BD FACService™ TECHNOTES

Customer Focused Solutions
Vol. 8 No. 4 December 2002

Measuring Bacterial Disinfectant Efficacy Using Flow Cytometry

Flow cytometry provides a rapid method to quantitate live and dead bacteria in a sample.[1-4] It can be used to measure the efficacy of antimicrobial compounds by exposing organisms to the disinfectant and subsequently staining with permeant and impermeant DNA dyes. Viability of microorganisms can be measured using a 30-minute flow cytometry assay. This flow cytometry assay exhibits comparable sensitivity to a standard 48-hour plate count assay. This flow cytometry application provides a rapid and quantitative method to determine the efficacy of disinfectants by counting live, dead, and injured microorganisms.

Methodology

Current methods to assess disinfectant efficacy are growth-based and rely on exposure of a test organism to disinfectant, with growth generally observed after 48 hours. These methods require substantial labor and time and can be susceptible to inaccurate results. In contrast, a flow cytometry assay can be completed in less than one hour and can provide rapid quantitation of live, dead, and injured microorganisms. The test organism is exposed to a disinfectant and then assayed for viability by flow cytometry. All cells containing DNA are stained with the permeant dye, thiazole orange* (TO), and fluoresce yellow to orange. Simultaneously, cells with damaged membranes are stained with the impermeant dye, propidium iodide (PI), and fluoresce orange to red.

Treatment and Staining

The disinfectant SPOR-KLENZ™ can be used as a model system. The active ingredients in SPOR-KLENZ are hydrogen peroxide and peroxyacetic acid. Bacteria are exposed for 10 minutes to SPOR-KLENZ. Treated bacteria are then split into two groups. One group is plated on BD RODAC™ D/E Neutralizing Agar to neutralize residual SPOR-KLENZ and checked for growth after 48 hours. The second group is prepared for flow cytometric analysis by adding TO and PI. Samples are vortexed and analyzed after a 5–10 minute incubation.

*US Patent Nos. 4,883,867 and 4,957,870

BD Cell Viability Kit Components

Cat No. 349483
Thiazole Orange
Propidium Iodide

BD Cell Viability Kit Components with BD Liquid Counting Beads

Cat No. 349480
Thiazole Orange
Propidum Iodide
BD Liquid Counting Beads

For more information on the BD™ Cell Viability Kit, contact your local BD Biosciences representative.

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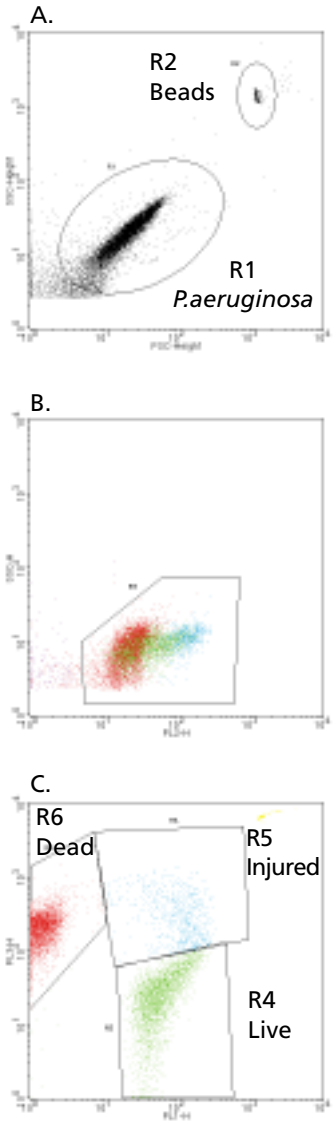

Figure 1. Gating and analysis strategy for bacterial populations, using a sample of P. aeruginosa stained with TO and PI in a tube containing BD Liquid Counting Beads. Panel A: FSC vs SSC dot plot with the bacterial population on-scale, with region R1 set liberally around the target population, region R2 set around the beads. Panel B: FL2 vs SSC dot plot with region R3 set around the stained bacteria Panel C: FL1 vs FL3 dot plot gated on (R1 OR R2) AND R3, with regions set around the live, injured, and dead bacterial populations

