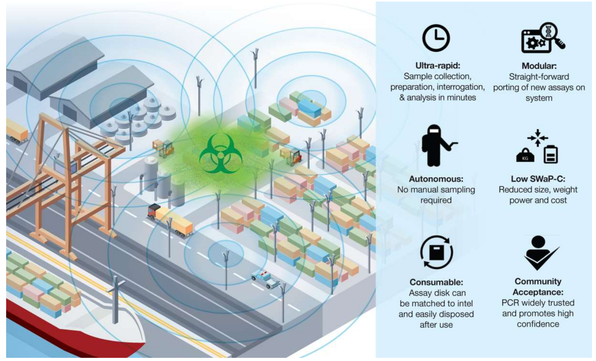
X-BIRD: Extreme PCR Bio-Identification Rapid Detector

Kim Berk[1], John Biondo[2], Johanna Bobrow[3], Jason Jong[3], Jordan Wynn[3], Todd Thorsen[3], Joe Lacirignola[3], Jude Kelley[3], Isabel Smokelin[3], Dave Walsh[3], Bryan Rivers[1]

[1]U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD, [2]Excet, Inc., Springfield, VA, [3]MIT Lincoln Labs, Lexington, MA

The New Age of PCR

Extreme PCR (xPCR) is a recently developed method that leverages plasmonic heat generated by the interaction of laser light energy with gold nanorods to thermocycle much faster than Peltier based methods commonly included in laboratory instruments. PCR systems are the gold standard for definitive bio-identification but take much longer to complete than lower sensitivity, antibody-based methods. Current device workflows require time measured in hours to complete sample preparation, amplify the target through thermocycling, and interpret the results. They also often require both a stable environment and trained staff to produce accurate results. Environmental sensors are often paired with an aerosol collection system that requires an operator to recover the filter in a remote location and transport the sample to the laboratory for analysis. These tasks risk the operator becoming contaminated with a biology hazard, are often performed while wearing bulky PPE, and further lengthen the sample-to-answer timeline.



This program couples xPCR with advancements in microfluidics, machine learning, and component miniaturization to shrink the time for sample-to-result to minutes. In collaboration with MIT LL, this program is developing a system capable of performing sample processing, plasmonic PCR, sample detection, and reporting in an autonomous device. X-BIRD is a pioneering system that will fully automate aerosolized biothreat detection in a small puck-style form factor that minimizes SWaP (size, weight, and power) to reduce logistical burdens. The puck is modular and can be attached a variety of systems including UAVs and perimeter sensing networks through the ACoRNS III interface.

Assay Development

Over 25 biothreat assays have been developed for the program using the Quant Studio 6 Flex as the base system. The system was operated at the fastest ramp speeds and shortest cycle times possible while still enabling florescent monitoring of the reactions.

Each assay was screened against performance metrics evaluating amplification efficiency (90-100%), limit of detection (≤ 40 GE/uL or 40,000 GE/mL sample), and linearity (R^2 of standard curve ≥ 0.995). 10 of the 25 assays have passed and moved forward into additional validation including inclusivity and exclusivity testing and porting onto the xPCR prototype. Select assays are shown below.

Assays developed and validated include those targeting: *B. pseudomallei*, B. *mallei*, *B. anthracis*, *Y. pestis*, *F. tularensis*, and *E. coli*. The *E. coli* assays were developed to de-risk aerosol release testing. All assays were evaluated for inclusivity and exclusivity and passed.

Continued assay development is being performed using alternative polymerases that have more flexibility for customization, have lower concentrations of glycerol, and are more amenable to lyophilization. The polymerases are being evaluated for use in the prototype system.

Organism	Assay	% Efficiency	LOD (GE/mL)
E. coli	ybbW	103.9	4,000
	23S	97.6	400
B. pseudomallei	BURK11	98.9	40,000
	BURK15	92.4	4,000
	Bp3	86.2	40,000
	Bp4	88.1	40,000
B. pseudomallei and B. mallei	BURK12	98.1	40,000
	Bpm	93.4	4,000
B. mallei	Bm	80	40,000
B. anthracis	BA Assay 1	94.4	40,000
	BA Assay 2	86.3	40,000
	BA Assay 3	92.4	4,000
	BA Assay 4	88.7	4,000
	BA Assay 5	86.4	4,000
	BA Assay 6	92.2	4,000
Y. pestis	YP Assay 1	90	400
	YP Assay 2	92.7	400
	YP Assay 3	87.3	400
	YP Assay 4	97	4,000
F. tularensis	FT Assay 1	86.8	4,000
	FT Assay 2	89.5	4,000
	FT Assay 3	88.4	4,000

Sample Prep

Incorporation of sample prep into the final device is critical for automated detection. The method must be simplistic in order to integrate into a microfluidic workflow while effectively removing PCR inhibitors. Chemical, mechanical, and physical methods were evaluated as well as combinations of methods. The top performers relative to untreated sample were: Biomeme lysis buffer, RIPA buffer, custom DTT lysis buffer, and 5 – 10% Triton X-100. Surprisingly, the most effective method was simply heating the sample to 95° C. This can be easily achieved on the device; however, methods will be needed to remove inhibitors from the aerosol sample.

