Bead Based Multiplexing for the Simultaneous Detection of *Salmonella enterica, Listeria monocytogenes, Escherichia coli*

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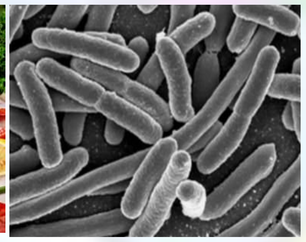

Introduction

As the human population increases food safety becomes an ever-increasing concern. *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* are three of the pathogens that can cause serious harm. *Salmonella enterica* and *Listeria monocytogenes* are two of the most likely to cause death, 1st and 3rd respectively, according to the Center for Disease Control (see CDC website). *Salmonella* and *Ecoli* are 2 of the top 5 likely to cause hospitalization, 1st and 5th respectively, according to the Center for Disease Control (see CDC website). The detection of pathogens in food products is crucial to the prevention of food-borne illness. This type of research is critical in safeguarding food supplies; The current process for pathogen detection is in need of an overhaul, as it is a slow and tedious process.

Specific Objectives

1. Determine ideal media, grow, and amplify DNA of pathogens in question.
2. Establish and standardize an efficient testing protocol for multiplexing on the Magpix by Luminex.
3. Evaluate the limit of detection (LOD) of multiplexer for these organisms.
4. Use spiked juice samples to simulate real world application of protocol.

Methods

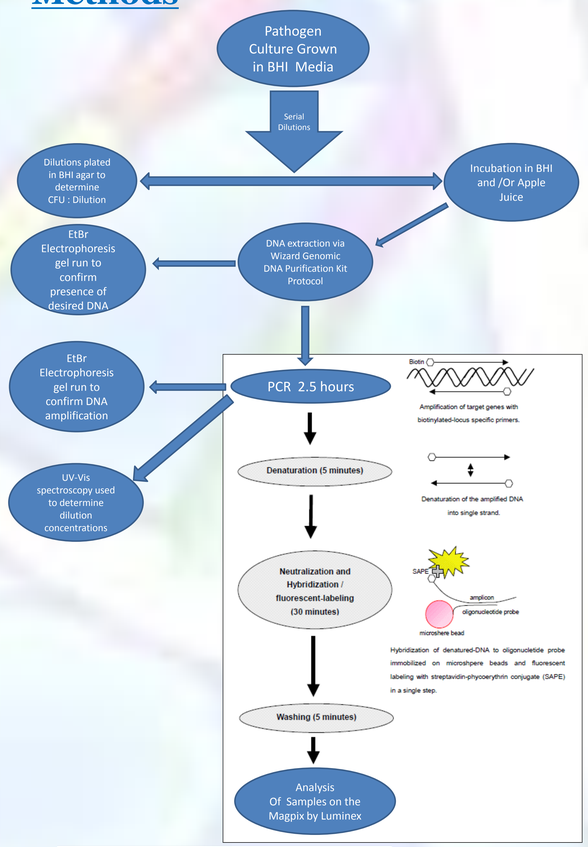

Figure 1: Schematic of the overall testing process.

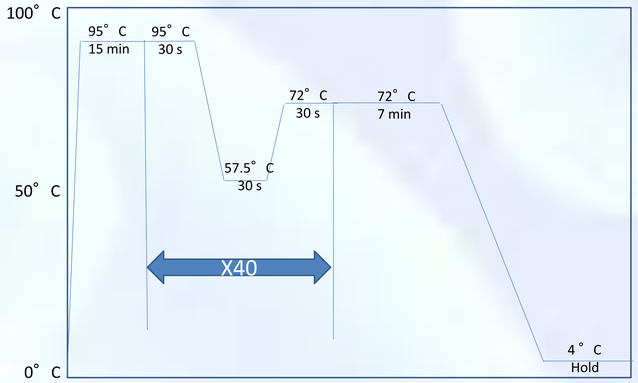

Figure 2: PCR program used in the amplification of DNA samples in the Eppendorf Thermo Cycler