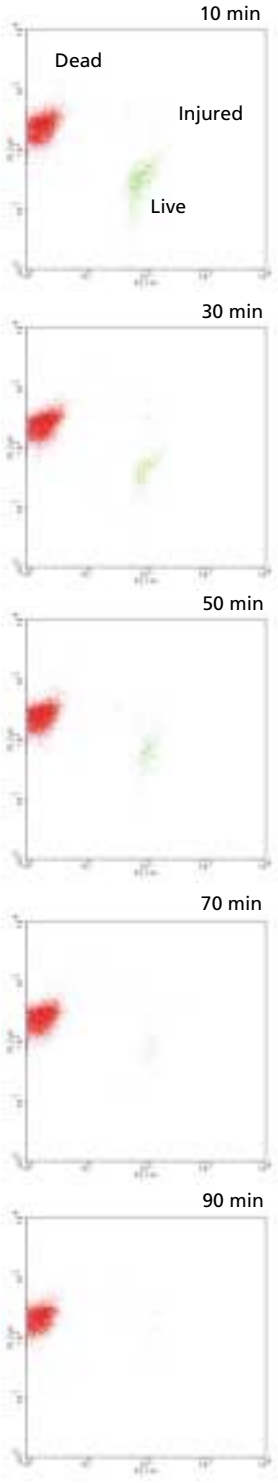

Figure 2. Change in live and dead populations of P. aeruginosa with 10–to 90–minute exposure to 3.1% SPOR-KLENZ

Acquisition and Analysis

A BD FACSCalibur™ flow cytometer is used for data acquisition and analysis. PMT voltages and threshold levels are adjusted using an unstained sample of diluted bacteria. The bacterial population is positioned so that it is entirely on scale on an FSC vs SSC plot (***Figure 1***). For fluorescence measurements, FL1, FL2, and FL3 PMT voltages are adjusted to place the unstained population in the lower left quadrant of two-parameter plots (data not shown).

Results and Discussion

Figure 2 shows the change in viability of Pseudomonas aeruginosa (*P. aeruginosa*) populations over 90 minutes of exposure to SPOR-KLENZ. ***Figure 3*** and ***Figure 4***, respectively, compare the effect of SPOR-KLENZ on Staphylococcus aureus (*S. aureus*) and *P. aeruginosa* by flow cytometry and plate counting. The observed change in viability with S. aureus is equivalent in both methods. P. aeruginosa shows a greater difference in response between the methods than S. aureus, perhaps due to differences in the cell envelope. Similar results are observed for Salmonella (data not shown). Maximal killing is observed with both methods at the same disinfectant concentration. Flow cytometry allows evaluation of disinfectant or preservative efficacy in as short as 30 minutes and allows the quantitation of live and dead cells. Results are comparable with standard microbiological plate counts. This approach can be applied to a variety of applications, including prediction of disinfectant stability and potency, and microbial studies where greater than 100 organisms per mL need to be detected, such as antimicrobial effectiveness, nutritional studies, and evaluation of non-sterile products.

Tips for Flow Cytometric Analysis of Prokaryotes

- TO fluoresces primarily in FL1 and FL2; PI fluoresces primarily in FL3. Therefore, the best discrimination of live and dead populations is on an FL1 vs FL3 plot. This method can be applied with a variety of buffer systems, but for optimal resolution, include some surfactant in the staining buffer.
- Set FSC and SSC on logarithmic amplification to ensure that a wide range of bacterial sizes appear on scale and present recognizable populations for gating.
- There will be differences between bacterial species in their abilities to take up TO and PI. The lipopolysaccharides (LPS) on gram-negative bacteria can interfere with the uptake of TO and other permeant dyes. Interference can be largely overcome by the inclusion of 1 mM EDTA in the staining buffer, which has been reported to remove LPS from the bacteria.[1]
- Adjust staining protocols for the bacteria being analyzed.
- Stain with TO for at least 2 to 5 minutes, although 15 minutes is necessary to achieve maximum intensity. PI stains very quickly, while TO enters the cells more slowly.
- Adjust the event rate to ≤1000 events per second to minimize coincidence and improve population resolution. High event rates can be corrected either by dilution or by decreasing the instrument flow rate.
- If the population of interest cannot be adequately resolved using an SSC threshold alone, a secondary threshold on FL1 can be used. An FL1 threshold alone might not be adequate due to the large amount of small fluorescent debris that can be present in a stained bacterial sample.
- Maintain an adequate concentration of bacteria. As the concentration of bacteria decreases, background noise will become progressively more prominent.
- If high background counts are observed on an instrument, check the staining buffer and sheath fluid for particles. An instrument cleaning cycle and a drain/fill cycle can also reduce noise.

