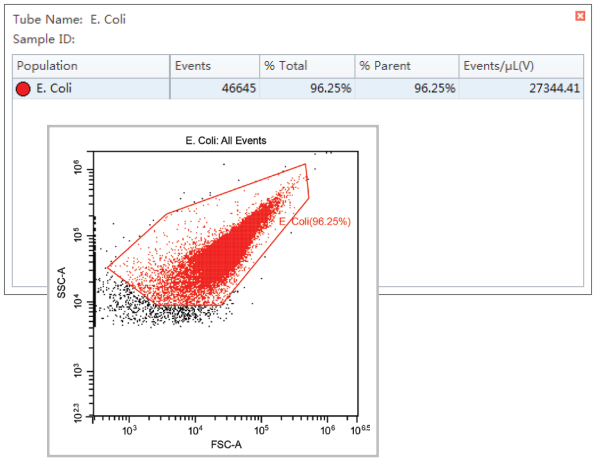
Counting *Eschericia coli* Using the CytoFLEX Research Flow Cytometer

Introduction

Often an enumeration of bacteria is required more quickly than a colony forming unit, CFU, assay can be completed. Here we describe a quick protocol to enumerate bacteria in a sample using the CytoFLEX* flow cytometer. The range of resolution can support the identification of bacteria by forward and side scatter parameters and does not require any fluorescent dyes or counting beads to enable detection or enumeration, respectively.

Materials and tools

E coli sample



Sample Preparation

1. Use PBS to dilute the *E. coli* sample if needed.
2. Record the dilution factor.

Data Acquisition and Analysis

1. Create a new experiment.
2. Draw a FSC/SSC plot, both axis are in log mode.
3. Run the diluted sample at Med or Fast rate settings.
4. Set the threshold on SSC channel.
5. Adjust the gains and threshold if needed.
6. Make sure that the abort rate is less than 10%. If not, increase the threshold or dilute the sample again.
7. Acquire at least 10µL sample
8. Create a gate on the *E. coli* population and check the cell count and concentration calculation in statistics window.
9. Use “Fit with Sample” function to show low signals.

Conclusions

Without using dyes or beads the bacterial sample can be enumerated using flow cytometry in a matter of minutes so that your research is not compromised or retarded while awaiting plating data. This is an inexpensive and rapid method for quantifying bacteria in suspension.

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