

Solutions for:

Finished Product and Raw Material **Screening for Bioburden**

Stock Culture **Enumeration**

**Monitoring** Microbial Fermentation

Purified and Process Water **Total Viable Organism Screening**

**Microbial Enumeration** for Probiotic Manufacturing



- Microbial enumeration in **4 minutes**
- **Pass / Fail** results for presence / absence of microbial contamination in **24 to 48 hours**
- Yeast, bacteria and mold detection in a **single run**
- **20 samples per hour** for qualitative analysis (presence / absence)
- Up to **15 samples per hour** for quantitative analysis (enumeration)
- Several reagents for enumeration of **live cells, dead cells or total biomass**
- **Graphical displays** include intensity, fluorescence, side scatter and counts vs. time
- **Data analysis** possible while the system processes other samples



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# BD FACSMicroCount™



*Confidence in microbial testing  
solutions to save you time.  
Time is money.*

*Rapid*  
Microbiology – it's what we do



Helping all people  
live healthy lives



## Rapid Detection of Bacteria, Yeasts and Molds

### Versatile and easy to use to save you time

- Easy to use with automated sample enumeration and screening, requiring minimal training
- Easy to validate with traditional methods
- Highly versatile with the ability to run various sample types

### BD FACSMicroCount™ System

Flow-cytometry based solution for rapid bioburden testing and rapid enumeration of microbes

Use the **BD FACSMicroCount** for:

**Product Release Validation** Meet regulatory requirements; direct correlation with traditional methods

**In-Process Quality** Earlier bioburden results allow early intervention and reduce risk of compromising future product

**Convenience** Multiple sample types and test protocols run simultaneously

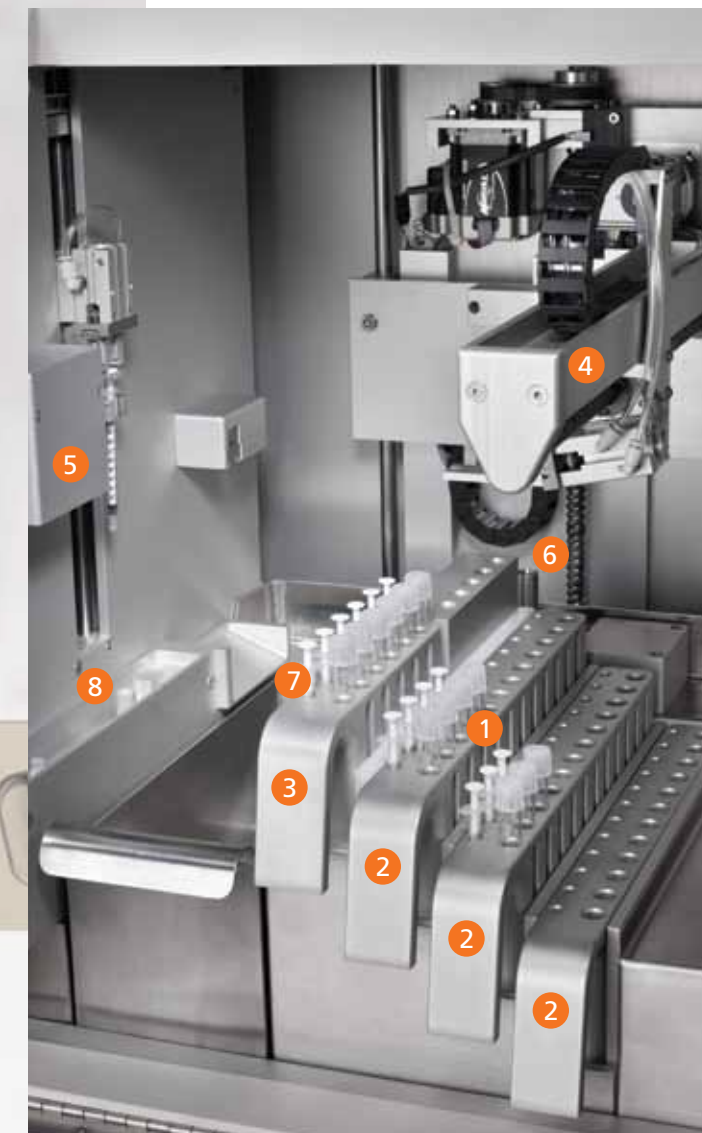
**Operational Cost Savings** Reduce inventory-related costs

**Labor Cost Reduction** Easy to use workflow and walk-away automation enables flexibility of resources

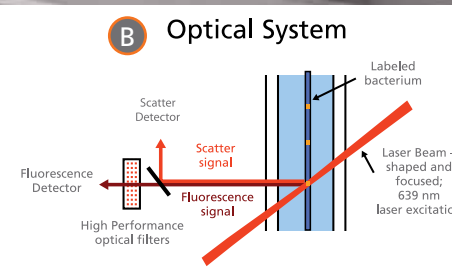
KNOW that your product contains the following microorganisms with the **BD FACSMicroCount** System:

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

## Fully Automated System: How it Works



- VIALS**
  - 3 mL sample in each vial
  - 42 samples can be loaded at a time
- SAMPLE TRAYS**
  - 3 Sample Trays for 12 samples each
  - Magnetized for automatic detection when loaded in system
  - Can be pulled out and loaded in the middle of a run to keep test going
- PRIORITY TRAY**
  - Holds 6 samples
  - Takes precedence over other samples if introduced in the middle of a run
- ROBOTIC ARM**
  - Enables Sample Gripper to move vials and syringes to desired ports
- REAGENT DISPENSER**
  - Up to 3 reagents dispensed
  - Microbes are labeled with nucleic acid dye to fluoresce
- VORTEXER**
  - Mixes sample with injected reagent
  - Vortexed sample incubated in Sample Tray
- SYRINGES**
  - Draws 1 mL aliquot from sample
- SAMPLE INJECTION PORT**
  - Between 0.1 mL – 0.5 mL sample is injected into this port for flow-cytometry based detection



