USACEHR TECHNICAL REPORT 0801

AN EVALUATION OF BLOOD CHOLINESTERASE TESTING METHODS FOR MILITARY HEALTH SURVEILLANCE

Paul L. Knechtges

May 2008

Approved for public release; distribution is unlimited

United States Army Center for Environmental Health Research
Fort Detrick, MD 21702-5010

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
30-05-2008	Technical	JUL 2007 - FEB 2008

4. TITLE AND SUBTITLE
An Evaluation of Blood Cholinesterase Testing Methods for Military Health Surveillance

5a. CONTRACT NUMBER
IPAAGR07KNECH1

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
PE 622787

6. AUTHOR(S)
Knechtges, Paul L.

5d. PROJECT NUMBER
PR FHZ

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
US Army Center for Environmental Health Research
568 Doughten Drive
Fort Detrick, MD 21702-5010

8. PERFORMING ORGANIZATION REPORT NUMBER
USACEHR-TR-0801

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
504 Scott Street
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Blood cholinesterase (ChE) testing is done to evaluate potential human exposure to chemicals that act as ChE-inhibitors, most often organophosphate and carbamates pesticides. The Model 400 Test-mate™ ChE kit (EQM Research, Inc.) is used for field blood ChE analysis within the Department of Defense. Suggested modifications to the Model 400 kit include displaying and recording acetyl-ChE activity uncorrected for temperature and using analytical standards for calibration or quality assurance purposes. The recent advent of a wide array of point of care devices may provide an opportunity for developing improved hand-held instruments field ChE analysis. If a hand-held device for field blood ChE analysis is developed for military health surveillance, a concept of operations for the device should be prepared as a first step. Some key performance areas for a field ChE device development are identified.

15. SUBJECT TERMS
cholinesterase, acetylcholinesterase, butyrylcholinesterase, biomarker, Test-mate, pesticides, blood, point of care device

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			David A. Jackson, Ph.D.
U	U	U	UU	49	19b. TELEPHONE NUMBER *(Include area code)* 301-619-3031

Abstract

Blood cholinesterase (ChE) testing is done to evaluate potential human exposure to chemicals that act as ChE-inhibitors, most often organophosphate and carbamates pesticides. An evaluation of techniques used within the Department of Defense to analyze blood ChE activity found that only one device has been validated for ChE testing in the field: the Model 400 Test-mate™ ChE kit by EQM Research, Inc. (Cincinnati, OH). Suggested future modifications to the Model 400 Test-mate™ ChE kit include displaying/recording of acetyl-ChE activity uncorrected for temperature and the use of analytical standards for calibration or quality assurance purposes. The recent advent of a wide array of point of care devices may provide an opportunity for developing hand-held instruments capable of improved field ChE analysis, but substantial technical challenges remain. Should it be determined that a hand-held device for field blood ChE analysis would be useful for military health surveillance, the first step should be developing a concept of operations for the device. Key performance areas for consideration in field ChE device development include sensitivity, accuracy and precision, cost, stability of reagents and consumable supplies, environmental conditions, portability, power requirements, speed and throughput, and operator training required.

Keywords: cholinesterase, acetylcholinesterase, butyrylcholinesterase, biomarker, Test-mate, pesticides, blood, point of care device

Table of Contents

List of Tables and Figures……...iv
Preface……v

1. Review of the Utility of Cholinesterase Testing Methods…….……..1
1.1 Introduction……..1
1.2 Relevance of Cholinesterase Testing for Medical and Public Health Purposes..……...……..……..2
1.3 Laboratory Testing Methods…….……5
1.4 Field-Testing Kits…..……...……..…7
1.5 Military Applications and Policies Regarding Cholinesterase Testing…….8
1.6 The Key Issue of Cholinesterase Testing versus Other Biomarkers..…….....11

2. Review of the Performance Characteristics of the Test-mate™ Cholinesterase Testing System……..13
2.1 Introduction..……..…..13
2.2 Commercially-Available Field Test Kits……..……13
2.3 Literature Review of the Test-mate™ Field Kit……...14
2.4 Conclusions…….....20

3. Survey of Handheld and Portable Devices for Measuring Biological Marker…...21
3.1 Introduction……...…….……..21
3.2 Currently Funded Projects..……..……..21
3.3 Leveraging Opportunities with Commercially-Ready and Available POC Devices..……..…….……..….26

4. Conclusions and Recommendations……..30
4.1 Leveraging the Growing Market in POC Diagnostics……..……..30
4.2 Suggested Ideal Performance Characteristics of a Field Cholinesterase Testing Device……...……..…31
4.3 Conclusions on the Status of Handheld or Portable Devices for Measuring Blood Cholinesterase……34

References……35

List of Abbreviations and Acronyms…….…….....42

List of Tables and Figures

Table 1-1: Summary and Comparison of Cholinesterase (ChE) Categories…..…….1

Table 1-2: Situations of Occupational Exposure and Nonoccupational Exposure to Organophosphates…….....3

