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A Hidden Gem: Highlighting the Indispensable Capabilities and History of the DoD Cholinesterase Monitoring Program and DoD Cholinesterase Reference Laboratory

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ABSTRACT The DoD Cholinesterase Monitoring Program and Cholinesterase Reference Laboratory have safeguarded U.S. government employees in chemical defense for over five decades. Considering Russia's potential deployment of chemical warfare nerve agents in Ukraine, it is critical to maintain a robust cholinesterase testing program and its efficiency presently and in future.

INTRODUCTION

Chemical warfare nerve agents[1–6] and organophosphorus pesticides[7] are potent cholinesterase-inhibiting substances (CIS). Cholinesterase-inhibiting substances disrupt normal acetylcholine synaptic concentration levels and cause overstimulation in the neuromuscular system. The resulting cholinergic crisis can lead to flaccid paralysis, respiratory failure, and even death.[8–10] Because depressed acetylcholinesterase (AChE) activity can serve as a valuable clinical biomarker for potential exposure to CIS, the U.S. Army established a robust nationwide testing network through the DoD Cholinesterase Monitoring Program (CMP) and Cholinesterase Reference Laboratory (CRL) in the 1970s. The CMP and CRL continuously monitor red blood cell (RBC) AChE activity for hundreds of thousands of government military and civilian employees involved in chemical nerve agents' storage and demilitarization operations.

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BEFORE 1970S

After World War II, the U.S. government started consolidating dated chemical weapon stockpiles for storage and destruction.[11] As a result, thousands of civilians were employed each year to assist the military. The Congress tasked the Army to establish the CMP and set up two major laboratories at Fitzsimmons Army Medical Center (FAMC) and Aberdeen Proving Ground. These two laboratories provided occupational AChE activity testing for the military service members and federal government civilian employees engaged in chemical defense missions. Early on, the CMP encountered several operational challenges. They included no standardization of sample collection and submission procedures, testing methods, testing frequency, or categorization of patients based on risk levels of potential exposure. Additionally, each testing facility was allowed to purchase the preferred testing equipment, utilize different testing methods, and significantly modify testing procedures. As a result, the CMP experienced alarming inconsistencies and deficiencies. The enormous interlaboratory testing result variations alone made it impossible to establish a valid normal range of human AChE activity or to conduct the long-term occupational AChE activity monitoring.

1970S TO 1990S

To combat the disappointing situation, the DoD standardized the optimized Michel method[12,13] as the sole acceptable testing method and limited the testing matrix to RBC only. A pilot quality control (QC) program initiated at FAMC, Dugway Proving Ground, and Tooele Army Depot in 1974 mandated regular quality assurance (QA) testing, periodic proficiency testing, and blind QC testing. Ultimately, this led to the

utilization of identical testing equipment and reagents across the three sites with FAMC functioning as the oversight for training all testing personnel. The results were encouraging, and the Army quickly incorporated the improvement measures into the first official DoD AChE activity testing doctrine, Technical Bulletin, Medical (TB MED) 292[14] in 1975. In 1977, the Army selected the FAMC laboratory as the CRL. The CRL was tasked to (1) prepare blind QC samples, (2) perform primary testing while re-test selected samples submitted by other sites for QA verification, (3) provide quarterly proficiency testing samples and monitor the results, (4) standardize testing personnel training, (5) purchase and maintain major testing equipment, and (6) offer technical support and conduct on-site compliance audits. Within 2 years, the efforts dramatically improved the testing precision and reduced interlaboratory testing variability. The pioneering QA measures later also demonstrated their broader effectiveness and importance through applications to other testing activities in and outside the DoD.

In 1986, the Congress passed the Public Law 99-145[15] and required all U.S. chemical weapon stockpiles to be destroyed. This significantly increased the number of personnel working in chemical weapon destruction and a rapid growth of the CMP. In the peak time of the program in the 1990s and 2000s, more than 25 satellite testing sites were established. The CMP provided annual occupational monitoring testing for more than 35,000 government employees. It crucially supported Army Support Teams for Weapons of Mass Destruction, DoD medical facilities, the Defense Threat Reduction Agency, DoD Chemical Surety Program, and numerous auxiliary personnel in missions supporting chemical weapon destruction. Most importantly, the program enabled the DoD to collect a large amount of testing data and establish a normal range of human RBC AChE activity for clinical screening.