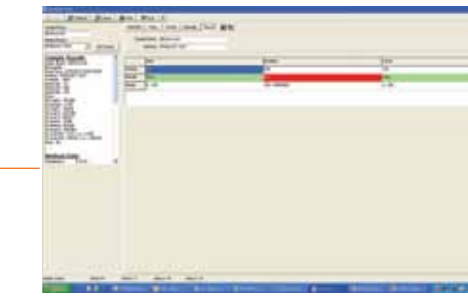
- FLOW CELL**
- Hydrodynamic focusing guides microbes in single file stream
  - Laser beam causes fluorescence
  - Optics system collects fluorescence and side-scatter information to confirm microbial contamination

A to D Area used on system  
1 to 8 Procedure



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

## How it Works



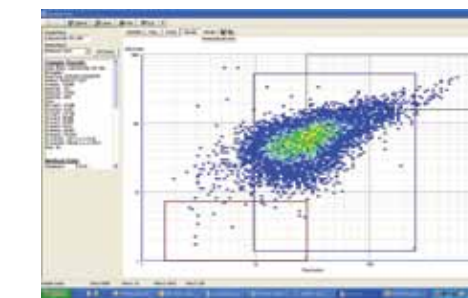
**DATA ANALYSIS**  
(is presented in tabular and graphical format)

### TABULAR FORMAT

- Can be reviewed while other samples are being processed
- Pass / Fail criteria can be specified to provide visual highlights (green for pass, red for fail, etc.)



D 3 reagent ports to dispense up to 3 reagents based on defined method

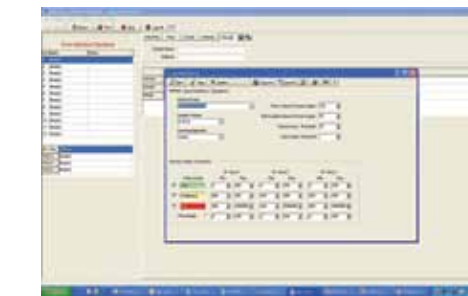


**DATA ANALYSIS**

### GRAPHICAL FORMAT

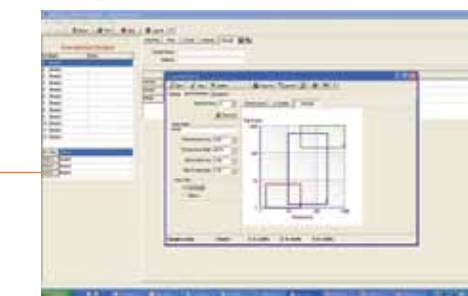
- Can be reviewed while other samples are being processed
- Detection of bacteria, yeasts and molds possible in the same run for product screening
- Total Viable Organism (TVO) count can be provided for specific samples

C



**OWN METHOD CREATION**

- STEP 1** Prepare sample for analysis using  $10^4 - 10^5$  cfu / mL concentration
- STEP 2** Create a new method file using Utilities and Methods links
- STEP 3** Set new method parameters including Sample Volume, Counting Algorithm, and Results Criteria on intuitive screen
- STEP 4** Define up to 3 areas of interest (e.g. mold, bacteria and yeast) per method
- STEP 5** Define up to 3 reagents per method



**EXISTING METHOD OPTIMIZATION**

- STEP 1** Use Utilities and Methods links to select desired method
- STEP 2** Use Area Definitions tab to add or modify new or existing area definitions through click and drag operations
- STEP 3** Use Settings tab to modify results criteria

# Microbial Enumeration of Probiotics

Rapid  
^ Microbiology – it's what we do

## APPLICATION OBJECTIVE

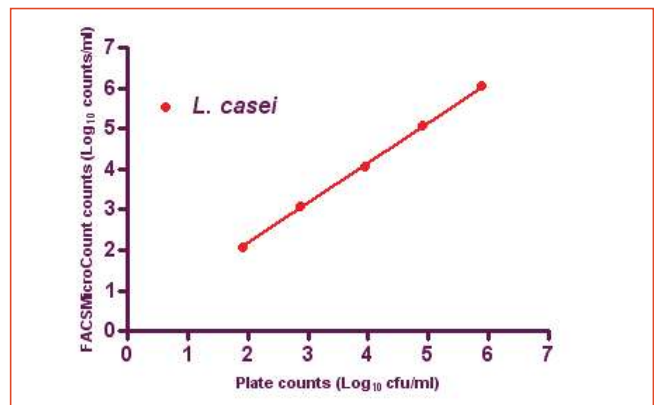
- Rapidly and accurately enumerate microbial levels in probiotic products
- Enables manufacturers of probiotics to monitor microbial content:
  - during growth through the entire fermentation process to optimize yield
  - at the time of product release to ensure compliance with label claims
  - over product shelf life to check for any drop in probiotic levels

## WHY THE BD FACSMicroCount SYSTEM?

- Release products to market faster by validating microbial content with label claim in minutes
- Reduce costs by eliminating need to warehouse products while label claim validations take place
- Optimize yields by monitoring microbial growth during the entire fermentation process
- Validate probiotic levels in product over shelf life; react faster to decreasing levels of microbial viability in product
- Generate consistency in results by eliminating operator-to-operator variability in manual counting

## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in 5 minutes instead of days with traditional methods
- Continuous sample loading with up to 42 samples at a time
- Analyze up to 15 samples per hour
- Correlates directly with traditional methods
- 21 CFR-Part 11 compliant data management system



Correlation of BD FACSMicroCount System counts/mL vs plate counts/mL for *Lactobacillus casei* ( $R^2 = 0.9997$ )