Table 1-3: Some Continuing Issues in Organophosphate Toxicology……..…4

Table 1-4: Cholinesterase (ChE) Testing in the U.S. Department of Defense……..11

Table 3-1: U.S. Army Medical Research Projects to Develop Handheld or Portable Devices for Measuring Biological Markers……...23

Table 3-2: Commercially-Available Point of Care (POC) Device Manufacturers and Biomarkers/Tests for Mobile, Emergency, Critical, and Primary Care Applications…….28

Table 3-3: Commercially-Available Point of Care (POC) Tests for Infectious Diseases…….....29

Figure 3-1: Point of Care Diagnostics Market by Application and Disease…….26

Preface

The U.S. Army Center for Environmental Health Research (USACEHR) is conducting research to identify key biomarkers associated with toxic industrial chemicals encountered by military personnel. The USACEHR is investigating the possibility of developing a hand-held biomarker detection device to identify not only novel biomarkers found through the USACEHR research program but also other biomarkers, such as cholinesterase activity, that are routinely monitored by the Department of Defense (DoD). Within DoD, definitive cholinesterase testing is conducted by the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), which operates the DoD Cholinesterase Reference Laboratory. Field testing for cholinesterase is conducted using the Test-mate™ ChE Cholinesterase Test System (EQM Research, Inc.), which, although small (28 cm x 18 cm x 25 cm; 4.5 kilograms), is not a hand-held device.

At a January 2007 meeting between senior USACHPPM and USACEHR personnel, it was agreed that further study was required to evaluate the current status of ChE testing in DoD and to determine the requirements for a hand-held ChE/biomarker detection device. Dr. Paul Knechtges, a visiting assistant professor with East Carolina University (ECU), was tasked with conducting the study under an Intergovernmental Personnel Act agreement between ECU and USACEHR. The study had four objectives:

1. Review the techniques used within the DoD to analyze ChE activity in blood and identify the major groups/individuals involved with developing ChE analysis methods and protocols.
2. Identify any potential performance issues with the current Test-mate™ assay. Evaluate use scenarios and essential performance criteria for a field assay for blood ChE activity.
3. Survey companies selling or developing hand-held devices capable of testing ChE activity (and other biomarkers) in blood, particularly those with DoD sponsorship. Develop a summary table of these devices, comparing characteristics important for field analysis of ChE and other biomarkers.
4. Prepare recommendations concerning next steps in the development of a hand-held biomarker device for ChE and other parameters.

This document is a compilation of four technical reports prepared by Dr. Knechtges to address these objectives. Thanks are due to several individuals for their review and comments on this report: MAJ Nizamettin Gull, COL Beverly Maliner, and Dr. Coleen Weese (USACHPPM); MAJ Lee Lefkowitz (Walter Reed Army Institute of Research); COL Brian Lukey (U.S. Army Medical Materiel Development Activity); and Dr. Thomas Gargan II and Dr. David Jackson (USACEHR). We also thank Ms. Linda Brennan and Ms. Melissa Knott for compiling and editing this document.

William H. van der Schalie, Ph.D.
Science and Technology Director
USACEHR

1. Review of the Utility of Cholinesterase Testing Methods

1.1. Introduction

Acetylcholine (ACh) is the most widespread and studied neurotransmitter involved with nerve transmission. Research in the early 1900s suggested that an esterase was involved with the inactivation of ACh and its removal from circulation. The first extract of cholinesterase (ChE) was prepared from horse serum and reported in 1932 by Stedman *et al*. Since that landmark publication, ChEs from different species have been identified and found to differ in substrate specificity and susceptibility to inhibitors (Giles, 2007). A number of schemes for naming the various ChEs was proposed, but the currently accepted one (Silver, 1974) is based on a family of enzymes with substrate preferences that fall into two broad categories: (1) Acetylcholinesterases (AChEs), which preferentially hydrolyze acetyl esters such as ACh, and; (2) butyrylcholinesterases (BChEs), which preferentially hydrolyze other esters such as butyrylcholine. Table 1-1 provides a summary and comparison of AChEs and BChEs.

Table 1-1: Summary and Comparison of Cholinesterase (ChE) Categories.

ChE Category	Synonyms	Substrate Preference	Biological Function
Acetylcholinesterases (AChEs)	Acetylcholine acetylhydrolase, Red Blood Cell (RBC) cholinesterase	Hydrolysis of acetyl esters.	Rapid hydrolysis of ACh at cholinergic synapses. Other roles in cell growth and adhesion.
Butyrylcholinesterases (BChEs)	Acylcholine acylhydrolase, pseudocholinesterase, non-specific cholinesterase, plasma cholinesterase	Hydrolysis of other types of esters, e.g., butyrylcholine.	Undetermined. No known specific, natural substrate. Possibly as a scavenging enzyme to detoxify natural compounds. Also capable of hydrolyzing ACh.

The primary biological role of AChE is the rapid hydrolysis of ACh at the cholinergic synapses in the Central and Peripheral Nervous Systems (CNS/PNS) and at neuromuscular junctions. The inhibition of AChE by certain xenobiotic chemicals (e.g., organophosphate pesticides) within synaptic clefts results in the dysfunction of nerve transmission by preventing the inactivation of ChE; this leads to excessive stimulation of the CNS/PNS.

