The Use of Flow Cytometry for the Rapid Detection of Fluorescent-tagged *Salmonella* spp. In Food and Environmental Samples

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Abstract

Green Fluorescent Protein (GFP)-tagged *Salmonella* strains are often utilized as positive controls for their distinguishability during a cultural confirmation by observing morphological fluorescence on an agar plate using UV light post incubation. Flow cytometry has the ability to distinguish GFP-tagged *Salmonella* without the need of additional labeling or manipulation. The purpose of this study was to demonstrate the ability of flow cytometry to distinguish a GFP-tagged *Salmonella* strain from other *Salmonella* spp. GFP-tagged *Salmonella enterica* subsp. *enterica* serovar Typhimurium SAL54 was propagated in Buffered Peptone Water, then transferred and grown up in RV Broth. An additional five wild-type *Salmonella* cultures were grown in a similar fashion. The cultures were then diluted and run on a flow cytometer. The instrument settings were adjusted to examine the voltage pulse height of the fluorescent emission through the BL1 530/30 band pass filter when excited by a laser with a wavelength of 488 nm. The BL1 histogram plot displaying the cellular events produced by the GFP-tagged *Salmonella* show a distinct cellular population emitting a stronger voltage pulse height due to the GFP excitation from the laser. Voltage intensity between 10^3 and 10^4 was observed in the GFP-tagged *Salmonella.* Wild-type *Salmonella* strains did not display the same cellular population and were easily distinguished from the GFP-tagged strain. The ability to distinguish GFP-tagged *Salmonella* control strains from wild-type *Salmonella* by flow cytometry allows for result confirmation in real-time. This discernment removes the need to grow cultures overnight to observe fluorescence, thus reducing the time to result.

Introduction

A flow cytometer uses laser light to detect particles in a fluid stream. Emissions scatter at different angles depending on particle size and internal complexity. The deflected light hits a series of detectors and the signals from these detectors are interpreted by computer software in real-time.

Figure 1. The Attune NxT flow cytometer.

A newer innovation, acoustic-assisted hydrodynamic flow cytometry, uses sound vibrations to align the particles and reduces the time to result.

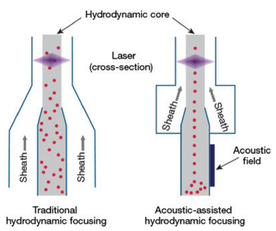

Figure 2. Rapid, improved alignment with acoustic focusing.

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Objective

Demonstrate the rapid detection of GFP-tagged *Salmonella* using flow cytometry and show the ability to discern GFP-tagged *Salmonella* from other *Salmonella* strains.

Materials and Methods

- Attune NxT Flow Cytometer equipped with Blue (488 nm)/Yellow (561 nm) laser configuration utilizing the BL1 530/30 band pass filter.
- *Salmonella enterica* subsp. *enterica* serovar Typhimurium SAL54 (Microbiologics, Cat. No. 01223UV-V) derived from FDA SAL 5694/ FDA SAL 5695.
- Wild-Type *Salmonella* spp. derived from Environmental Swab.
- Wild-Type *Salmonella* spp. derived from Whey Protein.
- Wild-Type *Salmonella* spp. derived from Dairy Powder.
- Wild-Type *Salmonella* spp. derived from Protein Shake (Chocolate).
- Wild-Type *Salmonella* spp. derived from Protein Shake (Vanilla).
- Diluted Rappaport Vassiliadis (RV) broth (Neogen, Cat. No. 7730) was used as a Negative Control to demonstrate the background inherent to the selective diluent.
- Cultures were propagated overnight in Buffered Peptone Water (Neogen, Cat. No. 7418).
  - Wild-Type strains were cultivated with their derived matrix.

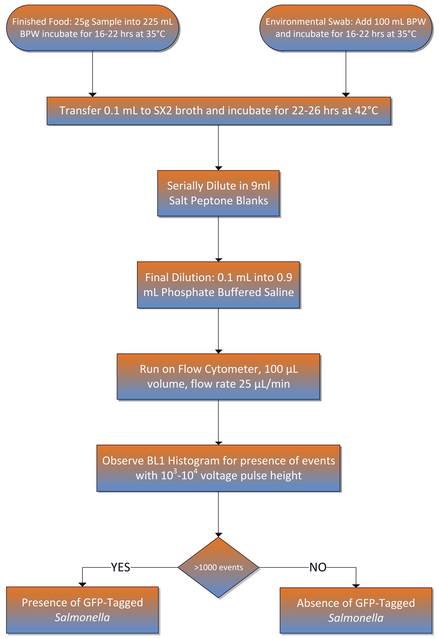

Figure 3. Flow diagram of flow cytometry method for detection of GFP-Tagged *Salmonella* spp. in finished product and environmental samples.