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live healthy lives



# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Mix probiotic with phosphate buffer



- Pipette 3 mL sample into a 5 mL sample tube

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

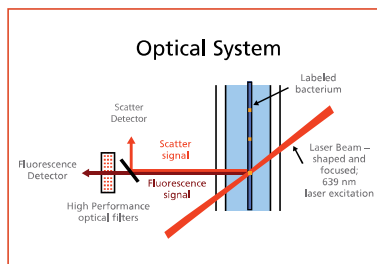


- Define probiotic analysis method
- Select method for the tray from drop-down menu

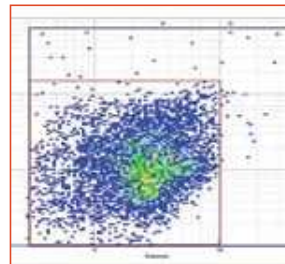
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Total Viable Organism (TVO) count is provided

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Probiotics provide a rapid and sensitive method for enumerating the total microbial content during various stages of the probiotic manufacturing process.



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# Screening Purified and Process Water for Consumer Products and Beverages

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## APPLICATION OBJECTIVE

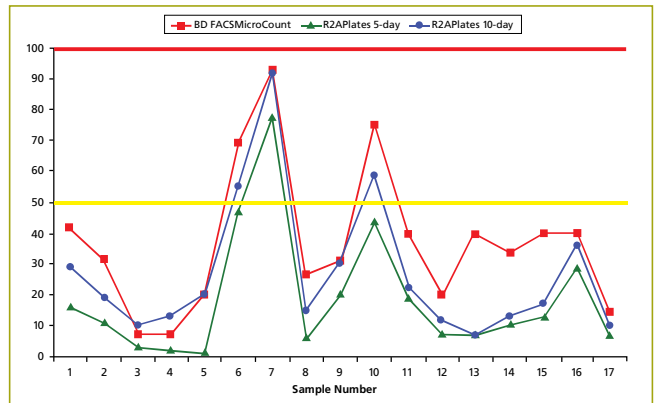
- Rapidly and accurately enumerate microorganisms in purified water and show correlation to traditional plate methods
- Enables manufacturers to monitor the microbial content of water to ensure:
  - product quality
  - compliance with regulatory methods like USP

## WHY THE BD FACSMicroCount SYSTEM?

- Mitigate risk with rapid monitoring (minutes not days) of microbial contamination levels at various points in the manufacturing process
- Evaluate water quality prior to production process
- Respond faster to contamination events
- Generate consistency in results by eliminating operator-to-operator variability in manual counting

## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in less than 10 minutes instead of days with traditional methods
- Continuous sample loading with up to 42 samples at a time
- Analyze up to 15 samples per hour
- Correlates directly with traditional methods
- 21 CFR-Part 11 compliant data management system



Comparison of water samples, from same sampling port, between the BD FACSMicroCount System and traditional testing using R2A plates incubated for 5 and 10 days. For each sample, results from BD FACSMicroCount correlated to traditional methods.

# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



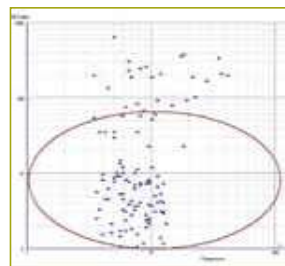
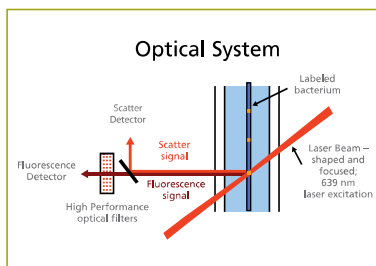
- Pipette 3 mL water sample into a 5 mL sample tube

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System
- Select water analysis method for the tray from drop-down menu
- Software activates the high-throughput option

## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection
- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms
- Total Viable Organism (TVO) count is provided

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Water Screening provide a rapid and sensitive method for enumerating the total viable microorganisms for purified and process water used in the manufacture of consumer products, beverages and household cleaners.



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# Microbial Enumeration of Industrial Fermentation Reactions

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## APPLICATION OBJECTIVE

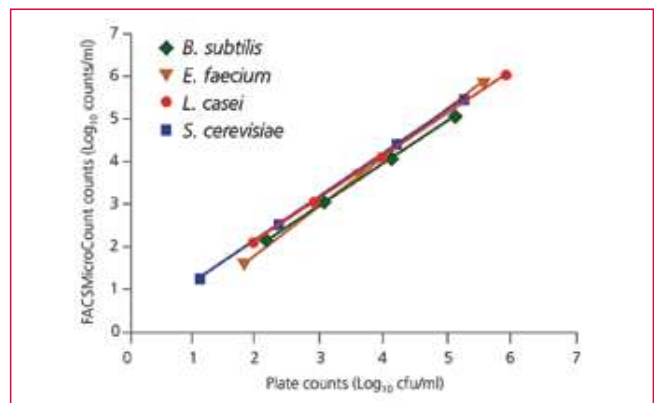
- Rapidly and accurately test total biomass, total number of viable microorganisms, or total dead cell count
- Enables manufacturers of enzymes, human and animal food additives, and other industrial products based on fermentation reactions to monitor microbial content:
  - during growth through the entire fermentation process to optimize yield
  - at the time of product release to ensure compliance with label claims

## WHY THE BD FACSMicroCount SYSTEM?

- Determine best time to harvest biomass with total biomass, viable and dead cell counts
- Release products to market faster by validating microbial content with label claim in minutes
- Accurately enumerate a broad range of microorganisms, including Mycoplasmas, gram-positive and gram-negative bacteria, yeasts, spores, fungi, spirochetes and anaerobes
- Generate consistency in results by eliminating operator-to-operator variability in manual counting
- Benefit with significant time savings over traditional testing methods that can take over 10 days

## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in 5 minutes instead of days with traditional methods
- Continuous sample loading with up to 42 samples at a time
- Analyze up to 15 samples per hour
- Correlates directly with traditional methods
- 21 CFR-Part 11 compliant data management system



Correlation of BD FACSMicroCount System counts/mL vs plate counts/mL for

- *Bacillus subtilis* ( $R^2 = 0.9995$ )
- *Enterococcus faecium* ( $R^2 = 0.9946$ )
- *Lactobacillus casei* ( $R^2 = 0.9997$ )
- *Saccharomyces cerevisiae* ( $R^2 = 0.9981$ )



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# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Mix fermentation sample with phosphate buffer

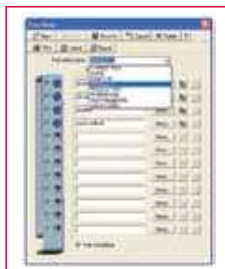


- Pipette 3 mL sample into a 5 mL sample tube

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

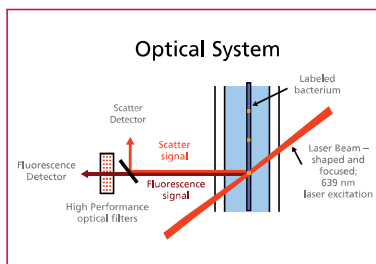


- Define fermentation analysis method
- Select method for the tray from drop-down menu

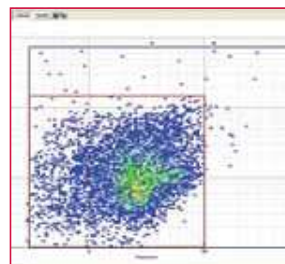
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Total Viable Organism (TVO) count is provided

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Fermentation Enumeration provide a rapid and sensitive method for enumerating the total biomass, viable microorganisms and dead cells for fermentation reactions used in the manufacture of industrial products like enzymes and food additives.



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# Microbial Enumeration of Fermentation Samples for Animal Health

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## APPLICATION OBJECTIVE

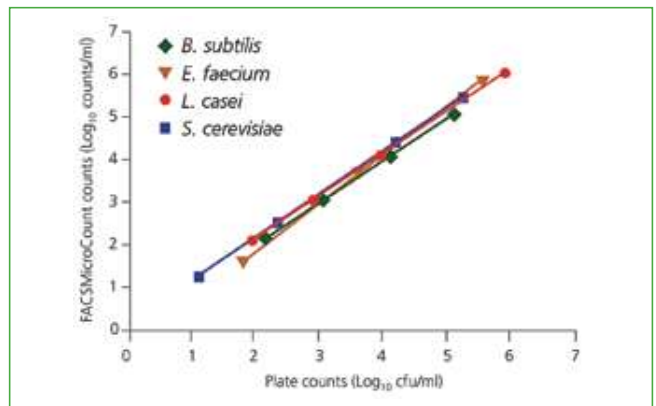
- Rapidly and accurately test total biomass, total number of viable microorganisms, or total dead cell count
- Enables manufacturers of drugs, enzymes, and vaccines, as well as human and animal food additives, to monitor microbial content:
  - during growth through the entire fermentation process to optimize yield
  - at the time of product release to ensure compliance with label claims

## WHY THE BD FACSMicroCount SYSTEM?

- Determine best time to harvest biomass with total biomass, viable and dead cell counts
- Release products to market faster by validating microbial content with label claim in minutes
- Accurately enumerate a broad range of microorganisms, including Mycoplasmas, gram-positive and gram-negative bacteria, yeasts, spores, fungi, spirochetes, and anaerobes
- Generate consistency in results by eliminating operator-to-operator variability in manual counting
- Benefit with significant time savings over traditional testing methods that can take over 10 days

## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in 5 minutes instead of days with traditional methods
- Continuous sample loading with up to 42 samples at a time
- Analyze up to 15 samples per hour
- Correlates directly with traditional methods
- 21 CFR-Part 11 compliant data management system



Correlation of BD FACSMicroCount System counts/mL vs plate counts/mL for

- *Bacillus subtilis* ( $R^2 = 0.9995$ )
- *Enterococcus faecium* ( $R^2 = 0.9946$ )
- *Lactobacillus casei* ( $R^2 = 0.9997$ )
- *Saccharomyces cerevisiae* ( $R^2 = 0.9981$ )



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# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Mix fermentation sample with phosphate buffer

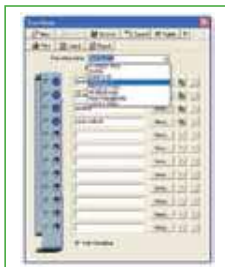


- Pipette 3 mL sample into a 5 mL sample tube

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

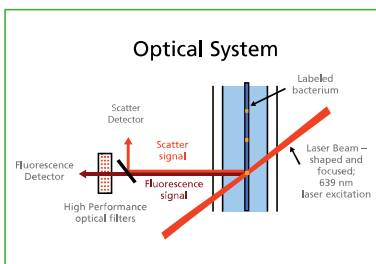


- Define fermentation analysis method
- Select method for the tray from drop-down menu

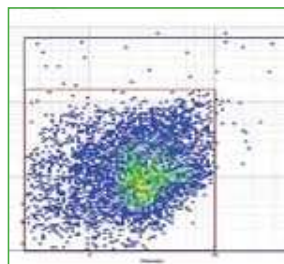
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Total Viable Organism (TVO) count is provided

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Fermentation Enumeration provide a rapid and sensitive method for enumerating the total biomass, viable microorganisms and dead cells for fermentation reactions used in the manufacture of animal health products like drugs, vaccines and food additives.