The AChE protein has three isoforms produced by alternative splicing of pre-mRNA, and each isoform also has non-catalytic roles such as cell growth and adhesion (Grisaru et al., 1999). The specific biological roles of these isoforms are not necessarily the same, and the distribution of AChE isoforms varies among different tissues and during different stages of development. Nonetheless, experiments have demonstrated that the different molecular forms of AChE have identical catalytic properties (Schwarz et al., 1995). The synaptic isoform (AChE-S) is produced and found primarily in brain and muscle tissues, while the "erythrocytic" isoform (AChE-E) is anchored to red blood cell (RBC) membranes and, hence, is also called RBC cholinesterase. Since obtaining the AChE-S from cholinergic synapses is very invasive and impractical for routine testing purposes, the AChE-E associated with RBCs is usually sampled as a surrogate indicator of AChE inhibition in the CNS/PNS.

Unlike AChE, the greatest concentration of BChE is found in the liver, although it is readily found in and sampled from the blood plasma (i.e., plasma cholinesterase). The biological role of BChE is not fully understood. Although BChE hydrolyzes ChE, the suggested role of BChE is a scavenging enzyme for the detoxification of naturally-occurring compounds (Grisaru et al., 1999). The ambiguous role of BChE is illustrated in rare individuals born with a mutated or missing BChE gene. These individuals live without any apparent physiological consequences. However, if they undergo surgery, post-operative apnea may result when tracheal intubation is used with the muscle relaxants succinylcholine or mivacurium (Darvesh et al., 2003). Research also suggests that BChE is in some way involved in a number of neurodegenerative diseases (Darvesh et al., 2003), and BChE levels are inversely related to risks for cardiovascular disease (Calderon-Margalit et al., 2006).

1.2. Relevance of Cholinesterase Testing for Medical and Public Health Purposes

The primary reason for measuring ChE is related to human exposure from certain pesticides that act as ChE-inhibitors, most notably the organophosphates (OPs, and other phosphorous-based compounds) and the carbamates. More than 200 OPs and 25 carbamates have been formulated into thousands of different products (Kwong, 2002). The widespread and global use of pesticides, along with poor health surveillance statistics, makes it difficult to estimate the actual number of exposures, illnesses, and deaths resulting from pesticides. Nevertheless, some estimates of the global health problem include more than 3 million poisonings and 200,000 deaths per year, with the majority of these incidents reported as intentional (Jeyaratnam, 1990). Based on a literature survey by Jaga and Dharmani (2003), the most likely situations involving exposures to OPs in the U.S. are outlined in Table 1-2.

Table 1-2: Situations of Occupational Exposure and Nonoccupational Exposure to Organophosphates (Jaga and Dharmani, 2003).

Occupational exposure	Environmental or nonoccupational exposure
Agricultural workers Manufacturing industry workers Pesticide exterminators Greenhouse workers and florists Office workers Health care workers Veterinary employees Personnel performing autopsies Store employees Gulf War Veterans	Residential exposure ⇒ Resident use, exterminator use ⇒ Dietary exposure ⇒ Accidental exposure[a] Agricultural worker take-home exposure Close proximity to farms Aerial spraying Public places Contaminated organ donor Suicidal (intentional) poisoning[a] Chemical warfare

[a]Accidental poisoning and suicidal poisoning are possible in both occupational and nonoccupational exposures.

The health consequences of ChE inhibition can range from immediately life-threatening to subtle. Although a great number of symptoms can occur from acute, high exposures to OPs, death is believed due to the inhibition of respiratory centers in the brainstem, resulting respiratory in failure (Lotti, 2001). Another clinical manifestation of OP toxicity is called the "intermediate syndrome," which is characterized weakness of respiratory, neck and proximal limb muscles (Costa, 2006). This intermediate syndrome is not a direct effect of ChE inhibition and occurs several hours after the signs and symptoms of severe ChE-inhibition have occurred (Senanayake and Keralliedde, 1987). Some OPs can produce a form of toxicity known as organophosphate-induced delayed polyneuropathy (OPIDP); in this case, the neuropathy occurs one to three weeks following exposure and involves weakness of the extremities, and neurodegeneration progressing to ataxia and paralysis. OPIDP appears to result from OP inhibition of neuropathy target esterase, an enzyme involved with lipid metabolism in the axonal membranes. (Glynn, 2006). There are also many unresolved issues regarding OP toxicity, some of which are listed in Table 1-3.

Table 1-3: Some Continuing Issues in Organophosphate Toxicology (Costa, 2006).

Issue	Question
Low chronic exposure	Does it result in behavioral abnormalities in humans?
Genetic susceptibility	Are certain individuals more sensitive to OP toxicity?
Developmental toxicity and neurotoxicity	Are children more sensitive to OP toxicity?
Common mechanism of action	Do all OPs have the same mode of action?
Delayed neurotoxicity	What are the precise molecular events involved in axonal degeneration?
Additional OP targets	Are additional targets relevant for some aspects of OP toxicity?

