Application Note

Microcapillary Flow Cytometry for Bacterial Enumeration, Viability, and Gram Typing

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Introduction

Bacterial analysis requires the routine and frequent determination of characteristics such as concentration (counts/mL), viability, or Gram typing. However, the determination of these characteristics has remained slow, cumbersome, and labor intensive particularly with reliance on methods such as traditional plate-based counting. Flow cytometry has been shown to be a highly sensitive and rapid method for characterizing microbes regardless of their cultivability on agar. However, adoption of traditional, more complex flow cytometry platforms has been limited—especially when platforms require additional, expensive reference beads for enumeration, which can make it difficult to use these methods routinely.

Microcapillary flow cytometry (MFC) utilizes a flow cell with a precision syringe pump to provide information on cellular and/or subcellular particles without the use of sheath fluid. Multi-parametric information on scatter and fluorescence characteristics of cells/particles are collected. These features provide advantages in that they result in compact, simple, and easy-to-use cytometry systems which provide absolute counting of populations without needing to use external beads.

In these studies, we explore the application of microcapillary flow cytometry on the Cytek® Guava® easyCyte™ and Cytek® Guava® Muse® cell analyzer platforms for the analysis of bacterial strain concentration, viability, and typing determination. These platforms are compact with simple assay optimized software, and are well established to generate precise and accurate results for a variety of cellular measurements. Studies were performed using the Guava® Bacterial Count & Viability Kit. These kits include staining methods that utilize a combination of membrane-permeable and impermeable nucleic acid dyes for analysis by microcapillary flow cytometry. Count, viability, and precision data for a variety of bacterial strains were obtained, and then compared with traditional bacterial plate count methods.

In addition, bacterial typing approaches were also examined using MFC on a range of bacterial strains, and we demonstrate that these approaches can be used to identify and distinguish Gram-positive and gram-negative bacteria.

Our results show that MFC can provide new capabilities for bacterial analysis, and can greatly simplify and accelerate the determination of aspects such as bacterial count, viability, and Gram typing analysis.

Methods

For the analysis of count and viability, bacterial samples were mixed and stained for 30 minutes with the Guava® Bacterial Count & Viability Kit. The kit includes a mix of membrane permeable and impermeable dyes. Samples were then analyzed on the Guava® platforms, which provided the necessary population counts and easy to interpret results of live and/or dead bacteria. For Gram staining, bacterial samples were stained with the Guava® Bacterial Gram Staining Kit for 30 minutes and analyzed by microcapillary flow cytometry to obtain identification of Gram type.

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Figure 1.

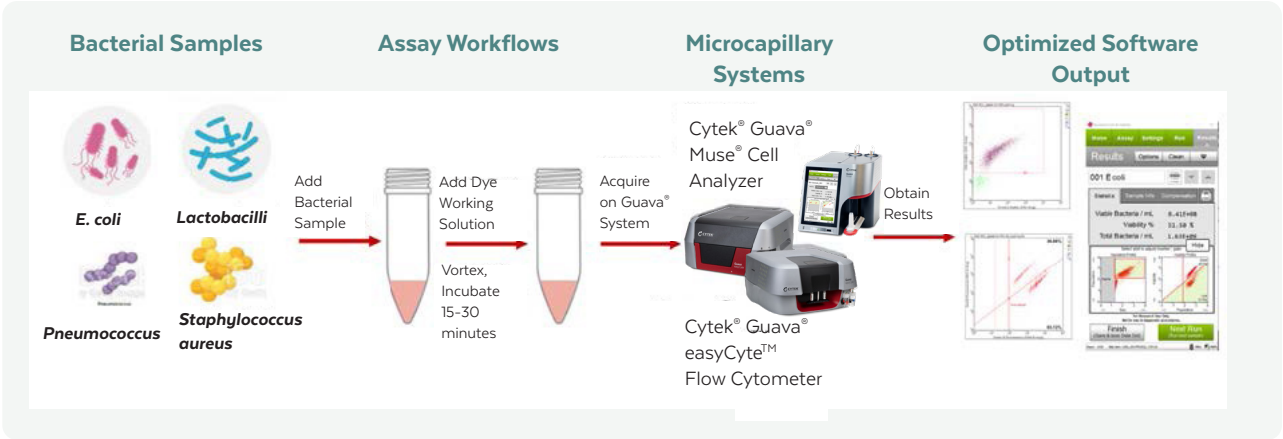

Figure 1. Microcapillary Flow Cytometry for Bacterial Analysis. Bacterial counting, viability, and Gram typing assessments are needed for various research, applied, and industrial applications.