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# Product and Raw Material Screening for Healthcare Products

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## APPLICATION OBJECTIVE

- Rapidly detect low levels of microorganisms in raw materials and finished products for pharmaceutical and other healthcare applications
- Enables manufacturers to meet regulatory needs (GMP, USP, etc.) through rapid screening of raw materials in process and finished goods for microbial contamination
- Reduce inventory needs and facilitate faster product release for manufacturers through rapid screening for microbial contamination

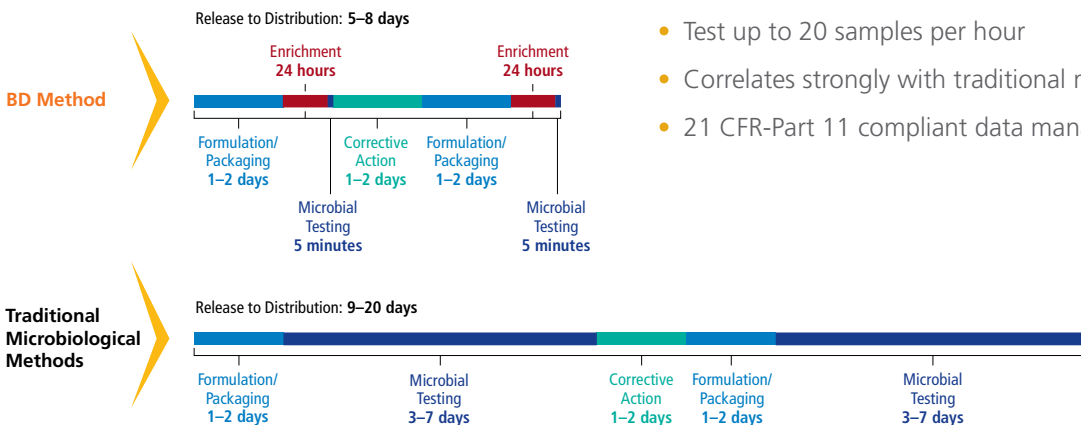
## WHY THE BD FACSMicroCount SYSTEM?

- Move products to market faster by cutting product release time with rapid product screening
- Reduce working capital with less inventory needs as rapid product screening for microbial contamination reduces inventory hold time
- Respond faster to contamination events and reduce the amount of product exposed to contamination
- Generate consistency in results by eliminating operator-to-operator variability in manual counting



## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in as little as 5 minutes, after 24 – 48 hour enrichment, instead of 3 – 7 days with traditional methods
- Detects bacteria, yeasts and molds in the same sample
- Continuous sample loading with up to 42 samples at a time
- Test up to 20 samples per hour
- Correlates strongly with traditional methods
- 21 CFR-Part 11 compliant data management system



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# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Create product suspension by suspending sample in Growth Enhancement Media (GEM) or other suitable media



- Transfer 1 mL of suspension to Tube A containing a swab and enrich for 24-48 hours



- Transfer swab from Tube A to Tube B and vortex
- Transfer 100 uL from Tube B to Tube C

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes (Tube C) and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

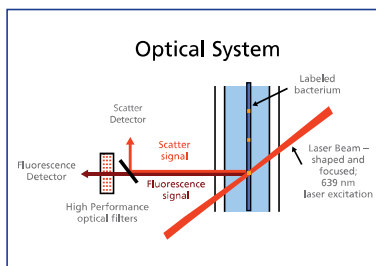


- Select product screening method for the tray from drop-down menu
- Software activates the high-throughput option

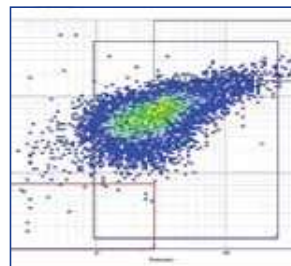
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Bioburden contamination presence / absence information provided in tabular format with color coded "Pass" or "Fail" result

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Product Screening provide a rapid and sensitive method for detecting the presence/absence of low level microorganisms in raw materials and finished products for pharmaceutical and other healthcare applications.



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# Screening Purified and Process Water for Healthcare Products

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Screening Purified and Process Water for Healthcare Products

## APPLICATION OBJECTIVE

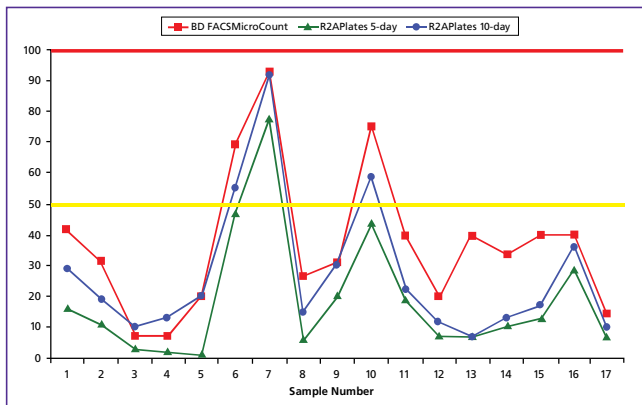
- Rapidly and accurately enumerate microorganisms in purified water and show correlation to traditional plate methods
- Enables manufacturers to monitor the microbial content of water to ensure:
  - product quality
  - compliance with regulatory methods like USP

## WHY THE BD FACSMicroCount SYSTEM?

- Mitigate risk with rapid monitoring (minutes, not days) of microbial contamination levels at various points in the manufacturing process
- Evaluate water quality prior to production process
- Respond faster to contamination events
- Generate consistency in results by eliminating operator-to-operator variability in manual counting

## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in less than 10 minutes instead of days with traditional methods
- Continuous sample loading with up to 42 samples at a time
- Analyze up to 15 samples per hour
- Correlates directly with traditional methods
- 21 CFR-Part 11 compliant data management system



Comparison of water samples, from the same sampling port, between the BD FACSMicroCount System and traditional testing using R2A plates incubated for 5 and 10 days. For each sample, results from BD FACSMicroCount correlated to traditional methods.