The measurement of ChE to estimate the exposure or effects from ChE-inhibitors has been proposed and studied since the landmark publication of Stedman et al. in 1932. However, the first formal guidance in the U.S. for using ChE testing was in 1971 by the American Medical Association's Committee on Occupational Toxicology, Council on Occupational Health (Milby, 1971). Another authoritative document was published in 1976 by the National Institute for Occupational Safety and Health (NIOSH) as a criteria document for occupational exposure to parathion (NIOSH, 1976). This document provided an extensive literature review that related ambient exposures to OPs with the depression of ChE activity and suggested that 70% inhibition of AChE activity in the RBCs is "significant." Over the years, many other documents have become available that provide guidance on the use of ChE testing for medical and public health purposes (Weese, 2005; California Environmental Protection Agency (Cal/EPA, 2002; Lessenger and Reese, 1999).

The overall importance of ChE testing is dependent on the types of decisions made by clinicians and public health professionals. For clinical decision-making that involves acute poisoning, the value of ChE test data for the diagnosis, treatment, and management of patients often depends on whether or not the poison is known or suspected to be a ChE-inhibitor, and whether the patient has baseline or pre-exposure ChE test data available (Lessenger and Reese, 1999). On the other hand, ChE test data is quite valuable for medical surveillance and/or biological monitoring of known or suspected exposures to OPs or carbamates.

In the latter situation, the decision usually involves whether to remove employees from workplace exposures to OPs or carbamates (Cal/EPA, 2002). In general, ChE testing is

considered more valuable for decision-making that involves medical surveillance or biological monitoring than for clinical diagnosis and treatment of acute poisoning.

Using ChE testing data for health decisions is inherently complicated for several reasons. Certain pesticides have preferential affinities for either AChE or BChE (Cal/EPA, 2002). In addition, great differences exist in the reactivation rates of either AChE or BChE after exposure to various inhibitors. For example, the OPs generally bind more strongly to AChE, and enzyme reactivation is slow compared with the carbamates. Even among the OPs, the binding capacity to AChE can differ because of different molecular structures of the OPs (Kwong, 2002). Some OPs bind irreversibly to AChE and chemically alter the enzyme over different periods of time, a process called "aging," which completely inactivates the enzyme (Costa, 2006). Another reason is the variable timing of AChE and BChE inhibition/reactivation due to the toxicokinetics of different pesticides (Kwong, 2002). Some of the factors influencing the toxicokinetics are the pesticide's chemical structure and individual variation in pesticide metabolism (Furlong et al., 2000).

Other reasons that make using ChE testing data complicated include the inter- and intraindividual variability of ChE activity and its relationship to clinical signs and symptoms. The variability of AChE activity between individuals based simply on genetics, sex, race, or age is estimated as high as 23% (Lessenger and Reese, 1999). This underscores the importance of having individual baseline values of ChE activity when assessing exposures to ChE-inhibitors, although some methods of developing post-exposure baselines have been proposed (Lessenger and Reese, 1999). The extent of intraindividual variability is controversial and can be influenced by a number of factors such as disease, medications taken, and unknown exposures to ChE-inhibiting substances (Lefkowitz et al., 2007; Costa, 2006; Mason and Lewis, 1989). The variability of ChE activity among individuals confounds clinical diagnosis, because the signs and symptoms of CNS/PNS poisoning do not necessarily correlate with the level of ChE activity in the blood (Weese, 2005; Lessenger and Reese, 1999).

Finally, an important source of variability that complicates decision-making for ChE testing is the sampling, handling and storage, preparation, and analysis of blood specimens. This analytical variation is particularly important when meaningful comparisons of serial ChE tests are needed (Cal/EPA, 2002). In addition, with various methods of performing ChE testing available, the results between laboratories using different methods are not directly comparable. This subject and the most commonly used methods of ChE testing are discussed in the next section.

1.3. Laboratory Testing Methods

At least six methods for ChE testing have been used in laboratories (Vandekar, 1980). Various novel and improvised methods for ChE testing are reported and proposed in the scientific literature. Nonetheless, the most commonly used assays for routine ChE testing are limited to three categories (Wilson, 2005):

1. The delta pH method by Michel (1949).

2. The radioactive ACh method of Johnson and Russell (1975).

3. The kinetic assays pioneered by Ellman et al. (1961).

The delta pH method of ChE testing is considered a kinetic assay that measures the change of pH in a specified period of time (e.g., one hour) and is usually reported in units of delta pH per hour (hr). The basic principle of the test is based on the addition of the substrate ACh bromide to either separated RBCs or plasma; the ACh is hydrolyzed by the ChE to produce acetic acid and choline, which results in pH changes. The U.S. Army's Cholinesterase Reference Laboratory uses a modification of the delta pH method for AChE so that testing can be done within 17 minutes of reaction time, and the results are then converted to delta pH/hr (USACHPPM, 2005).

