CRISPR/Cas cell-free sensors for rapid detection of pathogenic *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* in complex food and environmental samples

Helena de Puig Guixe[1], Devora Najjar[1], Michael S. Wiederoder[2], Dominique Reilly[2], Shannon K. McGraw-Manza[2], and James J. Collins[1]

[1] The Wyss Institute for Biologically Inspired Engineering, 3 Blackfan St, Boston, MA, USA

[2] US Army Combat Capabilities Development Command – Soldier Center, 10 General Greene Ave., Natick, MA, USA

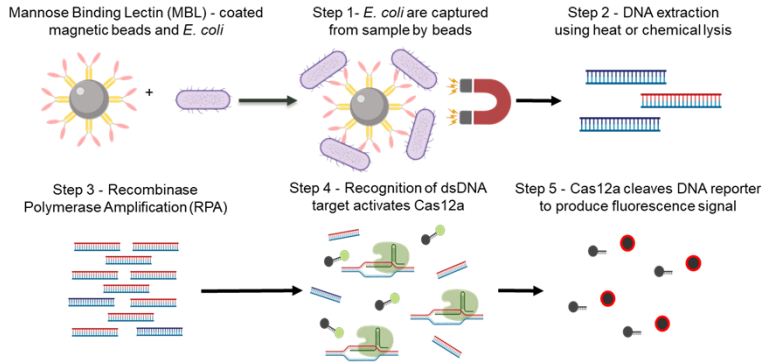
PROBLEM

- Consumption of food and waterborne pathogens is a significant hazard to military personal and civilians
- Standard testing methods such as culture counting and PCR require long wait times (24-48 hrs), expert personnel, and centralized labs
- Cell-free sensor systems freeze dried in tubes can enable rapid, portable, sensitive, low-cost, and easy-to-use diagnostics that overcome these limitations

BACKGROUND

- Prior work demonstrates detection of Zika [1] and malaria [2] from clinical samples on paper-based sensors using CRISPR/Cas-based Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK)
- Unfortunately, complex food samples contain acids, fats, carbohydrates, and debris that inhibit these assay chemistries and reduce diagnostic performance
- Prior work demonstrates isolation/concentration of numerous pathogen species (*S. aureus, E. coli, P. aeruginosa*, etc.) from clinical samples [3-5] using magnetic beads coated with mannose-binding (MBL)
- This study demonstrates non-specific pathogen isolation/concentration using MBL-conjugated magnetic beads with highly specific DNA molecule cell-free paper sensors (SHERLOCK) for detection of common food pathogens: shiga toxin producing *Escherichia coli, Salmonella enterica, and Listeria monocytogenes* in complex food and water matrices.

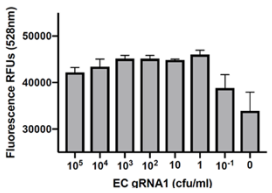
FcMBL + SHERLOCK EXPERIMENTAL DESIGN



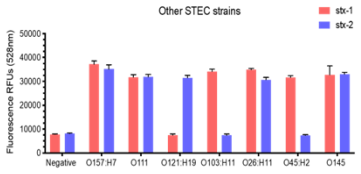
The system consists of five sequential steps that take 1.5-2 hrs at 30-41 °C

1. Target is isolated/concentrated from complex matrices using MBL-magnetic beads or polyethersulfone (PES) membranes.
2. Nucleic acids are extracted from captured bacteria using thermal lysis.
3. Target dsDNA sequences are amplified using recombinase polymerase amplification.
4. Target dsDNA is recognized by complimentary guide RNA which activates Cas12a, an endonuclease.
5. Activated Cas12a cleaves ssDNA reporter probes with a fluorophore and quencher to generate fluorescent signal.

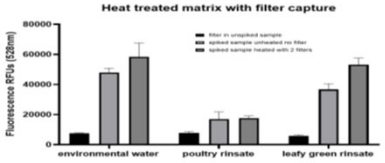
RESULTS



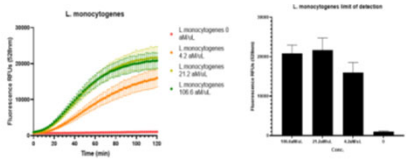
Fluorescent response for stx1 assay. *E. coli* O157:H7 diluted in 50 mL test water, concentrated with MBL magnetic beads, followed by simultaneous RPA and SHERLOCK in microwell. The LOD is 1 CFU/mL or 50 bacteria in 50 mL.



Designed system can detected two different shiga toxin producing sequences (stx-1 and stx-2) to identify seven unique pathogenic strains of *E coli*.



Fluorescent response for 10^4 cfu/mL *S. enterica* diluted in 1 mL of sample, concentrated with a PES filter. The sample was incubated with EGTA and DTT at 95°C for five minutes. The matrices were then flown through PES membranes and run in an RPA-CRISPR reaction.



The 2- pot CRISPR assay for L. monocytogenes detection using synthetic DNA target. It was shown to be sensitive down to 4.2aM/µL of target with clear signal differentiation by 30 minutes. The endpoint was taken at 120 minutes.

CONCLUSIONS AND FUTURE WORK

- Limit of detection is 1 CFU/mL in 2 hrs for *E. coli* O157:H7 in test water standard demonstrating high potential for rapid, low-cost sensors for food pathogen detection
- Assay was shown to be sensitive down to 10aM/µL of synthetic *S.enterica* DNA target over 60 minutes.
- Assay was found to be sensitive down to 4.2aM/µL of synthetic *L. monocytogenes* DNA target in 30 minutes.
- Using genomic DNA the *S. enterica* sensor showed a detection sensitivity of 50 cfu/mL in 60 minutes.
- A PES membrane added for target concentration and capture had a detection limit of 5cp/mL for *S. enterica* genomic DNA in a 50mL water sample over 120 minutes.
- When the assay was tested in complex matrices (environmental water, poultry rinsate, leafy green rinsate) however, the matrices were found to significantly inhibit the membrane's ability to concentrate the target. It was determined that the samples need to be lysed before filter capture in order see a successful assay reaction.

ACKNOWLEDGEMENTS

This work was funded directly by the DoD Combat Feeding Research and Engineering Program.

REFERENCES

[1] Gootenberg, J.S., et al., "Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6," *Science*, **360**, 439, 2018.
[2] Lee, Rose A., et al. "Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria." Proceedings of the National Academy of Sciences 117.41 (2020): 25722-25731
[3] Bicart-See, A., et al., "Rapid Isolation of Staphylococcus aureus Pathogens from Infected Clinical Samples Using Magnetic Beads Coated with Fc-Mannose Binding Lectin," *PLOS ONE*, 11, e0156287, 2016
[4] Seiler, B.T., et al., "Broad-spectrum capture of clinical pathogens using engineered Fc-mannose-binding lectin enhanced by antibiotic treatment," *F1000Research*, **8**,108, 2019
[5] Cartwright, M. et al., "A Broad-Spectrum Infection Diagnostic that Detects Pathogen-Associated Molecular Patterns (PAMPs) in Whole Blood," *EBioMedicine*, 9, 217-227, 2016

FOR FURTHER INFORMATION:

U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND SOLDIER CENTER:
DEVCOM.ARMY.MIL

POINT OF CONTACT:
Shannon McGraw-Manza
shannon.k.mcgraw-manza.civ@army.mil
508-206-3355

Approved for Public Release, PR2023-278

U.S. ARMY