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BD FACSMicroCount™

# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Pipette 3 mL water sample into a 5 mL sample tube

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

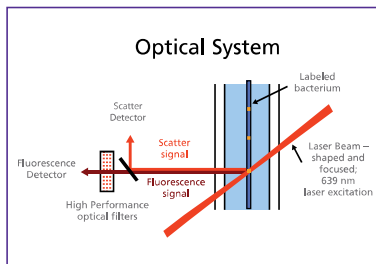


- Select water analysis method for the tray from drop-down menu
- Software activates the high-throughput option

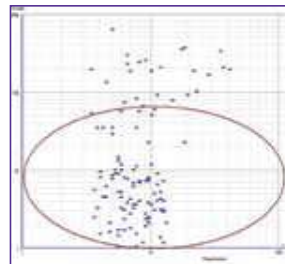
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Total Viable Organism (TVO) count is provided

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Water Screening provide a rapid and sensitive method for enumerating the total viable microorganisms for purified and process water used in the manufacture of healthcare products.



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# Product and Raw Material Screening for Personal Care and Household Products

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## APPLICATION OBJECTIVE

- Rapidly detect low levels of microorganisms in samples such as personal care, cosmetics, over-the-counter, household cleaning and beverage products
- Enables manufacturers to meet regulatory needs (GMP, USP, etc.) through rapid screening of raw materials in process and finished goods for microbial contamination
- Reduce inventory needs and facilitate faster product release for manufacturers through rapid screening for microbial contamination

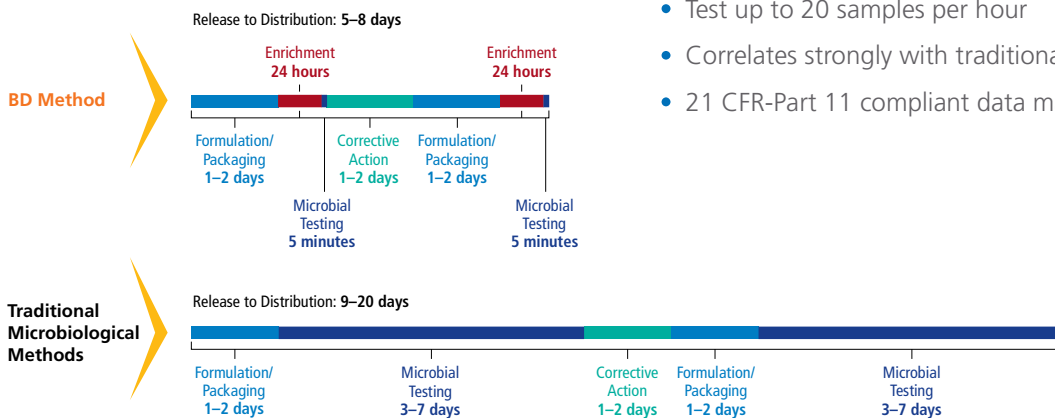
## WHY THE BD FACSMicroCount SYSTEM?

- Move products to market faster by cutting product release time with rapid product screening
- Reduce working capital with less inventory needs as rapid product screening for microbial contamination reduces inventory hold time
- Respond faster to contamination events and reduce the amount of product exposed to contamination
- Generate consistency in results by eliminating operator-to-operator variability in manual counting



## WHAT THE BD FACSMicroCount SYSTEM DOES

- Provides results in as little as 5 minutes, after 24 – 48 hour enrichment, instead of 3 – 7 days with traditional methods
- Detects bacteria, yeast and mold in the same sample
- Continuous sample loading with up to 42 samples at a time
- Test up to 20 samples per hour
- Correlates strongly with traditional methods
- 21 CFR-Part 11 compliant data management system



Helping all people  
live healthy lives

# Simple 3-Step Sample Analysis Process

## 1 SAMPLE PREPARATION



- Create product suspension by suspending sample in Growth Enhancement Media (GEM) or other suitable media



- Transfer 1 mL of suspension to Tube A containing a swab and enrich for 24-48 hours



- Transfer swab from Tube A to Tube B and vortex
- Transfer 100 uL from Tube B to Tube C

## 2 LOADING THE BD FACSMicroCount™ SYSTEM



- Place sample tubes (Tube C) and needle-less syringes in desired sample tray
- Load sample tray into the BD FACSMicroCount System

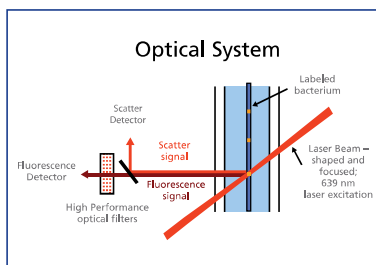


- Select product screening method for the tray from drop-down menu
- Software activates the high-throughput option

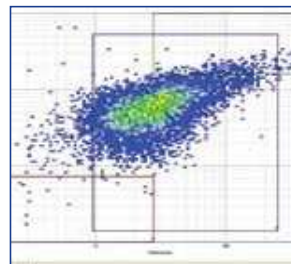
## 3 AUTOMATED SAMPLE ANALYSIS



- Automated reagent addition, mixing and sample injection



- Sample is injected into flow cell
- Laser causes fluorescence in labeled microorganisms



- Bioburden contamination presence / absence information provided in tabular format with color coded "Pass" or "Fail" result

### CONCLUSION

The BD FACSMicroCount System and the BD FACSMicroCount Media Kit for Product Screening provide a rapid and sensitive method for detecting the presence/absence of low level microorganisms in various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products.



