Evaluation of DNA/RNA Protect Swabs with *Escherichia coli* Spiked Stool Samples

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Summary

In order to evaluate the applicability of DNA/RNA Protect™ Swabs (Sierra Diagnostics, Inc., Sonora, CA) to transport and store stool samples at room temperature, normal stool from healthy donors was spiked with Shig toxin-producing *Escherichia coli* (STEC) O157:H7 at two inoculation levels.

High Inoculation level of 10^7 cells/g is comparable to the excretion level of an individual with an acute illness. Low levels of 10^5 cells/g is traditionally regarded as the limit of detection using PCR directly from stool.

Procedure

Thirty four swabs with high and low inoculation levels, respectively, were prepared and stored at room temperature. One to three swabs from each inoculation level were tested on days 0-7, 11, 14, 18, 21, 29, 36, and 43.

The swabs were first streaked on sorbitol-MasConkey agar and then used to extract DNA with MagnaPure (Roche Molecular Dignostics, Mannheim, Germany) automated DNA extraction system.

The overnight growth from sorbitol-MasConkey plates and the DNA extracted directly from the swabs were tested for the presence of genes responsible for Shiga toxin production (*stx*$_1$ and *stx*$_2$) using hybridization probe-based real-time PCR assay.

Results

Results for the first 21 days: all high inoculation level swabs grew on sorbitol-MasConkey plates with high number of colonies typical for STEC O157:H7 (lactose negative, colorless colonies). All but four (days 1, 4, 5, and 18) low inoculation level swabs yielded a culture with few isolated colonies typical for O157:H7.

The colonies picked from the plates were confirmed to be O157:H7 by PCR. All DNA samples extracted directly from the swabs were positive by PCR, including DNA from the four swabs that did not yield a culture. Even the low inoculation level swabs were strongly PCR-positive, suggesting that the limit of detection could be at least one log lower than 10^5 cells/g.

Test results for days 29, 36, and 43 are pending.

Conclusion

Preliminary results suggest that DNA/RNA ProtectTM swabs are very well suited for transportation and storage of stool samples for enteric pathogens even for prolonged period of time.

More importantly, they do not only facilitate fast diagnosis by PCR but can also be used to obtain a culture and isolate for further subtyping.