Field evaluation of microfluidic paper-based analytical devices for microbial source tracking

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Summary

We have developed a method to determine the contamination of lettuce and collection flags exposed to airborne fecal matter around animal operations. The team collected 1,800 samples of lettuce and collection flags from Salinas, California, and analyzed these samples using quantitative polymerase chain reaction (qPCR) assays to detect *Bacteroidales* as markers of fecal contamination. The analysis demonstrates that background levels of *Bacteroidales* are extremely low in clean low-risk fields. We are in the process of conducting similar measurements in the field by using assays based on loop-mediated isothermal amplification (LAMP) and implementing them on paper-based devices. We have tested a preliminary form of this system for on-farm LAMP assays in a field close to an animal operation in Indiana.

Objectives

1. **Establish background levels of fecal and pathogenic contamination in the field to determine the limits of detection that are needed for a field-based assay.**
   - Determine the contamination of lettuce leaves exposed to airborne fecal matter from different animal sources, including cattle, swine, and poultry.
   - Determine the levels of fecal contamination in low-risk lettuce fields.
2. **Design and test a portable microfluidic paper-based analytical device (µPAD) that can detect contamination and provide results within an hour in the field.**
   - Design and fabricate a fluid delivery system for precise transfer of DNA samples to the LAMP reaction sites.
   - Design, fabricate, and test a heating system and integrate it with the fluid delivery and imaging units for on-farm LAMP assay.

Methods

For lettuce leaves and collection flags (made using a piece of plastic and a wooden stick), the Purdue team has developed a swabbing protocol in which a sterile swab was dipped in ultrapure water, and then the entire surface area of the leaf/flag was continuously swabbed. The collected matter (settled bioaerosols) was re-suspended in 200 µL of water. For sample collection in the field in Salinas, the collection flags are placed in the field for a week before shipping them back to Purdue and storing at –20 °C until processed. For conducting on-farm experiments, the team deployed 36 flags around an animal operation in Indiana for a week. The team conducted LAMP assays on these flags in the field using µPADs and a custom-built fluid delivery and heating system.

Results to Date

The team has designed a LAMP assay for detecting *Bacteroidales* as a marker for fecal contamination. The team demonstrated that the levels of this marker are low in low-risk fields (**Figure 1**) and high in high-risk situations (**Figure 2**). The assays in the liquid medium are now well established **(Figure 3**) and the team has started performing LAMP assays on paper in the field (**Figure 4**).

Benefits to the Industry

This project is developing and is testing a procedure to determine contamination of fresh produce by aerosolized fecal matter from various sources. Such a procedure is useful for assessing risk of planting crops around animal feeding operations. The measurements conducted here demonstrate that the levels of fecal contamination vary by 3–4 orders of magnitude when comparing the low-risk and high-risk extremes. The tools developed here could support site-specific risk-assessment if thresholds for risk are established.

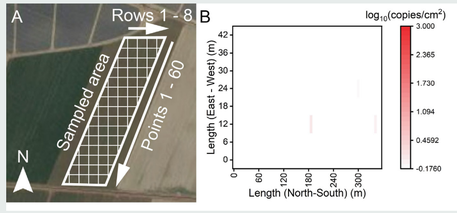

Figure 1. Risk assessment heatmap in Salinas, California. **A)** Satellite image of the sampling site with 8 rows of lettuce planted. 480 collection flag samples were collected from an area of ~360 m (North South) x 48 m (East West). **B)** Heatmap with 480 data points generated using qPCR indicates extremely low levels of fecal indicator *Bacteroidales* DNA in a field that is anticipated to be safe (away from animal operations). Only 3 out of 480 samples show Bacteroidales DNA that is measurable above the theoretical limit of detection of 0.667 copies/cm ^{2}, which corresponds to 1 copy/ reaction. These 3 samples are at the following coordinates in the plot for North South (m), East West (m) locations: (186, 12); (300, 24); (348, 12).

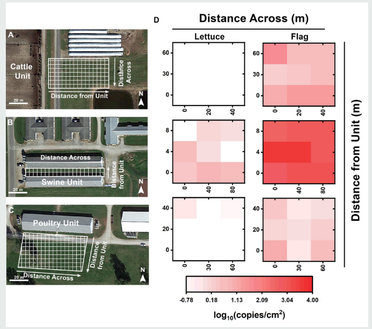

Figure 2. Risk-assessment heatmap near animal operation units. **A, B, C)** Satellite images of the sampling sites. 9 leaves/ flags were collected in each site. **D)** Heatmaps with 9 data points generated using qPCR indicate extremely high levels of fecal indicator *Bacteroidales* DNA in a field that is close to animal operations. The results obtained from collection flags are more consistent compared to those obtained from lettuce leaves.

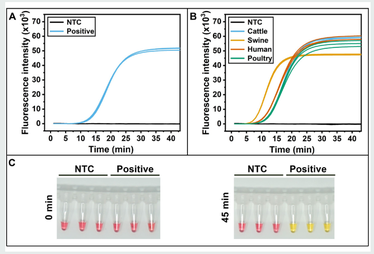

Figure 3. Characterization of LAMP primer (Universal. *Bacteroidales*.16s rRNA.1). **A)** Fluorometric result from LAMP primer set using genomic DNA extract from pure culture of *Bacteroides fragilis*. **B)** Fluorometric performance of LAMP primer set using stool extractions. **C)** Colorimetric results from LAMP primer set using genomic DNA extract from pure culture.

Figure 4. Comparison of two different methods for conducting paper-based LAMP assay on site in the back of a pickup truck: one method uses a scanner, and the other uses a camera.