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) probably ranks first in the world for the number of tests performed using the delta pH method. Each year over 15,000 personnel are biologically monitored by the USACHPPM's Cholinesterase Reference Laboratory (Wilson, 2006). The USACHPPM also has a highly refined and consistent quality assurance program. In a recent report by Lefkowitz et al. (2007), the variability of AChE activity in individuals was analyzed by USACHPPM and determined to be negligible over several decades. This suggests that much of the controversy surrounding intraindividual variability may be due to differences in analytical methods and techniques among laboratories. Despite its successful use by the USACHPPM, the delta pH method is slow and has low throughput, which limits its feasibility by commercial laboratories.

The radioactive ACh method is a micro-assay that measures hydronium products produced from 3H-labeled ACh following enzymatic reaction and organic/water extraction. Although this method is accurate and suitable for multiple determinations of ChE activity, it is expensive and has radioactive waste disposal problems (Wilson, 2005). Consequently, this method is not commonly offered by commercial laboratories. Several different kinetic assays use thiocholine substrates to measure ChE activity, but the most common assay is the Ellman method (Wilson, 2005). This method measures hydrolysis of the substrate acetylthiocholine (ATCh) by either BChE or AChE (depending upon blood sample preparation) to yield acetate and thiocholine. The latter product reacts with 5, 5'-dithiobis-2-nitrobenzoic acid to produce a yellow-colored acid anion (5-thio-thionitrobenzoic acid) that can be measured with a spectrophotometer. The results are typically expressed as micromoles per minute per milliliter (μmol/min/mL) or international activity per milliliter (U/mL). Several commercial laboratory kits are produced based on the Ellman method, and many variations of the method have been published.

A problem with using different methods for ChE testing comes from comparing the results of periodic or serial testing. As mentioned previously, the baseline or trend of ChE activity for an individual is important for making meaningful assessments of exposure to and recovery from ChE-inhibitors. However, direct comparisons between the between the

various methods are not possible. In an effort to generate conversion factors for comparing the results between delta pH and Ellman methods, the U.S. Army Medical Research and Materiel Command (USAMRMC) contracted with the University of California at Davis (UC Davis) to study the variability and reliability of these methods by using split samples, and by "spiking" samples with ChE-inhibitors (Wilson, 2006). The early results of the UC Davis study showed that the Ellman method is more sensitive at higher AChE activity levels than the delta pH method (Wilson, 2006). This suggests that the Ellman method is better at detecting early warning signs of exposure to AChE-inhibitors, i.e., low levels that slightly suppress AChE activity. Conversion factors were also developed to permit comparison of the two methods, and a study is ongoing using a variety of OPs to inhibit ChE in volunteer blood samples.

Several new laboratory methods for analysis of ChE levels have been developed and reported in recent years. Gordon et al. (2004) developed a method at the Walter Reed Army Institute of Research (WRAIR) that measures both BChE and AChE in whole blood. This method uses three substrates with different affinities for BChE and AChE and an algorithm that derives activity levels for both types of enzymes. The system uses microtiter plates and is automated with robotic processing, a spectrophotometer, and computer processing. Comparison testing has been performed and conversion factors developed for the WRAIR, delta pH, and Ellman methods (Haigh et al., 2006). Analytical methods that quantitatively measure ChE enzymes have also been developed. Sun and Lynn (2007) developed a proteomics method using mass spectrometry (Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) that quantitatively measures peptides in BChE that have been chemically modified by OPs and carbamates. The testing results using this method were much more sensitive compared with the Ellman method (3% vs. 20% inhibition detection). Despite the impressive results, the authors admit that the current method has limited clinical utility but suggest that it may lead to the development of other methods.

1.4. Field-Testing Kits

The most widely used field-testing system for ChE is called the Test-mate™, which is manufactured by EQM Research, Inc. (Cincinnati, OH). Although a new model of Test-mate™ is planned for production, the latest commercially-available system is the Model 400 Test-mate™ ChE (Wilson, 2006). This unit requires only 10 microliters (μL) of blood, and the entire assay can be completed in less than 4 minutes (min). The Test-mate™ is the only known field-testing kit for ChE that is "approved" by the U.S. Food and Drug Administration (FDA) (see Section 2.2).

The Test-mate™ ChE system is a variation of the Ellman method. It can specifically measure either AChE or BChE, depending on which assay kit with ChE-inhibiting reagents is used with the system. The system also measures hemoglobin and an internal reagent blank to refine photometric measurements; the ChE activity is measured with a compact photometric analyzer. The results are normalized to 25°C, and also to the hemoglobin concentration for AChE (Taylor et al., 2003). The results are usually

expressed in U/mL, or alternatively for AChE as activity per gram of hemoglobin (U/g Hgb).

Through the contract mentioned previously with the USAMRMC, the performance of the Test-mate™ system was compared with the laboratory-based Ellman and Delta pH methods (Wilson, 2006). In general, the Test-mate™ has a good agreement with the Ellman and Delta pH methods over most of AChE activity range. However, the Ellman method appears to be more sensitive at highest levels of AChE activity, i.e., less exposure and consequently less AChE depression. Regression formulas were also derived to permit the conversion of results among the three different methods.