Results

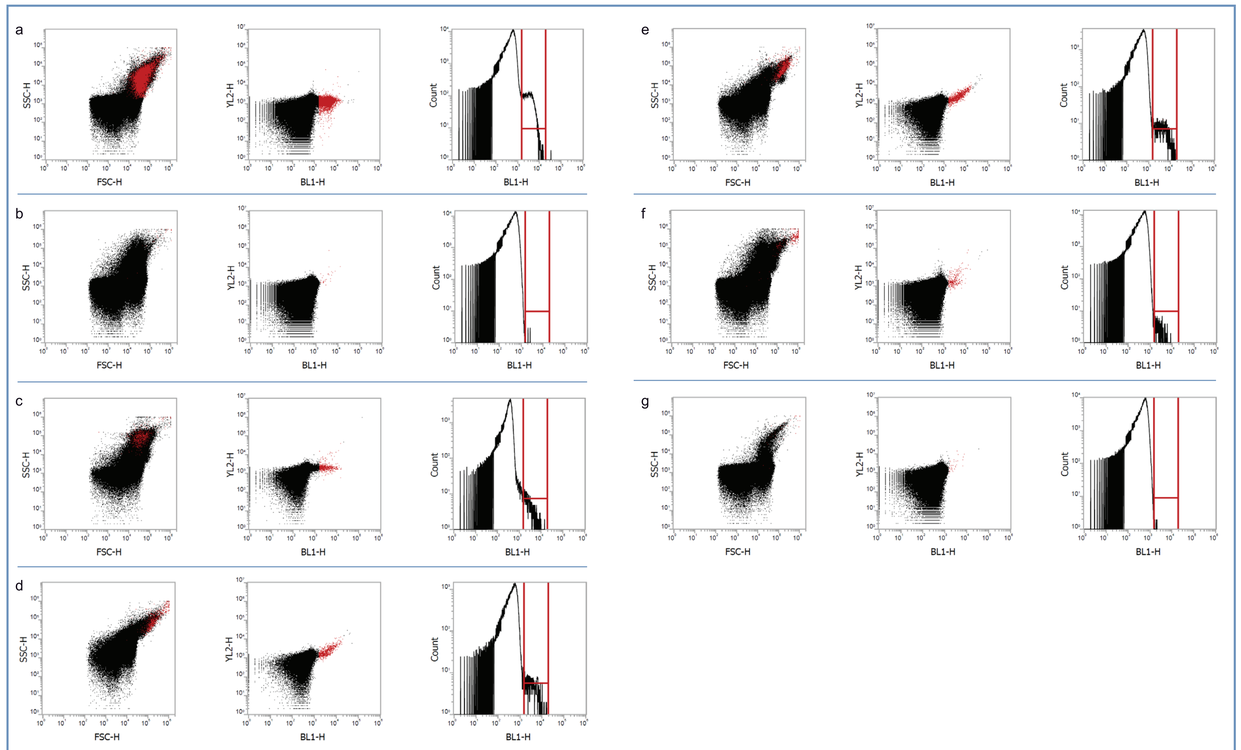

Figure 4.(a) GFP-Tagged *Salmonella enterica* subsp. *enterica* serovar Typhimurium SAL54 demonstrates excitation from the blue laser as shown by gated population on the BL1-H histogram (right). Excitation due to the fluorescent tag shows a distinct cellular population based on the voltage pulse height of the emission captured through the BL1 band pass filter (530/30). The gated population is identified on the BL1-H/YL2-H dot plot with events in red. **(b)** Negative control shows absence of events in the histogram gate and evidence of background events from RV media and particle debris. **(c)-(g)** Wild-Type *Salmonella* strains (c: environmental swab, d: whey protein, e: dairy powder, f: protein shake (chocolate), g: protein shake (vanilla)) without the addition of a fluorescent label do not produce the same emission spectra captured by BL1-H. Some cellular events are captured within the same gate due to natural fluorescence of the organism, but significantly less compared to the GFP-tagged strain.

Conclusion

The use of GFP-tagged *Salmonella* strains as positive controls for rapid qualitative *Salmonella* detection testing provides assurance during the confirmation process the presumptive positive sample result obtained on the instrument was not due a positive control contamination event. Historically, this distinction occurs after presumptive cultures are streaked for isolation and grown on agar plates overnight.

Flow cytometry has the ability to distinguish GFP-tagged *Salmonella* from non-fluorescing strains in real time. Non-fluorescing strains did not show the same emission properties and can be easily distinguished on the histogram. Real-time distinguishability of GFP-tagged *Salmonella* by flow cytometry can confirm a presumptive positive signal obtained by the instrument is not due to a positive control contamination event immediately after the signal is produced.

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