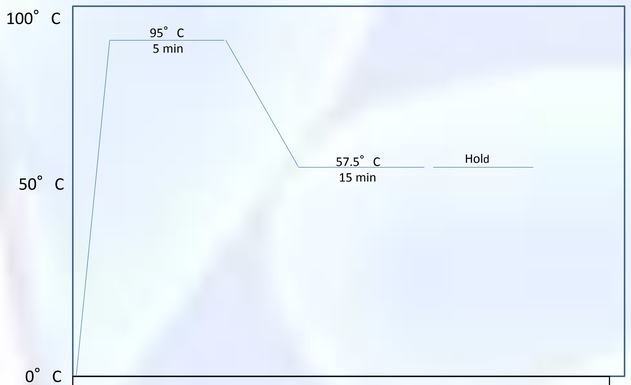

Figure 3: Bead hybridization program used on the Eppendorf Thermo Cycler

The bacterial genes and strains subjected to this process include: *invA* gene of *Salmonella* typhimurium (ATCC14028), *hlyA* gene of *Listeria monocytogenes* (ATCC7644), and the *hlyA* gene of *Escherichia coli* (ATCC43889)

Results

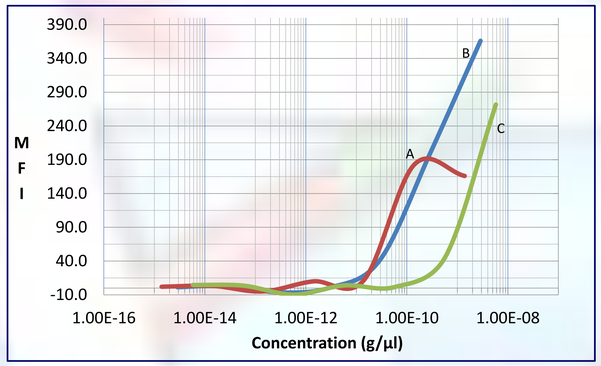

Figure 4: Median Fluorescence Intensity (MFI) is the unit of measure of the Magpix by Luminex apparatus . **(A)** *Listeria monocytogenes (ATCC7644)* **(B)** *Salmonella enterica* **Typhi** (ATCC14028) **(C)** *Escherichia coli (ATCC43889)*

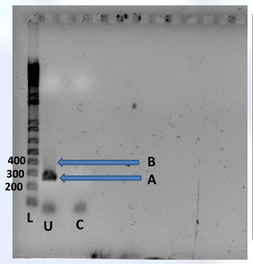

Figure 5: (L) DNA Reference Ladder
(U) Unknown Sample containing pathogen DNA
(C) Control containing primers , no DNA

(A) Evidence of DNA presence in the 250-300 bp.
- *Listeria monocytogenes hlyA* gene (271 bp)
- *Salmonella enterica* Typhi invA gene (280 bp)

(B) The streak is evidence of presence of DNA 300-400 bp in length, though amounts are minute. Ethidium Bromide (Et-Br) agarose gel has a lower limitation of ~20 ng of DNA.
- *Escherichia coli hlyA* gene (363 bp)

The process has not been as expedient and efficient as we had hoped for, but the results clearly show the multiplexing capabilities of this apparatus. The MFI data represented in Figure 4 shows the detection of the 3 pathogens in a single sample and their presence in the sample is supported by the Et-Br agarose gel. The two also demonstrate that the LOD can be less than 1 ng concentration of pathogen DNA.

Conclusions and Future Work

Preliminary results are promising but a minimum of spiked apple juice samples have been run. More samples with spiked juice will need to be run to further support existing results. Moving on, samples should include other types of food and increase the number of pathogens detected in each sample. The Magpix by Luminex has a multiplexing capacity of 50 pathogens per analyte and it will be necessary to develop protocols for as many as possible before putting it to use in commercial settings. The results are promising. Nevertheless and the future for its use in commercial settings, even more; limiting food borne illness should be greatly improved.

References

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http://www.cdc.gov/foodsafety/facts.html

This material is based upon work supported by the Howard Hughes Medical Institute (HHMI). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of HHMI.

This work was administered by the Cal Poly Center for Excellence in Science and Mathematics Education (CESaME) and the Fresno State Science and Mathematics Education Center (SMEC) on behalf of the California State University.