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## Screening Products for Microbial Contamination Using the BD FACSMicroCount™ Detection System

### EXECUTIVE SUMMARY

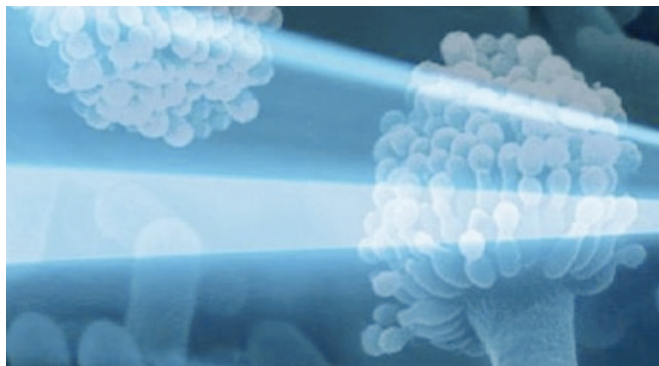
BD Diagnostic Systems, in keeping with USP <61> sample preparation, has developed a procedure to rapidly screen products for the presence of microbial contamination. Various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products were tested with the Product Screening Procedure using the BD FACSMicroCount™ Detection System and BD FACSMicroCount™ Media Kit. Fifty-five different product samples were inoculated with <100cfu of bacteria, yeast and mold. All inoculated samples were positive for microbial contamination within 24-48 hours. Of these, 87% were positive for microbial contamination within 24 hours, 91% were positive within 30 hours, and 100% were positive within 48 hours. Additionally, the BD FACSMicroCount software allowed differentiation between bacteria, yeast and mold contamination in a single sample.

### OBJECTIVE

Demonstrate the ability of the Product Screening Procedure using the BD FACSMicroCount and BD FACSMicroCount Media Kit to detect low-levels of microorganisms in various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products.

### MATERIALS AND METHODS

Various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products were tested with the Product Screening Procedure using the BD FACSMicroCount Detection System and BD FACSMicroCount Media Kit. Products were diluted 1:10 in Phosphate Buffer (PB) or the Growth Enhancement Media (GEM) and mixed to achieve a homogeneous suspension. One milliliter was then



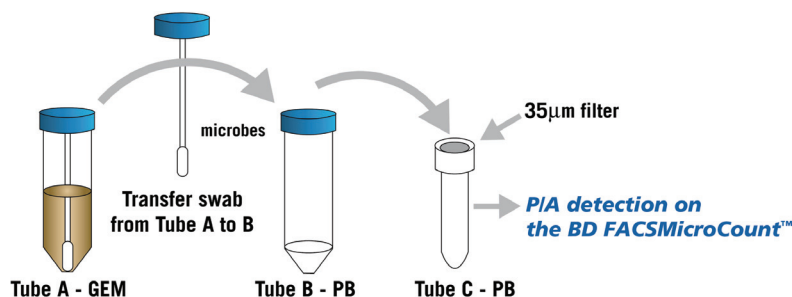
transferred to 19mL of GEM + substrate (Tube A in the BD FACSMicroCount Media Kit) and neutralized for 30 minutes. *Pseudomonas aeruginosa* ATCC™ 9027, *Escherichia coli* ATCC 25922 or 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 were inoculated individually into Tube A for each product (<100cfu/volume). *Bacillus subtilis* subsp. *spizizinii* ATCC 6633 was substituted for *E. coli* in the excipient samples. Inocula were spread-plated on Tryptic Soy Agar (TSA) for confirmation of inoculum levels. Negative control (non-spiked) product samples were analyzed using the above protocol to determine product baselines. Samples were enriched at 30°C with shaking for a minimum of 24 hours. After enrichment, the substrate was transferred to 2mL of PB (Tube B in the BD FACSMicroCount Media Kit) and vortexed. One hundred microliters was then added to 2.9mL PB through a filter cap (Tube C in the BD FACSMicroCount Media Kit) and the samples were analyzed on the BD FACSMicroCount Detection System. (See Figure 1 for an illustration of the BD FACSMicroCount Media Kit Product Screening Procedure). Samples with BD FACSMicroCount outputs  $\geq 3X$  the baseline failed, indicating that the sample was positive for microbial contamination.





## APPLICATION NOTE

**Figure 1.** Illustration of the BD FACSMicroCount Media Kit Product Screening Procedure



### ACCEPTANCE CRITERIA

Internal validation studies have been conducted to determine Pass/Fail criteria for the BD FACSMicroCount Detection System using product baselines. The baseline is calculated by taking the average non-spiked product results plus the standard deviation. A sample with a result  $\geq 3X$  the baseline is considered a Fail, and indicates that the sample is positive for microbial contamination. A sample with  $< 3X$  the baseline is considered a Pass, and indicates that the sample does not contain microbial contamination.

### RESULTS

A total of 55 different product samples were inoculated and tested using the above protocol. Of these, 87% were positive for microbial contamination within 24 hours, 91% were positive within 30 hours, and 100% were positive within 48 hours (Table 1). Inoculums ranged from 2-78cfu. All inoculated samples were  $\geq 3X$  the baseline post-enrichment. Area definitions were predefined in the BD FACSMicroCount software to distinguish between mold, bacteria and yeast contamination events. All inoculated samples were positive for microbial contamination in their predefined area (Figure 2).

**Table 1.** Product Screening results showing detection of low-levels of microorganisms in various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products using the BD FACSMicroCount Detection System.

