Detection and identification of the ‘big six’ STECs in nanoliter droplets using isothermal DNA amplification

Carlos Duarte-Guevara[1], Eric Salm[1], Anuradha Nandyala[1], Atul K Singh[2], Bobby Reddy Jr.[1], Vilasur Vikhram[1], Arun K Bhunia[2], Rashid Bashir[1]

[1]*University of Illinois at Urbana-Champaign*
[2]*Purdue University*

Shiga toxin-producing Escherichia coli (STEC) strains are virulent agents responsible for thousands of illnesses in the United States. The most common and notorious STEC serotype is O157:H7; however, other serotypes, such as the O104:H4 strand that was responsible for the May 2011 HUS outbreak in Germany, account for one third of STEC-related illnesses. Beginning in June of 2012, the U.S. Department of Agriculture initiated a zero tolerance policy for six non-O157 STEC groups (the ‘big six’) that cause over 70% of total non-O157 illnesses. In order to detect these 6 serotypes (O26, O45, O103, O111, O121, and O145) the food safety and inspection service has outlined a detection protocol that requires two steps of quantitative PCR (qPCR). The new requirements make the screening process more expensive and labor intensive. By combining micro-fabrication and DNA isothermal amplification techniques it is possible to reduce complexity and cost of the ‘big six’ detection and identification process.

Using an array of silicon oxide micro-wells, a primer dehydration technique, and a microinjection system, we have demonstrated a method for parallel detection and identification of the ‘big six’ group in nano-liter droplets. The primer dehydration protocol enables the detection of multiple genes in a single assay. The use of the microinjector to fill the microwells reduces required sample volumes, minimizing the consumed reagents per reaction. The complexity of required equipment is significantly reduced by the use of loop-mediated isothermal amplification (LAMP), a highly specific amplification technique that eliminates the need for precise thermo cycling control required by more common amplification techniques such as PCR. Furthermore, the use of a platform microfabricated utilizing standard semiconductor processes enables the possibility of future integration with bio-chemical field effect transistors for smaller and cheaper detection systems. Characterization experiments demonstrate that is method has a detection limit of a few templates per micro-well, specificity that enables robust multiplexed identification of pathogens, and detection times under one hour. These results demonstrate significant steps towards affordable, fast, and automated on-site detection of food pathogens.