Rapid and Automated Identification of Aerosolized Biothreats

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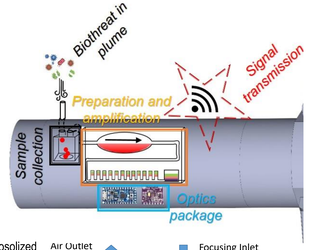
Abstract

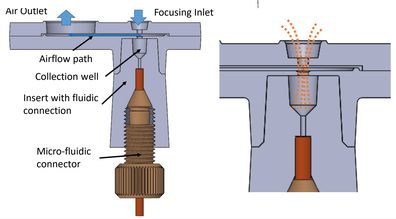
Automated aerosolized biothreat identification technologies are needed to reduce risk to the warfighter and improve decision making during a biological attack/incident. Current technologies used to detect and identify these biological aerosols are cumbersome and require human intervention to not only process, but analyze and identify the biological agent in question. Recently, with the understanding that SARS-CoV-2 is transmitted via aerosols, portable biological identification devices coupling miniaturized aerosol collectors and isothermal amplification have become a new area of research and development, but developing an automated prototype that combines these two processes is still elusive. Here we present a prototype that has the ability to not only automatically collect, but elute and identify various aerosolized biological agents. To add to its functionality, this prototype is made in a modular fashion such that the collection and detector components can operate independently from one another; this allows for more flexibility in its uses rather than a limited single use device. Currently, as the prototype is designed, sample collection and elution is triggered remotely after which the sample is prepared using Arcis reagents followed by isothermal amplification with our uniquely designed primers. Fluorescence from the assay is measured using the optics package, and positive/negative results are transmitted to the end user. This future-forward technology will provide new capabilities to survey the battlefield and operational environments remotely, providing real-time data for integrated early warning.

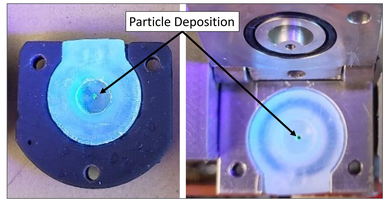
Approach

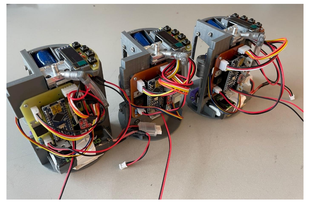
Critical functions of the prototype were separated as modules and each function was then subsequently integrated together. The critical functions of this prototype are: sample collection, preparation and amplification, and finally, signal transmission.



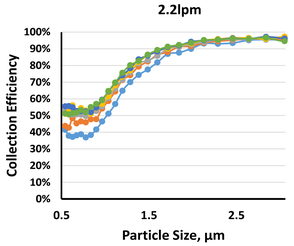
To collect the aerosolized biological threat, we developed an automated collector/elution module that uses a microwell impactor to collect aerosols. This collector can concentrate any biological aerosol to a small volume which is subsequently eluted using a microfluidic connection. The eluent is then moved to the detector module.

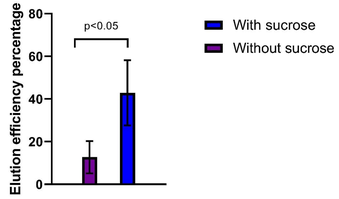




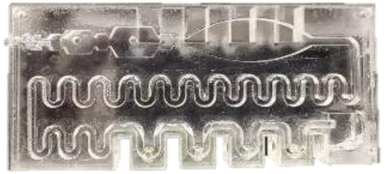


We were able to create and test our microwell impactor with two micron fluorescent beads and were able to show its ability to effectively concentrate sample. Also, we created multiple programmable collector/elution modules that we could not only integrate with our biological identification prototype, but also test their collection and elution efficiencies.