For application notes on prokaryotic analysis, click on the following links:
Bacterial Detection and Live/Dead Discrimination by Flow Cytometry and Bacterial Disinfectant Efficacy Using Flow Cytometry. For yeast analysis, see Evaluation of Yeast Viability and Concentration During Wine Fermentation Using Flow Cytometry. Additionally, the Microbial Cytometry References are a great resource.

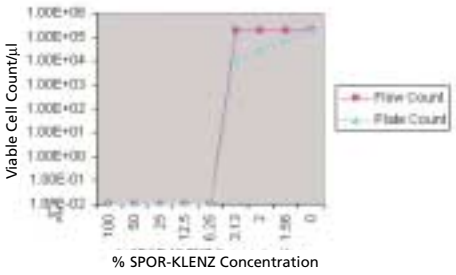

Figure 3. Comparison of flow cytometry and plate counting on *S. aureus*

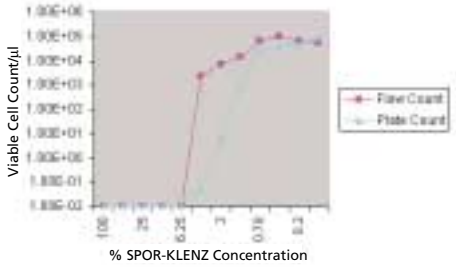

Figure 4. Comparison of flow cytometry and plate counting on *P. aeruginosa*

References

1. Davey HM, Kell DB. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single cell analyses. *Microbiological Reviews.* 1996;60:641-696.
2. Shapiro HN. Microbial analysis at the single-cell level: tasks and techniques. *Journal of Microbiological Methods*. 2000;42:3-16.
3. Nebe-von-Caron G, Stephens PJ, Hewitt CJ, Powell JR, Badley RA. Analysis of bacterial function by multi-colour fluorescence flow cytometry and single cell sorting. *Journal of Microbiological Methods*. 2000;42:97-114.
4. Nebe-von-Caron G, Stephens PJ, Badley RA. Bacterial detection and differentiation by cytometry and fluorescent probes. Proceedings of the Royal Microbiological Society. 1999;34:321-327.

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23-7143-00

From BD Biosciences Applications Support: Archiving Essential BD FACStation Information for Acquisition and Analysis of Flow Cytometric Data

BD Biosciences strongly encourages customers to routinely archive data to floppy, Zip®, or compact disks. Alternatively, data can be saved on an external drive or a network drive if it is configured to support Macintosh®, services. Data archival will save you time and minimize frustration if a computer malfunction occurs that requires the need to reformat the hard disk or reload software, or both. In a regulated environment, routinely archiving data also assists in complying with the record protection requirements of 21 CFR Part 11. We recommend archiving data to recordable compact disks (CD-R) or rewritable compact disks (CD-RW) since magnetic media can be erased and data can be lost.

We recommend that archived data be stored at two different locations. One location can be located within easy access in case it is needed immediately, and another in a secure place.

Along with the data archives, keep a copy of software serial numbers that were provided with the software products. These are generally provided with the software users guides. You will need to enter this information when launching the software after it has been re-installed. Routinely archive the files shown in ***Table 1*** for the software applications that you use. By archiving this information on a regular basis, you increase the probability that the information being saved is current. Additionally, instrument settings generated by BD FACSComp™ software,* BD CellQuest™ software,* BD CellQuest Pro™ software,* BD MultiSET™ software,* and BD SimulSET™ software* are generally saved in the BD Files > Instrument Settings Folder. Wherever you store these files, they should be archived on a regular basis. Feel free to back up any additional information that you feel is important to save.