Results

Rapid Analysis of Bacterial Count and Viability

The application of this approach to a mix of live and dead bacteria was examined first. Live and dead (alcohol killed) *E. coli* were mixed at ratios of 1:0, 1:3, 3:1, and 0:1 (Live: Dead), stained with the Guava® Bacterial Count & Viability Kit, and analyzed on the Guava® easyCyte™ system (**Figure 2. left panel**). The mix of dyes clearly identified both live and dead populations, and provided expected viabilities.

The approach was also applied to a mix of multiple gram-positive (*S. aureus, L. delbrueckii, B. subtilis*) and gram-negative (*K. pnemoniae*) strains (**Figure 2. right panel**), which provided a clear distinction of populations in all cases. Count of total events, live and dead populations, and viability information were obtained from the analyses as well.

Figure 2.

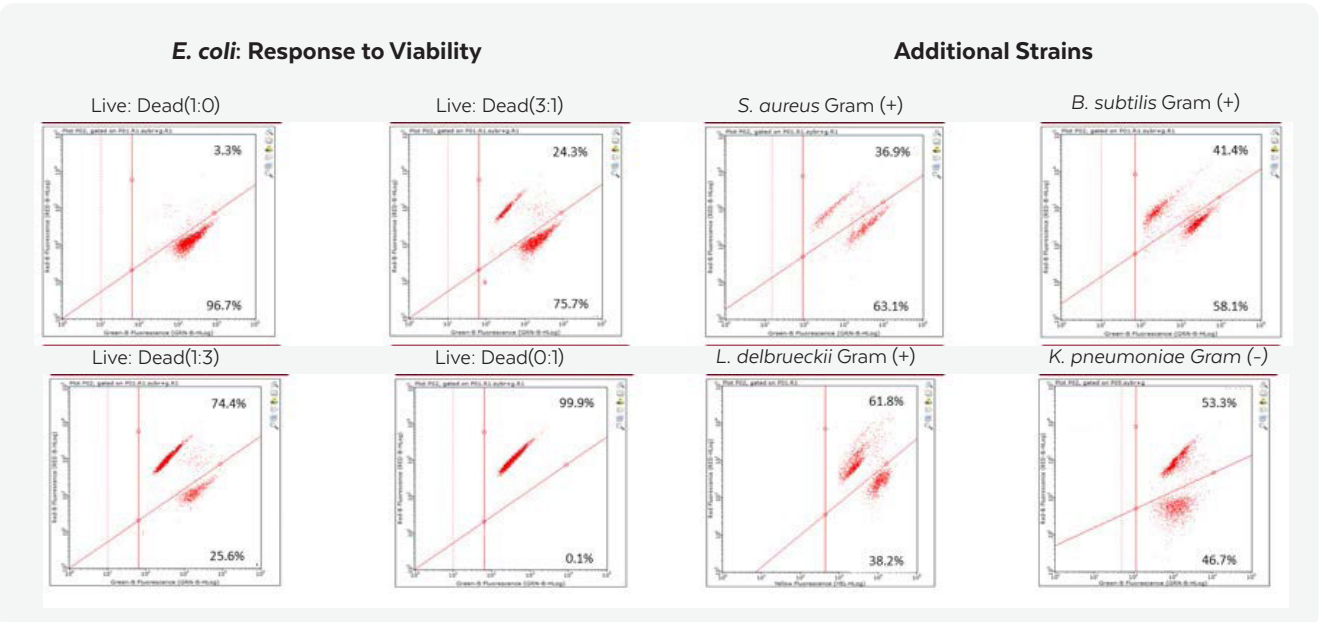

Figure 2. Example staining of live and dead bacterial samples using the Guava® Bacterial Count & Viability Kit.