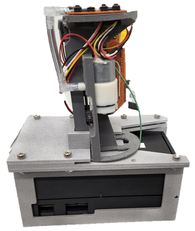
For our target particle size, we could obtain aerosol collection efficiencies close to 100% and our elution efficiencies were ~40% with the addition of a sucrose layer to our microwell. **Together, this shows us that we are able to create a portable and easily integrateable collector/elution module that can operate with and without our complete prototype.**



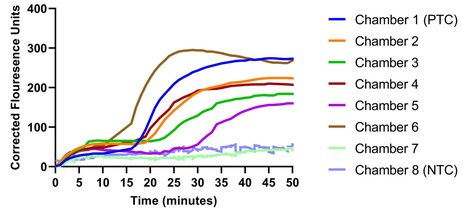
After biological aerosol is collected and eluted with our collector/elution module, the sample is mixed with sample preparation reagent through the delay channel and serpentine parts of our microfluidic cassette. It is then deposited for analysis in the loop mediated isothermal amplification (LAMP) chambers at the bottom of this cassette. Because the cassette is clear, and there are critical communications chips present in the detector module, **we are able to remotely monitor fluorescence in this more portable amplification device**.

Prototype development and testing

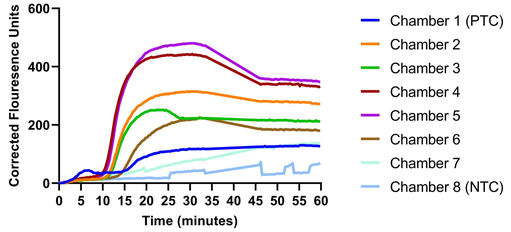
Upon designing and perfecting our modules, we integrated them into one device in which the collector/elution module and the detector module were connected to each other through PEEK tubing. In this format, the components of our prototype are encased in a light impermeable chamber and a TACBIO inlet is attached to aid in aerosol collection.

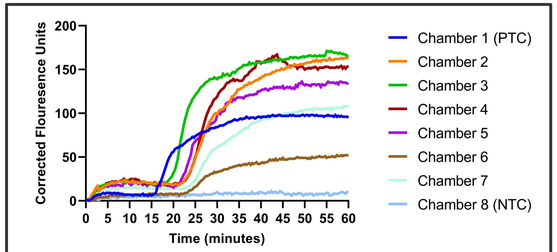


We initially tested this prototype with a Gram-negative biological surrogate in a Calliope aerosol test chamber. This was a more controlled setting compared to an outdoor testing event, so we would be more able to assess the detection limits of our prototype. For this experiment, LAMP chambers 2-7 contained specific primers to detect the biological surrogate and the entire process was fully automated. **Through this testing, we were able to achieve fully automated biological identification using the Calliope aerosol test chamber.**



After our successful Calliope testing, we then conducted open air testing in Dugway Proving Ground. **We first attempted biological identification of a Gram-negative biological surrogate and we were successful in automatically identifying this surrogate in a open-air setting.** Just like our Calliope aerosol testing, LAMP chambers 2-7 contained specific primers to detect the biological surrogate.





We also attempted a bacterial spore identification challenge at Dugway Proving Ground in an open-air release and we were able to identify the spores in a fully automated fashion. Just as in our previous experiments, LAMP chambers 2-7 contained specific primers to detect the biological spore surrogate.

Conclusions and Discussion

By dividing all of the critical parts of the prototype into modules, we were able to create a rapid, fully automated aerosolized biological identification device. The automated collection/elution module utilizes a microwell impactor that concentrates any biological aerosol into a small volume which is then eluted to the detector module using a syringe pump. After the eluent travels through the microfluidic cassette, an isothermal amplification assay known as LAMP reacts with the aerosolized biological sample's DNA to generate an amplification curve. Since the reaction will only proceed if primers specific to the biological sample are present, we can obtain rapid identification of sample.

This prototype serves as a proof of concept but we anticipate to modify it in several ways to enhance its capacity to identify biological aerosols. Currently, the prototype we have developed has eight separate chambers, so we can theoretically have six different primer sets for six separate biological organisms (one chamber must serve as a positive control and another chamber must serve as a negative control), but we anticipate to expand our cassette to include more chambers to detect more biological organisms. We are also in the process of developing an automatic identification algorithm that can provide an end user with a positive/negative answer depending on the amplification curve and the specific LAMP chamber. Finally, we anticipate to fully integrate all of the modules together in a lower SWAP device that can be utilized in various formats.

Although future versions of our prototype will be more integrated, both the collector/elution and the detector modules can be operated independently of one another and can be utilized for different functions. For example, our collector/elution module can be used as a miniaturized aerosol collector since currently available biological aerosol collectors are more bulky and cumbersome. Also, our detector module serves as a smaller isothermal amplification device which is much more portable that an typical PCR machine.

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