Another commercially available field-testing method is the Lovibond Cholinesterase AF 267 Test Kit. This product is marketed as a rapid test of exposure to OP insecticides to alert users of overexposure and unsafe work practices. The company advertises that the kit is specified in the World Health Organization's technical information sheet No. 356 (WHO, 1967). Unlike the Test-mate™, this field testing method utilizes a color comparator rather than photometric analyzer (Da Silva et al., 1994). This suggests that the method is a relatively crude estimation of ChE activity compared with the Test-mate™.

1.5. Military Applications and Policies Regarding Cholinesterase Testing

All of the military services are required to comply with Department of Defense (DoD) policies regarding occupational safety and health programs (DoD, 2007). Cholinesterase testing is used routinely by the military for occupational medical surveillance. Military and civilian personnel who are biologically monitored for ChE activity include pest controllers at installations and bases (Navy Environmental Health Center (NEHC), 2007). For these occupational exposures, the procedures followed for ChE testing are very similar to those used by non-military organizations. The Navy and Air Force have contracts with Quest Diagnostics for clinical testing services, which includes tests for BChE and AChE. The method of ChE testing used by Quest Diagnostics is listed as kinetic spectrophotometric, which most likely is a variation of the Ellman method (Quest Diagnostics, 2007).

Another very important application of ChE testing by the military involves uniformed and civilian personnel who handle chemical warfare agents (CWAs) and munitions such as sarin, soman, and tabun. These situations have special requirements known as "surety" that emphasize safety, security, and personal reliability (U.S. AR 50-6). The chemical demilitarization program is particularly important because of its size and scope. The Cholinesterase Reference Laboratory (CRL) at USACHPPM has primary responsibilities for ChE testing of surety workers (DA PAM 40-8, TB MED 590). As mentioned previously, the CRL uses the Delta pH method, and for routine analyses, specifically measures AChE activity of the RBCs. The CRL maintains a massive database of AChE test results for over 15,000 personnel, and most of them have multiple test results in the database. The estimated annual number of AChE tests performed at the CRL is 25,000.

The historical test results for many individuals span decades over time (Lefkowitz et al., 2007).

Chemical warfare defense on the battlefield is a military-specific application of ChE testing, although recent concerns about terrorist attacks have gained the interest of civilian agencies in using ChE testing for homeland security. Since many of the CWAs are nerve agents that specifically inhibit ChEs, the depression of ChE is considered a quick and easy biomarker of exposure to these agents (TB MED 296). Following an incidental exposure of explosive ordinance disposal personnel to sarin in Iraq, the U.S. Army issued a policy regarding baseline and post-exposure AChE testing for soldiers at risk of CWA exposures (Office of the Surgeon General (OTSG) Memorandums 2004a and 2004b). This policy raised the issue of ChE testing to the forefront of a Working Group under the Assistant Secretary of Defense for Health Affairs. The Working Group developed a draft DoD policy on ChE testing across the military services. However, despite a year of meetings and debate on the subject of ChE testing, the draft DoD policy was abandoned following non-concurrences with the policy by the Navy and Air Force. Currently, no uniform policy exists across the services regarding when and whom to test for ChE activity, nor is there standardization of ChE testing methods; Army personnel at risk of CWA exposure have AChE testing performed at the CRL, while the other services rely mostly upon clinical laboratory contracts with commercial firms.

The U.S. Army is the DoD Executive Agent for research, development, testing and evaluation (RDT&E) regarding medical aspects of CWA defense. The Army also issues Technical Bulletins (TB MEDs, 1996 and 2001) on this subject, which the other services often use as guidance or reference but lack the authority of policy. An important document that provides guidance on using ChE testing for CWA exposures is Chapter 3 of TB MED 296: "Verification of Nerve Agent Exposure – Monitoring Blood Cholinesterase Activity with the Test-mate™ OP Kit." This TB reflects the informal adoption of the Test-mate™ for field verification of CWAs that are ChE-inhibitors. The chronology of the acquisition process for the Test-mate™ has been described in a separate report (Science Applications International Corporation, 1998).

Despite the lack of a uniform policy for ChE testing across the services for CWAs, a policy regarding ChE testing frequency and acceptable methods does exists for occupational medical surveillance of pesticide workers. According to the DoD Occupational Medical Examinations and Surveillance Manual (2007), a worker with potential exposures to cholinesterase-inhibiting pesticides must have both AChE and BChE activity levels determined at the following frequency:

1. Before starting pesticide work/spraying (baseline).

2. First in-season test at 45-60 days.

3. Quarterly thereafter if spraying continues.

The Manual permits the Ellman, Delta pH, and Test-mate™ methods and refers health professionals to the CRL at the USACHPPM for details on the inter-conversion of results, which must be expressed as international units per milliliter (U/mL) on the Ellman scale for purposes of record keeping. Furthermore, the Manual does not exclude the use of alternative methods of ChE testing provided that the alternative and reference methods have at least a 0.9 correlation coefficient squared (r^2). The Manual stipulates that methods are unsatisfactory if they do not provide separate measures for AChE and BChE. This stipulation does not apply to chemical "surety" workers who are covered under separate policies and directives.