1990S TO THE PRESENT

Currently, there are two general types of clinical CIS testing methods: (1) biological effects (BE) based, including the modified Michel method,[12,13,16] and (2) molecular fingerprint detection by mass spectrometry (MS).[17–19] The MS methods are superior in specificity and accuracy but suffer from major drawbacks. Besides high testing costs and technical requirements, the chemical structure of the analyte must be listed in the MS library for substance identification. In contrast, the BE-based methods are sensitive, less specific, easy to operate, and low cost with the potential for use in the field environment. As a result, the BE-based methods are ideal for early screening, especially when the CIS is unknown. It is also the reason why the CMP has used the modified Michel method[13] since the late 1970s.

Currently, the CRL is a part of the DoD Food Analysis and Diagnostic Laboratory under Public Health Command-Central at Joint Base San Antonio-Fort Sam Houston. The CMP and CRL are operated under the updated regulations, TB MED 590[20] and Department of the Army Pamphlet 40-8,[21] and serve federal, state, and local government employees under the Chemical Surety and Training Programs, government contractors, national laboratories scientists, private sector workers, and the general workforce in agricultural industries with potential exposure to CIS. Additionally, the program provides preventive testing services for new pilots in the U.S. Navy and Air Force.

As a high reliability and unique organization, the CRL has been accredited under the DoD Clinical Laboratory Improvement Program since the early 1990s and earned the prestigious ISO 15189/Clinical Laboratory Improvement Program dual accreditation through the American Association for Laboratory Accreditation in April 2021. The CRL successfully renewed its accreditation in December 2022.

Since the mid-2010s, several U.S. chemical weapon destruction sites have completed their mission,[11,22] and AChE testing demand has significantly decreased. In 2009, the CMP had 16 testing sites in support of over 25,000 personnel compared to 11 testing sites in 2017. Currently, the CMP manages seven network laboratories across the DoD and Department of Homeland Security. They serve around 7,500 personnel every year and support the DoD's Chemical Surety Program for nerve agents, which directly supports the chemical weapon demilitarization mission and the Chemical Weapons Convention Treaty. Over the past 30 years, the CMP was able to detect approximately 25 cases of potential exposure to CIS at chemical warfare nerve agent destruction sites and agricultural facilities with the use of pesticides. This resulted in those affected individuals to promptly follow up with their primary care providers for comprehensive examinations that prevented disease sequelae from the exposures.

FUTURE

Although the CMP has downsized a bit, the recent use of chemical weapons in the Syrian civil war and Russia's potential deployment of nerve agents in Ukraine underscore an undeniable reminder that it remains urgent for the U.S. military to maintain a reliable and robust AChE testing and monitoring program for the future.

Russia continues to develop, weaponize, and stockpile highly toxic nerve agents even after it signed the Chemical Weapon Convention.[23] Its newest fourth-generation "Novichoks" agents were designed with significantly enhanced toxicity compared to the traditional G- and V-series nerve agents.[6] Because of the lack of transparency, little to no information on Russian chemical weapon programs has been disclosed. As a result, Russia is still posing as a major chemical threat to the rest of the world.

Because many "Novichoks" do not have confirmed structures yet, it is impossible to develop practical MS-based testing methods. Consequently, the CMP will remain critical for the early detection of U.S. and allied troops' potential exposure to these new CIS.

CONCLUSION

The DoD CMP and CRL have served as a critical and powerful tool for screening human potential exposure to CIS for five decades and have demonstrated their critical potential and enduring value in future military and civilian applications.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY

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CLINICAL TRIAL REGISTRATION

Not applicable.

INSTITUTIONAL REVIEW BOARD (HUMAN SUBJECTS)

Not applicable.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

Not applicable.

INDIVIDUAL AUTHOR CONTRIBUTION STATEMENT

P.K., R.A.S., and A.M.F. collected the information and drafted the original manuscript. M.S.C., M.D.W., and S.L.M. reviewed and edited the manuscript. All authors read and approved the final manuscript.

INSTITUTIONAL CLEARANCE

Institutional clearance approved.

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