* For In Vitro Diagnostic Use.

Table 1

SOFTWARE	FILE NAME	LOCATION	DESCRIPTION
General (applies to all acquisition software)	Instrument Settings	BD Files > Instrument Settings folder	Flow cytometer instrument settings
BD Attractors™ software †	Attractor Set	Any	Dot plots, population attractors, attractor settings, and legend
BD CellQuest or	Experiment document	Any	Contains plots, regions, gates and stats
BD CellQuest Pro software	CellQuest or CellQuest Pro Preferences	System Folder > Preferences	Panel and Reagent information
	FCS data files	Any	Data
BD FACSComp software	Target files	BD Applications > FACSComp folder	Instrument setup information
	FACSComp Preferences	System Folder > Preferences > BD Preferences	BD FACSComp preferences
	FACSComp Print Setup	System Folder > Preferences > BD Preferences	BD FACSComp printing preferences
	LJ Data	BD Applications > FACSComp folder	Historical lyse/wash data from BD FACSComp runs
	LJ Data.LNW	BD Applications > FACSComp folder	Historical lyse/ no-wash data from BD FACSComp runs
BD FACSComp software Levey-Jennings QC application†	LJ FCMP QC 30-runs or LJ FCMP QC 60-runs	Any	Macros and spreadsheet files that contain historical BD FACSComp data
BD MultiSET™ software Levey-Jennings QC application†	MSET LJ QC v.1.0	Any	Macros and spreadsheet files that contain historical BD TruCOUNT Control tube data generated by BD MultiSET software
BD MultiSET™ software	3CPanelReagentFile	BD Applications > MultiSET folder	Default and any user-defined panels
	3CBDReagent File	BD Applications > MultiSET folder	Default and any user-defined reagents
	MultiSET.WM.sch or other MultiSET Schedule files	BD Applications > MultiSET folder	Reagent and BD TruCOUNT lot IDs and Bead count values
	LJ MSET Data	BD Applications > MultiSET folder	Current runs of BD MultiSET data for LJ plots
	UserDefined Reagents	BD Applications > MultiSET folder	User-defined BD Attractors templates
	FCS Data Files	Any	Data
	MultiSET Preferences	System Folder > Preferences > BD Preferences	BD MultiSET preferences
	MultiSET Print Setup	System Folder > Preferences > BD Preferences	BD MultiSET printing preferences
BD PAINT-A-GATE PRO™ software †	PAINT-A-GATE Set	Any	Plots, populations, analysis method, legend
BD ProCOUNT™ software*	ProCount.WM.sch or other ProCOUNT schedule files	BD Applications > ProCOUNT Folder	Reagent and BD TruCOUNT lot IDs and BD TruCOUNT Bead count values
	ProCOUNT PanelReagent File	BD Applications > ProCOUNT folder	Panel and reagent information
	FCS data files	Any	Data
BD ProCOUNT software *	ProCOUNT Preferences	System Folder > Preferences > BD Preferences	BD ProCOUNT preferences
	ProCOUNT Print Setup	System Folder > Preferences > BD Preferences	BD ProCOUNT printing preferences
BD SimulSET software	Panelreagent File	BD Applications > SimulSET Folder	Panel and reagent information
	SimulSET.WM.sch or other SimulSET schedule files	BD Applications > SimulSET Folder	Reagent lot ID
	SimulSET Preferences	System Folder > Preferences > BD Preferences	BD SimulSET preferences
	SimulSET Print Setup	System Folder > Preferences > BD Preferences	BD SimulSET printing preferences
	FCS Data Files	Any	Data
BD™ WorklistManager software	WorklistManager Files	BD Files > *WorklistManager Files	Patient ID information from historical BD WorklistManager runs
	WorklistManager Preferences	System Folder > Preferences > BD Preferences	BD WorklistManager preferences
	Worklist Print Setup	System Folder > Preferences > BD Preferences	BD WorklistManager printing preferences