A final application of ChE testing in the military is associated with RDT&E. As mentioned earlier, new methods of ChE testing have been the subject of research at the WRAIR (Gordon et al., 2004), although method development for ChE testing within the military is not a major research effort. Other applications of ChE testing involve research to study the effects of nerve agents and the efficacy of nerve agent scavengers or treatments. A summary of ChE testing applications in the military is provided in Table 1-4.

Table 1-4: Cholinesterase (ChE) Testing in the U.S. Department of Defense.

Scenario or Situation	Primary Purpose	Laboratory Facilities Utilized	Decision Makers or Users	References
Surety Program for Chemical Warfare Agent (CWA) Handlers and Demilitarization Workers, Usually CONUS	Biomonitoring of Exposure and Effects, Occupational Medical Surveillance, Compliance with Regulations/Policies, Exposure Documentation.	Cholinesterase Reference Laboratory (CRL) at USACHPPM.	Occupational Health Clinicians, Database Curators for Future Claims and Health Effects Research.	U. S. AR 50-6, TB MED 590
Operational Exposures to CWAs, Primarily OCONUS for Deployed Units and Possibly CONUS for Homeland Defense.	Rapid Determination of Exposure to ChE-inhibiting Nerve Agents, Medical Treatment and Management, Return-to-Duty Evaluations, Exposure Documentation.	CRL (Army), Commercial Laboratories (Navy, Marines, Air Force) Test-mate™ Kit is used in Theater/Field Laboratories by the Army, but not mandatory for the other services.	Operational Commanders, Chemical, Biological, Radiological, Nuclear and Explosive Incidents (CBRNE) Defense Teams, Medical Officers with Operational Support Roles, Military Preventive Medicine or Public Health Personnel.	OTSG Memorandums dated 10 Sep 2004, TB MED 296
Occupational Exposures to Organophosphate and Carbamate Pesticides (e.g., Pest Controllers), Primarily CONUS and OCOUNS Bases and During Deployments.	Biomonitoring of Exposure and Effects, Occupational Medical Surveillance, Compliance with Regulations/Policies, Exposure Documentation.	Commercial Laboratories (all services) Test-mate™ Kit is optional but not mandatory under deployed situations during vector control activities.	Occupational Health Clinicians, Military Preventive Medicine and Public Health Personnel.	DoD 6055.05-M, NEHC Medical Matrix
Military Biomedical Research Applications, CONUS.	Development of New ChE Testing Methods, Biomarker of Dose and/or Effect in Research involving Laboratory Animals or Human Trials.	In-House Laboratory Facilities (AFRL, WRAIR, USAMRICD, Naval Medical Research Center (NMRC), USACHPPM), Commercial Laboratories, Academic/Company Research Partners.	Principal Investigators, Food and Drug Administration (for Drug approval).	

1.6 The Key Issue of Cholinesterase Testing versus Other Biomarkers

While ChE inhibition has been a biological marker, or biomarker, of exposure and effect for decades, it may not be advantageous for some purposes. For example, the verification of a specific agent for forensic evidence is circumstantial when using either ChE activity or enzyme levels. Many substances and physiological conditions can alter individual ChE activity, and the specific ChE-inhibitor can only be identified by analyzing the parent compound or unique metabolites. This type of identification is particularly important in incident responses involving CWAs where non-medical decisions require robust forensic evidence.

Among the methods for confirming CWA or ChE-inhibiting nerve agent exposures are the following (Worek et al., 2005):

1. Qualitative and quantitative analysis of nerve agent in the plasma that is unbound to ChE using chromatography and mass spectrometry (GC-MS or LC-MS/MS);

2. Qualitative and quantitative analysis of decomposition products such as phosphonic acids in the plasma and urine;

3. Fluoride-induced methods of reactivating the inhibited AChE and BChE and regeneration of the nerve agent followed by analysis by GC-MS;

4. Analysis of the proteins in plasma (BChE, albumin) using tryptic digestion and LC-MS/MS to identify adducts formed from the binding of nerve agent metabolites with peptides.

Using ChE inhibition as a biomarker also has limitations in epidemiologic studies for health risk assessments involving pesticides. Among these limitations are some of the issues discussed earlier, such as inter- and intraindividual variation and the suppression of ChE caused by health conditions or from unknown inhibitors. In addition, AChE levels in children may be different from adults, and relatively large doses of pesticides are required for significant AChE inhibition (Wessels et al., 2003). The ideal biomarker of exposure and/or effect for epidemiologic studies is easy to analyze and sensitive and specific to the agent of interest. To this end, many potential biomarkers for pesticide exposures have been compiled, but none of them is considered ideal (Wessels et al., 2003). Despite the shortcomings of ChE activity as a biomarker, the methods of ChE analysis are relatively inexpensive and easy to perform. The U.S. Environmental Protection Agency (USEPA) also considers AChE inhibition data useful for deriving reference doses for pesticide toxicity – provided the quality of data are considered (USEPA, 2000). However, for the issues presented earlier in Table 1-3, ChE inhibition may not be very helpful for health risk assessments.

