COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Comparing Cost & Efficacy Of Antibody Sequencing Using Microwave-assisted Acid Hydrolysis (MAAH) Digestion Vs Enzymatic Digestion

Gabrielle Rizzo U.S. Army DEVCOM Chemical Biological Center **Elizabeth Dhummakupt** U.S. Army DEVCOM Chemical Biological Center **Conor Jenkins** U.S. Army DEVCOM Chemical Biological Center **E. Randal Hofmann** Excet, a Precise Systems Company

The Defense Biological Product Assurance Office (DBPAO) antibody repository is an invaluable resource to the Warfighter, maintaining a stockpile of antibodies to a range of biological threat agents. Should any of these antibodies become compromised, either through damage to the repository itself or to the antibody-producing cell lines, it could render them useless as a potential treatment against those biological threats. It is therefore imperative that the antibody sequences are known to engineer new antibody. This can be accomplished by leveraging de novo antibody sequencing, which is typically achieved by using a cocktail of enzymes to digest an antibody. The resulting overlapping peptide fragments are pieced together to establish the full antibody sequence. However, the cost of the enzymes used to perform de novo sequencing is steep, and only increases with a higher variety of enzymes used. In this work, we explored the efficacy and cost-effectiveness of an alternative method, microwave-assisted acid hydrolysis (MAAH), in place of enzyme digestion. We used two antibodies to perform this comparison: a commercially available NIST antibody standard, and an antibody provided by the DBPAO antibody repository. Both antibodies were digested using either a 6-enzyme cocktail or microwave-assisted acid hydrolysis. Total sequence coverage, cost, and time to perform the digestion per sample were assessed. The results of this comparison suggest that MAAH can be a viable method of achieving de novo antibody sequencing, particularly when cost or time is a limiting factor. The enzyme cocktail achieved higher sequence coverage, but required almost 25x the up-front costs, in addition to requiring longer prep time and additional time spent waiting for enzymes to arrive due to supply chain issues. However, both methods still failed to achieve sufficiently high sequence coverage of the repository antibody. To improve sequence coverage of the repository antibody, we combined the spectral data from both digestion methods and performed a supplemental canonical database search against an in-house antibody sequence database. With this method, we were able to achieve full antibody sequence coverage of the heavy chain. This level of sequence coverage has proven this combined method is a promising tool in safeguarding the antibodies maintained in the DBPAO repository, thus bolstering the repository's ability to protect the Warfighter in the face of biological threats.

This research is funded by the Defense Biological Product Assurance Office (DBPAO).