INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Bridging Study On Transition Of Dbpao Assays To The Biomeme Platform And Additional Assay Development

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The Defense Biological Products Assurance Office (DBPAO) produces forty-six PCR assays targeting twenty-one biological warfare (BW) agents produced under ISO 17034 accredited standards by Naval Medical Research Center (NMRC). The assays were developed by Department of Defense (DoD) laboratories in a standardized DTRA CB56 data package. The standardized assays are available to the US Government and their partners in research and development efforts or operational use for detection of BW agents. The result was a set of well characterized and validated assays formulated for three widely used platforms at the time. These reagents have been shown to function with no loss of performance on newer benchtop platforms such as the ABI StepOne, QuantStudio5, and the MIC qPCR cycler. The Biomeme Franklin series of instruments are fast to result, battery operated, and highly portable with an open architecture allowing the end-user to design and run their own assays including multiplexed assays. Open architecture is critical to the DoD as the platform is not reliant on manufacturer assay availability and performance, or subject to manufacturing timelines and profitability which allows for rapid response to pandemics and novel emerging pathogens. The objective of this study was to demonstrate DBPAO reagents function with no loss of performance on the Biomeme platform expanding the range of ISO certified assays available to entities employing the platform. To accomplish this, previously produced master mixes that have passed acceptance criteria were evaluated on the Biomeme platform following similar production and conformance testing protocols. The resulting data was compared to original production data. Acceptance criteria data from standard curve analysis includes Ct values, r2 value, slope, intercept, efficiency, and approximate limit of detection. Non-specific amplification was evaluated through multiple no template control samples. Aggregate historical production data was compiled and used for comparison of performance across the assay history. Results indicated that all DNA based assays (29 total) in the DBPAO portfolio functioned with no loss of performance and could be ISO certified for use on the Biomeme platform. Estimated limit of detection results for RNA based assays (17 total) did not meet original acceptance criteria in several cases. However, increasing the RT incubation step in the protocols overcame the issue and the RNA based assays could be ISO certified for use on the Biomeme platform with slight adjustments to cycling conditions. Pilot studies on multiplexing existing singleplex assays indicated that multiplex formulations exhibit acceptable performance and opens a pathway to transitioning existing assays to agent specific virulence detection assays or broad screening panels that would greatly enhance the flexibility and function of the platform. It has been demonstrated that DBPAO PCR reagents are platform agnostic and function across a wide variety of instruments in operational use by JSTO, Joint Forces laboratories, and deployed units. Having a portfolio of well characterized and validated assays free from commercial manufacturer limitations that function across multiple platforms enhances the confidence in results and supports national security efforts for biological agent detection and identification.

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