantitative results from the BD FACSMicroCount system correlate and trend with traditional plate count methods.



This graph demonstrates the trend among BD FACSMicroCount and traditional testing using R2A plates incubated for 5 and 10 days for 17 samples. In this test, the alert limit was set to 50 cfu/mL (yellow line). For each sample, results from the BD FACSMicroCount correlated to traditional methods. Importantly, for samples #6 and #10, the traditional method took 10 days to reflect the microbial content of the sample, yet the BD FACSMicroCount produced the same result in just 5 minutes.



Results from BD FACSMicroCount and R2A 5- and 10-day Plate Count.

# Monitoring Microbial Fermentation and Enumerating Stock Cultures

Microbial fermentation is an important component of industrial microbiology used to manufacture drugs, enzymes, and vaccines, as well as human and animal food additives. Accurate and timely testing of the total biomass, total number of viable microorganisms, or total dead cell count can help prevent batch loss and more accurately determine the best time to harvest to optimize yield.

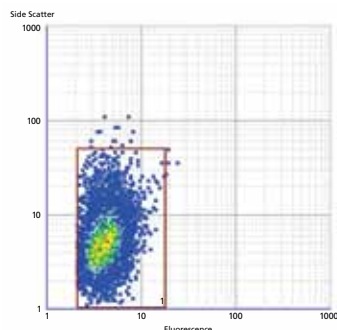


The BD FACSMicroCount can produce reliable, quantitative results in 4 to 5 minutes, accurately enumerating a broad range of microorganisms, including fastidious organisms, *Mycoplasmas*, gram-positive and gram-negative bacteria, yeasts, molds, spores, spirochetes, and anaerobes. This delivers significant time savings over traditional testing methods that can take over 10 days.

By enabling rapid testing, the BD FACSMicroCount empowers manufacturers to realize “real-time” monitoring across all phases of the fermentation process. Constant monitoring allows for optimization at each phase:

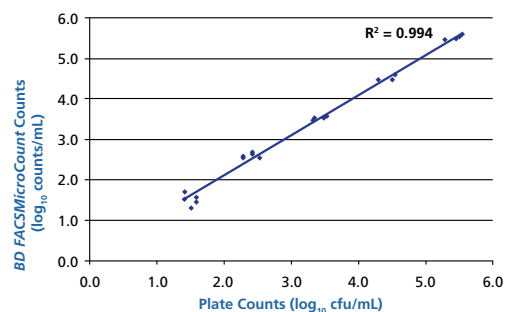
- **In upstream testing** the system can detect media contamination and determine starter culture titer. Early detection of raw material contamination can minimize the impact of a downstream contamination event.
- **During fermentation monitoring** total biomass, viable, and dead cell counts can be enumerated. This total count (including dead cell counts) is unique to the BD FACSMicroCount system and offers valuable information to help determine the best time to harvest the biomass.
- **Downstream testing** can determine cell concentrations in both in-process and in-final product samples such as inactivated bulks and concentrates.

## Animal Health



**BD FACSMicroCount scatter plot showing counts of *Streptococcus equi*.**

Each dot on the plot represents an *S. equi* cell counted by the BD FACSMicroCount. The result was  $3.92 \times 10^9$  counts/mL on the BD FACSMicroCount with a corresponding plate count result of  $3.40 \times 10^9$  cfu/mL.



**Correlation of BD FACSMicroCount counts versus plate counts for *Streptococcus equi*.**

Here the BD FACSMicroCount results directly correlate with results from traditional methods (eg, plate count, color changing unit, hemacytometer). Time to result was less than 5 minutes per sample.

The BD FACSMicroCount reagent kits are available for enumeration of biomass, viable organisms, and dead cells. The system automatically performs reagent additions, mixing, and sample injections. The system holds a maximum of 42 samples, but also allows for continuous operation utilizing a batch process.

By eliminating manual counting steps that can lead to variable results, the BD FACSMicroCount system provides greater consistency than traditional test methods. Studies that compared counts/mL from the BD FACSMicroCount analysis and cfu/mL by traditional methodology showed excellent correlation and agreement.

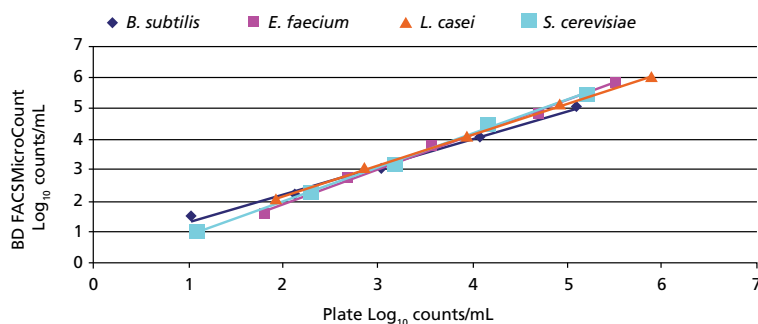


### Correlation of BD FACSMicroCount Viable Counts vs. Plate Counts of *B. atrophaeus* 9372, *C. albicans* 10231, *E. coli* 25922, and *M. bovis* 25025.

This figure shows the correlation of results from the BD FACSMicroCount with the traditional plate method throughout the dynamic range of 10 to 10<sup>6</sup> cfu/mL.

Microorganism	Number of samples	R <sup>2</sup>
<i>Bacillus atrophaeus</i>	25	0.986
<i>Candida albicans</i>	30	0.976
<i>Escherichia coli</i>	34	0.993
<i>Mycoplasma bovis</i>	23	0.974

### Fermentation and Probiotic Manufacturing



BD FACSMicroCount counts versus plate counts for *Bacillus subtilis* (R<sup>2</sup>= 0.9931), *Enterococcus faecium* (R<sup>2</sup>= 0.9944), *Lactobacillus casei* (R<sup>2</sup>= 0.9997), and *Saccharomyces cerevisiae* (R<sup>2</sup>= 0.9966).

# Services and Support

**BD instruments and reagents are backed by a world-class service and support organization with unmatched experience in microbiology and flow cytometry.**

For over 25 years, BD has actively worked with researchers to develop tools that help improve workflow, ease of use, and performance. With in-depth knowledge and experience, BD delivers comprehensive training, application expertise, excellent technical support, and world-class field service.

## **Training**

Held at BD training centers worldwide, training courses combine theory and hands-on practice to provide participants with the skills and experience they need to take full advantage of the capabilities of their instruments.

## **Technical application support**

BD technical application support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, BD technical application specialists are well equipped to address customer needs in both instrument and application support.

## **Field service engineers**

BD's field service engineers are located across the world. When instrument installation or service is required, a BD field service engineer can be dispatched to the customer site. On-site service and maintenance agreements are available to provide long-term support.

## **IQ/OQ**

An Installation and Operational Qualification (IQ/OQ) certifies that an instrument performs according to its specifications in the environment where it is installed. BD provides IQ/OQ validation for its instruments. This service should be quoted and scheduled in advance of the installation.



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BD flow cytometers are Class I (1) laser products.

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