ON-CHIP PARALLEL DETECTION OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Shiga toxin-producing *Escheria coli* (STEC) strains are virulent agents responsible for thousands of illnesses in the United States [1]. The most common and notorious STEC serotype is O157:H7 but other serotypes, like O104:H4 responsible for May 2011 HUS outbreak in Germany [2], are responsible for one third of STEC related illness. Beginning on June of 2012 the U.S. Department of Agriculture started a zero tolerance policy against six non-O157 STEC groups (the 'big six') that cause over 70% of the total non-O157 illnesses. The new regulation requires a 2 step quantitative PCR (qPCR) that makes the screening process more expensive and labor intense [3]. We are creating a biological microchip that will run parallel amplification of target DNA reducing time and cost of detection assays.

The biological chip for detection of STEC groups will have several advantages over the conventional methods. First, the bio-chip will be capable of running multiple assays in parallel. This will allow simultaneous detection of multiple genes reducing screening costs. Second, reaction samples will be an order of magnitude smaller. Instead of using microliters per reaction the bio-chip will only need nano-liters reducing cost of reagents. Finally the biochip assay will automate the DNA detection process. Using already reported techniques of pre-dried primers and automation of microfluidic tools like a capillary micro injector, the screening process will be simpler and automated reducing required labor and training.

We have performed DNA amplification based detection of *E.coli* using a system that creates a nano-droplet array and then amplify target DNA. Instead of the standard PCR we are using a novel nucleic acid amplification technique named LAMP [4]. This new technique is isothermal, reducing system's hardware and software complexity, and is also more specific than PCR due to a larger number of annealing primers. So far we have created arrays of ~50nL droplets targeting a single gene (*eae*) and including negative controls (Figure 1). Future steps include primer dehydration to easily target multiple genes in a single assay. This will take us closer to an automated bio-chip for detection of non-O157 STEC groups that will ease new regulation compliance by making screening of virulent agents in food faster, cheaper and easier.

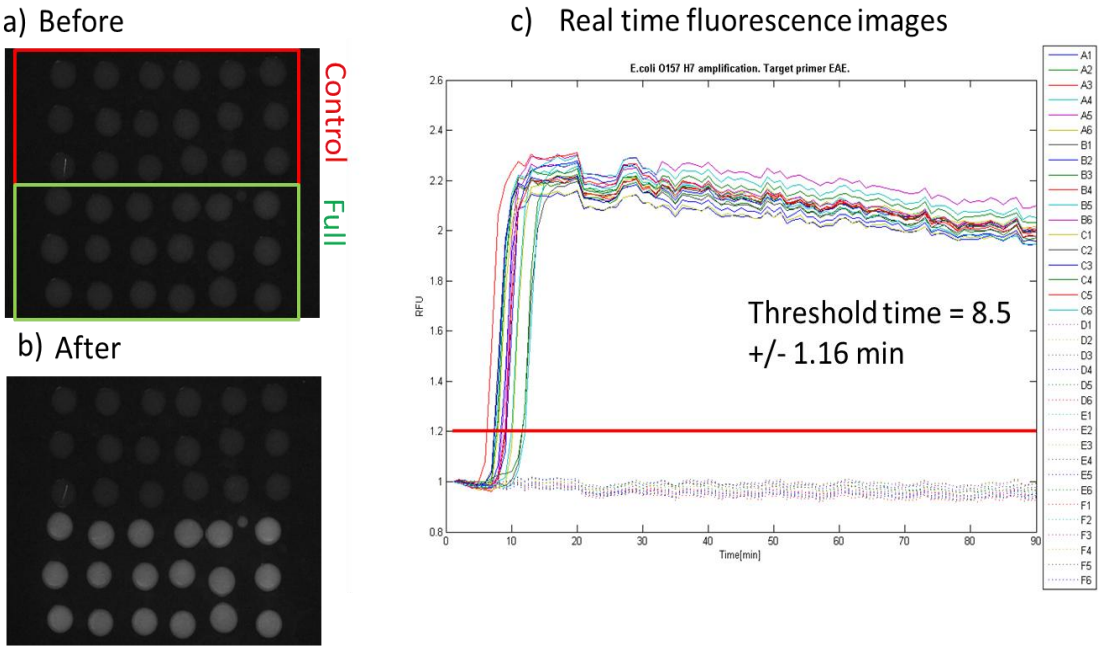

Figure1. 6x6 array for on-chip detection of *E.coli* O157:H7 using LAMP amplification targeting *eae* gene. The 6x6 array contain 18 full samples and 18 negative control samples. a) Fluorescence image prior amplification. b) Fluorescence image after amplification (90min). c) Real time fluorescence measurements for each reaction. Rows are labeled with literals and columns with numerals. Dotted lines are for negative controls and full lines for positive. No false positives or negatives were observed

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