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Precision Data and Measurement Comparison to Plate Counting

Precision of concentration measurement by microcapillary flow cytometry (MFC) and plate counting was evaluated by comparing the coefficient of variation (%CVs) of triplicate samplings for three bacterial strains at multiple concentrations (**Figure 3. left panel**). Results demonstrated that high precision with %CVs of <10% were observed for all strains and samples by MFC, while the %CV from plate counting was variable and much higher for equivalent samples. Viable cell counts from the microcapillary systems were also compared to plate counting (**Figure 3. right panel**). Viable cell counts showed correlation between the two methods, as indicated by the slope and R^2 value. Results indicated that microcapillary flow cytometry provides both accurate and precise results for bacterial concentration measurements.

Figure 3.

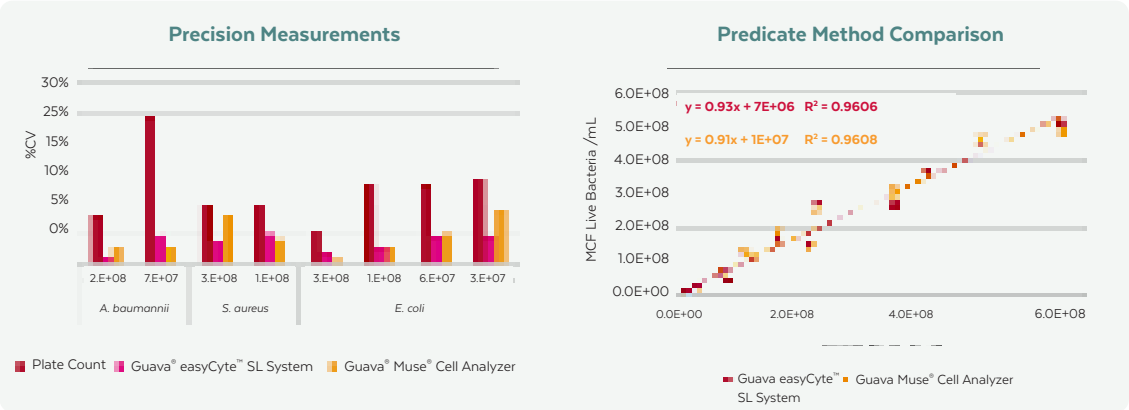

Figure 3. Comparison of counts and %CVs for Guava® Bacterial Count & Viability Kit and plate counting.

Applications to Antibiotic Susceptibility Studies

Antibiotic susceptibility of an *E. coli K12*, FMP strain was evaluated with the Guava® Bacterial Count & Viability Kit and microcapillary flow cytometry. This strain has been shown to be resistant to kanamycin (Kan), but susceptible to ampicillin (Amp). Two mL of log phase *E. coli K12* were treated at 100 µg/mL for 0.5 to 26 hours. Aliquots were removed at indicated time points and analyzed with the Guava® Bacterial Count & Viability Kit. Data from viability and concentration analyses are shown (**Figure 4**). The viability data shows a clear reduction in viability upon ampicillin treatment, but viability is sustained upon treatment with kanamycin. In addition, cell concentration exhibits a decrease with ampicillin treatment, but gradually increases with treatment by kanamycin. Our results indicate that studies such as these can be greatly facilitated using MFC.

Figure 4.

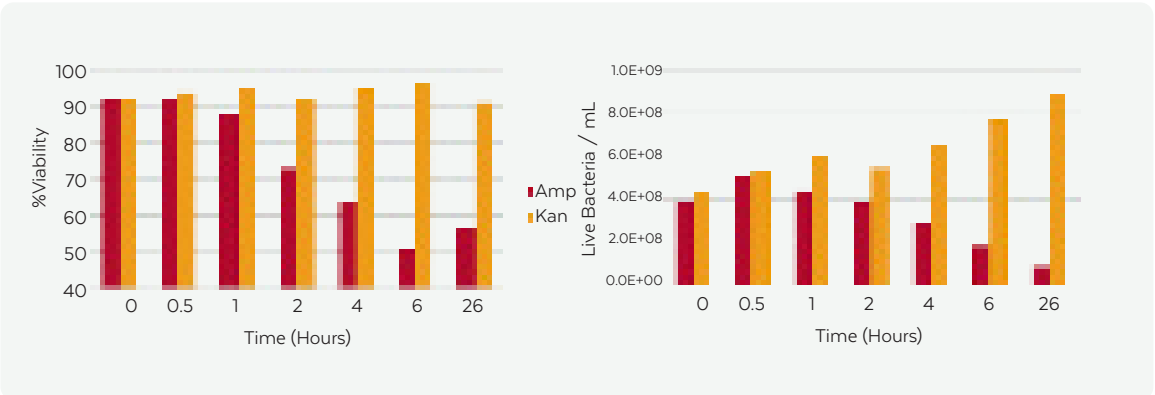

Figure 4. Results from *E. coli K12*, FMP treatment with kanamycin or ampicillin and staining with the Guava® Bacterial Count & Viability Kit.