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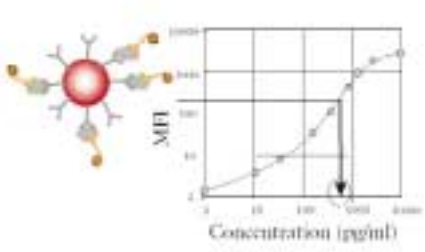
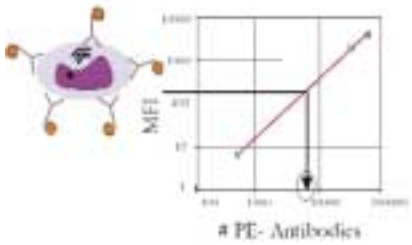
Quantitation Tools for Flow Cytometry





What is the BD QuantiBRITE System?

The BD QuantiBRITE™ system offers the first completely standardized approach to fluorescence quantitation. BD QuantiBRITE PE beads are lyophilized pellets of beads conjugated with four levels of PE. These beads calibrate the FL2 fluorescence axis of a flow cytometer in terms of PE molecules. All of our BD QuantiBRITE reagents are guaranteed ≥95% one PE molecule per antibody, making your flow cytometric quantitation studies more dependable, efficient, and flexible.

What is BD CBA?

The BD™ Cytometric Bead Array system (CBA) employs a series of particles with discrete fluorescence intensities to simultaneously detect multiple soluble analytes. Each bead in a CBA provides capture-surface for a specific protein and is analogous to an individually coated well in an ELISA plate.

Schedule	Feb. 7, Mansfield, MA May 23, San Jose, CA
Tuition	$495

Course Content

- Quantitate soluble cytokine levels using the BD CBA kit.
- Calibrate the flow cytometer using BD QuantiBRITE PE Beads.
- Report antibody-binding capacity (ABC) as a cell-surface quantitation unit.
- Troubleshoot sample prep, data acquisition, and analysis problems.

Prerequisites

- basic understanding of immunology and flow cytometry
- proficiency in BD FACSCalibur/ BD FACScan™/ BD FACSort™ operation
- experience with data acquisition and analysis using BD CellQuest™ software

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BD™ CBA with BD™ Multiwell AutoSampler

A Multiplexed Immunoassay System You Can Count On

The BD™ Cytometric Bead Array (CBA) system provides reproducible data and reliable performance you can count on.

- Get more results from a single small volume sample
- Run one standard curve for all your analytes
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- Achieve quantitative results with less time and labor
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Prerequisites	A basic understanding of immunology and completion of an operator course, or equivalent experience that includes data acquisition and analysis using BD CellQuest software	Completion of an operator course, or equivalent experience that includes proficiency with BD CellQuest and ModFit™ *LT* software	Basic Macintosh® skills and a basic understanding of flow cytometry, and the analysis of flow cytometry data	Completion of a BD FACSVantage SE™ operator course, or equivalent experience
Who Should Attend	Users proficient in BD FACSCalibur/ BD FACScan/ BD FACSort operation and who want to gain experience in preparing and analyzing specimens used in studying immune function	Users proficient in BD FACSCalibur/ BD FACScan/ BD FACSort operation and those who want to expand their repertoire of DNA analysis techniques	Users who have upgraded to BD FACStation™ system, users who deal mainly with data analysis rather than cytometer operation, or users who are familiar with BD CellQuest software but require more in-depth training	Users who have a working knowledge of the BD FACSVantage SE™ instrument and who want to obtain more experience with cell sorting
Duration	1 day	2 days Contact hours * -13	1 day	2 days
Schedule	Feb. 6, Mansfield, MA May 22, San Jose, CA	Feb. 4 – 5, Mansfield, MA May 20 – 21, San Jose, CA	Feb. 3, Mansfield, MA May 19, San Jose, CA	TBA
Tuition	$495	$990	$495	$990

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