Evaluation of additional methods of sample preparation that meet both the needs of the end-user and the constraints of the microfluidic device is still on-going.

To simultaneously evaluate sample recovery from the collection filter and inhibitor removal, a sample recovery device that mimics the workflow of the final xBIRD system was developed.

ACORNS Integration

xBIRD is designed for full integration with the existing infrastructures using the ACoRNS platform. This allows modular deployment and the ability to meet multiple CONOPs. Because of the low size, weight, and power of xBIRD, it can easily be integrated with drones, vehicles, UGSs (unmanned ground system), and stationary perimeter defense mounts to meet the situational needs of biothreat detection. Integration with existing networked sensors would also allow it to be activated by an alert sent by lower sensitivity trigger detectors or algorithms analyzing for the presence of aerosol anomalies.

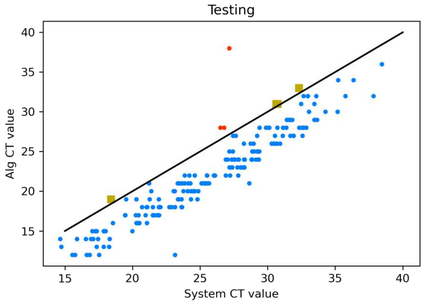
Machine Learning Automates Assay Evaluation

We are leveraging an machine learning (ML) algorithm to automate the interpretation of fluorescent amplification plots obtained during xPCR. A Support Vector Machine (SVM) algorithm was created to establish a decision boundary between positive and negative fluorescence data and determine the point where the sample becomes positive. The algorithm was trained using data from over 5,000 individual samples run on the Quant Studio 6 Flex and ABI 7900HT qPCR systems. Overall, the algorithm performs better than the OEM software that is used to evaluate the data after collection.

Blue: Alg C_T better than QuantStudio C_T

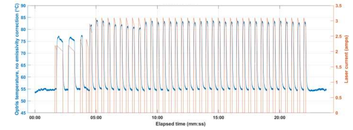
Red: Alg C_T worse than QuantStudio C_T

Orange: Alg C_T equal to QuantStudio C_T



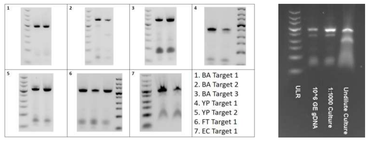
X-BIRD Prototype Testing

A subcomponent of the prototype, developed at MIT LL, was transitioned to DEVCOM CBC which allowed iterative testing of the xPCR reactions. At this time, 7 assays were successfully transitioned to prototype with amplicon generation (including hot start) completed in as little as 8 minutes. Additional assays are being transitioned sequentially. MIT LL is developing the microfluidic, microimpactor, and fluorescence detector and will transition updates to CBC iteratively as prototype matures into its final form factor. A recent update included a hot start procedure incorporated into the controller for the prototype and uses a lower temperature than is typically required on standard thermocyclers.



The LOD of assays evaluated on the prototype system is approximately 6000 GE/mL or better using an input of 4 – 6 µL genomic DNA. This is on par with the established LODs on the Quant Studio 6 Flex system.

Initial testing using a saturated E. coli culture that was placed directly in the prototype system reaction well, without processing, proved successful. Additional testing is underway against gram positive and negative organisms as well as spores.



Conclusions

- Extreme PCR is a viable method for biodefense-relevant assays.
- Biodefense amplification reactions can be completed in as little as 8 minutes using laser-induced rapid heating and cooling cycles.
- Adoption of ACoRNS III interface into device expands use-cases to perimeter defense as well as UGS and UAS platforms.
- Proof of concept for utilizing an SVM machine learning algorithm to automate the interpretation of fluorescence data was realized.
- Continued development of this device will provide:
  - Automated bioaerosol testing and reporting utilizing existing network infrastructures.
  - Specificity and sensitivity on par with currently fielded molecular assays.
  - Sample to result time equivalent to for faster than Hand Held Assays (HHAs).
  - No requirement for trained personnel or laboratory space to do so.

DEVCOM CBC @ DTRA CBD S&T Conference
Scan the QR Code to view all of CBC's
2022 DTRA CBD S&T Conference materials
https://cbc.devcom.army.mil/cbdst-conference/

Acknowledgements:
This project is funded by DTRA JSTO CBA, program lead Dr. Steve Francesconi, and is a collaboration with MIT Lincoln Labs. This project includes important contributions from Technical Staff at MIT-LL including Dr. David Walsh and Todd Thorson (co-PI's), as well as Jason Jong, Johanna Bobrow, and Jordan Wynn. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government