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Easy Identification of Bacterial Gram Typing

Bacterial typing assays use distinctions based on membrane structure and permeability of gram-positive and gram-negative bacteria (**Figure 5. left, top, and bottom panels**) to provide identification of Gram type. Bacteria strains were stained with the Guava® Bacterial Gram Typing Kit and analyzed with microcapillary flow cytometry. This approach was applied to a range of gram-positive and gram-negative bacteria, and the results are summarized in the dot plots in the panel on the right. Gram-negative bacteria showed general bacterial staining and appear in the lower right region (**Figure 5. bottom panel**). Gram-positive bacteria showed staining with both dyes in the mix and appear in the upper right of the plot profile (**Figure 5. top panel**). Results demonstrate that bacterial typing can be easily performed with microcapillary flow cytometry.

Figure 5.

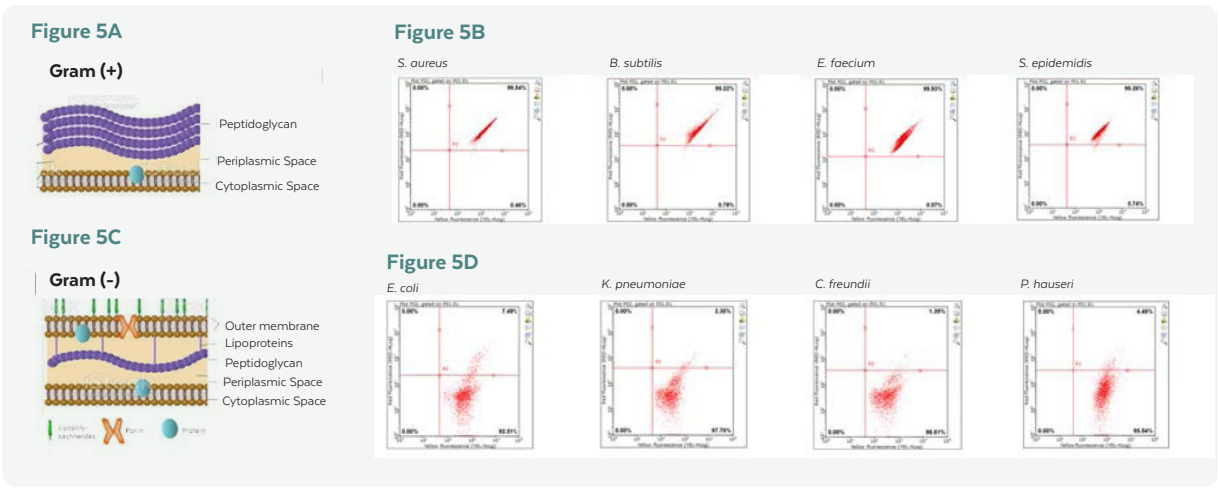

Figure 5. Example staining for the Guava® Bacterial Gram Typing Kit on Gram (+) **5B** and Gram (-) **5D** bacterial strains.

Conclusions

Determination of bacterial count and viability by traditional methods has numerous challenges: slow and time-consuming, exhibits high variability, and inability to detect non-culturable bacteria. Several platforms/technologies are challenging due to the small size of bacteria and the need to distinguish them from debris.

In these studies, we demonstrate the application of microcapillary flow cytometry to bacterial characterization with fluorescent dyes. The capability to achieve absolute count information on these platforms without adding additional beads is useful to easily obtain bacterial count and viability information. These studies demonstrate applicability to a variety of gram-positive and gram-negative bacterial strains, low variability results, and good correspondence to viable bacterial count. Gram typing results could also be obtained with microcapillary flow cytometry and corresponded well with traditional methods.

Microcapillary flow cytometry platforms thus present bacterial researchers with new capabilities for the rapid and simplified study of a range of bacterial characteristics.

APPNOTE437952
July 2023

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