A153 Detection of Pathogen Mixtures Using Luminex® Technology

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After attending this presentation, attendees will gain some insight into how Luminex® technology may be used to address the perennial threat of bioterrorism.

This presentation will impact the forensic science community by demonstrating that the Luminex® technology is capable of detecting pathogens with a celerity and specificity that surpasses alternative methods, thus allowing for a swift and appropriate response to potential cases of bioterrorism.

Luminex® technology is a bead-based liquid array platform that relies on small (5.6 µm diameter) polystyrene microspheres that are each internally labeled with their own unique dye combination. DNA probes unique for a particular bacterial species were attached to the microsphere surface. Target DNA (*i.e.*, the unknown) was prepared by PCR and labeled with a reporter fluorophore. Hybridization between the DNA probes and the target DNA causes a fluorescent emission which is then detected using Luminex® xMAP technology and MiraiBio MasterPlex® software. The instrument classifies the microspheres by using a red diode laser (635 nm) to detect the fluorescence emitted by the internal dyes of the microspheres, and a green diode laser (532 nm) to detect and quantify the target DNA by measuring the intensity of the fluorescence emitted by the reporter.

It was hypothesized that the robustness of Luminex® technology would be validated not only when screening for individual pathogens, but also when screening mixtures of several pathogens. First, the ability to detect each of four individual pathogens (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, or *Salmonella enterica*) was evaluated using probes complementary to sequences in the respective 23S ribosomal RNA genes, *rrl*. The specificity of the assay was confirmed, although some cross-reactivity was observed between *E. coli* and *S. enterica*. Adjustments made to the buffer salt concentration and hybridization temperature did not remedy the problem.

The ability to detect DNA in mixtures was then evaluated. To begin, all possible binary, ternary, and quaternary mixtures of pathogen DNA were examined using 10 ng DNA from each respective pathogen. In all mixtures, the highest median fluorescent intensity (MFI) values always corresponded to the bacterial DNA that was present. However, the MFI values did not always meet the minimum threshold (> 2X background MFI), and thus there were several instances of false negatives. No false positives were observed. Most of the false negatives involved the probe for *S. enterica*, which exhibited MFI values below the threshold even when *S. enterica* DNA was present. This was possibly due to cross- reactivity with the *E. coli* probe, which exhibited 85% sequence homology with the *S. enterica* probe.

A subsequent mixture study examined all possible binary mixtures of the four pathogens' DNA when present in various ratios (1:1, 1:2, 1:5, 1:10, and all converse ratios). Again, the highest MFI values always corresponded to the bacterial DNA that was present, even when the minor component was present at 1/10 the concentration of the major. As before, there were several instances in which the MFI values unexpectedly failed to meet the minimum threshold, thus yielding a false negative. All false negatives involved the *S. enterica* probe, possibly due to the cross- hybridization described above. No false positives were observed.

Future studies will include specificity, sensitivity, and mixture studies on DNA from additional pathogens that are more closely related. The use of markers with minimum sequence homology is expected to reduce potential problems with cross-reactivity. Multiple markers for each pathogen will also be sought, so that the likelihoods for false positives among closely related pathogens can be minimized.

Luminex®, Pathogens, Bioterrorism

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