Emulsions (3)	Inoculum Range (cfu)	Time to Result*
Silicone Emulsions (1 and 2)	15 - 46	24 hrs
Silicone Emulsion (3)	15 - 46	48 hrs
Excipients (14)	Inoculum Range (cfu)	Time to Result*
Clear Solutions	8 - 66	24 hrs
Personal Care Products (12)	Inoculum Range (cfu)	Time to Result*
Baby Wipes	15 - 75	24 hrs
Body Wash	8 - 66	24 hrs
Conditioner	13 - 57	30 hrs
Face Scrub	9 - 49	24 hrs
Hair Gel	11 - 78	24 hrs

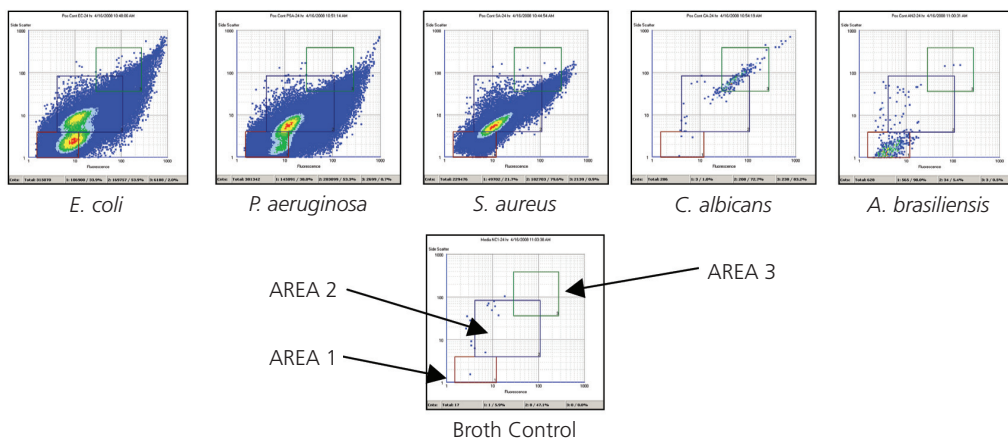
**Table 1 Continued**

Hand Lotion	6 - 70	24 hrs
Hand Soap – Antibacterial	6 - 70	24 hrs
Mouthwash	2 - 52	24 hrs
Shampoo	14 - 57	24 hrs
Shave Gel	2 - 41	24 hrs
Sunscreen	8 - 70	24 hrs
Toothpaste	7 - 48	24 hrs
<b>Over The Counter Products (14)</b>	<b>Inoculum Range (cfu)</b>	<b>Time to Result*</b>
Acetaminophen – Children's Liquid	12 - 18	24 hrs
Allergy – Children's Liquid	11 - 78	24 hrs
Antacid Tablets	15 - 64	24 hrs
Anti Itch	11 - 78	24 hrs
Cold/Flu Liquid – Night Time	9 - 62	24 hrs
Cough Syrup	15 - 64	24 hrs
Fiber Capsules	13 - 33	24 hrs
Fiber Powder	15 - 40	24 hrs
Ibuprofen – Children's Liquid	12 - 18	24 hrs
Laxative, Saline	9 - 49	24 hrs
Nasal Spray	9 - 49	24 hrs
Sleep Aid, Liquid Caps	15 - 64	24 hrs
Vitamins – Multi, Children's Chewable	9 - 62	24 hrs
Vitamins – Multi, Adult	15 - 66	30 hrs
<b>Household Products (6)</b>	<b>Inoculum Range (cfu)</b>	<b>Time to Result*</b>
Dish Soap	2 - 58	24 hrs
Dishwasher Rinse Agent	9 - 51	24 hrs
Disinfecting Wipes	15 - 75	48 hrs
Fabric Refresher	11 - 36	48 hrs
Household Cleaner	2 - 56	24 hrs
Laundry Detergent	11 - 36	24 hrs
<b>Beverage Products (4)</b>	<b>Inoculum Range (cfu)</b>	<b>Time to Result*</b>
Antioxidant Water	12 - 18	24 hrs
Cranberry Juice	6 - 41	24 hrs
Lemonade	6 - 41	24 hrs
Vegetable Juice	6 - 41	24 hrs
<b>Cosmetic Products (2)</b>	<b>Inoculum Range (cfu)</b>	<b>Time to Result*</b>
Eye Shadow	12 - 61	48 hrs
Mascara	12 - 61	48 hrs

\*Maximum enrichment time required to detect the presence of the microorganisms listed in USP <61> in the respective products.

## APPLICATION NOTE

**Figure 2.** Examples of BD FACSMicroCount intensity plots for positive control samples. Area 1 (red) encompasses mold populations, Area 2 (blue) encompasses bacterial populations, and Area 3 (green) encompasses yeast populations.



## DISCUSSION

All 55 product samples inoculated for this study were positive for microbial contamination in 24-48 hours.

The BD FACSMicroCount software contains 3 analysis boxes which allow the user to differentiate between mold, bacteria and yeast contamination in a single sample. One analysis box (Area 1) encompasses mold populations, one analysis box (Area 2) encompasses bacterial populations, and one analysis box (Area 3) encompasses yeast populations. The ability of this procedure to yield results in <48 hours is due in part to the ability of the BD FACSMicroCount Detection System to detect mold filaments, which increases the sensitivity of the method.

**NOTE:** Due to the nature of several of the products tested, it was necessary to modify the above procedure. One milliliter of each excipient was added directly to Tube A without a 1:10 dilution. This was possible because these samples lack preservatives and had low backgrounds. The fiber capsules and fiber powder required 1:20 and 1:100 pre-enrichment dilutions, respectively. This was due to the absorbent nature of the fiber, and a 1:10 product dilution was not possible for these samples. The eye shadow and mascara required an additional 1:10 dilution post-enrichment to reduce product background. One baby wipe/disinfecting wipe was stomached in 90mL PB or GEM for 30 seconds and the liquid was decanted. One milliliter was then added to Tube A.

## CONCLUSION

The BD FACSMicroCount Detection System and BD FACSMicroCount Media Kit Product Screening Procedure provide a rapid and sensitive method for detecting the presence / absence of low-levels of microorganisms in various emulsions, excipients, personal care, OTC, household, beverage and cosmetic products.



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