As discussed earlier, the value of ChE activity as a biomarker depends foremost on the needs of the decision maker. For biological monitoring and routine medical surveillance of workers with established baselines and a known inventory of exposures to ChE-inhibiting chemicals, ChE activity provides valuable information for decisions regarding the elimination of unnecessary exposures to specific chemicals. On the other hand, the use of ChE activity for forensic investigations as “smoking gun” or *prima facie* evidence of exposure to specific agents is very limited, albeit in combination with other supporting evidence it could be convincing. In short, the use of ChE activity for decision making should follow well-defined guidelines of interpretation that are aimed at the specific purpose of the decision.

2. Review of the Performance Characteristics of the Test-mate™ Cholinesterase Testing System

2.1 Introduction

Agricultural workers with exposures to organophosphate (OP) and carbamate pesticides comprise the major population that benefits from field testing of cholinesterase (ChE) activity, though residents in rural areas may also benefit from a portable and rapid screening test for ChE activity. In most cases where pesticide exposures are biologically monitored for ChE activity, the individuals are tested in a clinical setting, or a blood specimen is collected in the field and transported to a laboratory facility. Both of these approaches to biological monitoring can be time consuming and expensive. Furthermore, the time between receiving the test results and taking necessary actions to reduce pesticide exposures and/or removing the worker can be excessive. Hence, the need for a field-portable test for ChE activity has long been recognized, particularly for the lesser developed countries (Jeyaratnam, 1990 and Edson, 1950).

2.2 Commercially-Available Field Test Kits

During the literature search for this report, only two field-portable systems were identified as commercially available for ChE testing. The oldest is the "tintometric" field kit, which has been available for over 50 years (Edson, 1950). The current version is the Lovibond® Cholinesterase AF267 Test Kit marketed by Tintometer Limited (London, UK). Early studies of the tintometric method concluded that the kit is adequate for determining whether "dangerous" amounts of pesticide exposure have occurred to workers (Miller and Shah, 1982). Subsequent studies have shown that the tintometric method has insufficient agreement with the Ellman method and lacks precision to reliably establish a baseline ChE activity for workers (McConnell and Magnotti, 1994). The main problem with the tintometric method is the use of a color comparator for semi-quantitative determination of ChE activity; this non-instrumental approach is subjectively determined by the kit operator (Magnotti et al., 1988). The most recent study of the Lovibond kit found that it performed poorly compared with available quantitative techniques (Carmona-Fonseca, 2007).

The other widely used field-portable system for ChE testing is called the Test-mate™, manufactured by EQM Research, Inc. (Cincinnati, OH). Although a new model of Test-mate™ is planned for production, the latest commercially available system is the Model 400 Test-mate™ ChE kit (Wilson, 2006). This system requires only 10 microliters (µL) of blood, and the entire assay can be completed in less than 4 min. It weighs 10 pounds and is relatively small (11" x 7" x 10") compared with laboratory-based apparatus. The key component of this system is a fixed-wavelength absorption photometer, which is compact (3 ½" x 5 ¾" x 2") and powered by a 9-volt battery. Other components include a hard-shell case, tube rack, and reagent assay kits.

The Test-mate™ ChE system's chemistry is a variation of the Ellman method. It can specifically measure either AChE or BChE, depending on which assay kit with ChE-

inhibiting reagents is used with the system (EQM Research, Inc, 2003.). The system also measures hemoglobin and an internal reagent blank to refine photometric measurements. The results are normalized to 25°C, and with the hemoglobin concentration for AChE (Taylor et al., 2003). The ChE activity is measured with the compact photometer, which also incorporates the electronics and algorithms to normalize the results to the other parameters. The results are usually expressed in U/mL, or alternatively for AChE as activity per gram of hemoglobin (U/g Hgb).

The Test-mate™ is the only known field-testing system for ChE that is "approved" by the U.S. Food and Drug Administration (FDA). This "approval" is actually a concurrence by the FDA that Test-mate™ is "substantially equivalent" to other cholinesterase test systems, which means it is a Class I medical device with a Premarket Notification and a 510(k) submission (21 CFR 862.3240). Unlike a Premarket Approval, the 510(k) is a Premarket submission to the FDA that must demonstrate the device is substantially equivalent to a legally marketed device. In other words, the manufacturer must have data to support the claim the device is substantially equivalent to a predicate device or method. After the claim is reviewed and the assertion of substantial equivalence is concurred by the FDA, a letter of determination is sent to the manufacturer that allows interstate marketing of the device without the need for Premarket Approval, a much more complex approval process.

2.3 Literature Review of the Test-mate™ Field Kit

Early published studies that laid the groundwork for the Test-mate™ kit were by Magnotti et al. (1987, 1988). The authors optimized the Ellman-based AChE and BChE chemistry for field use and employed a battery-operated Model 176 colorimeter by EQM Research, Inc. (Cincinnati, OH), the manufacturer of the current Test-mate™ kits. For comparison purposes, they also used the semi-quantitative Lovibond tintometric field kit. The two methods had only a fair agreement ($r^2 = 0.841$), and in some patient cases the tintometric method indicated normal ChE activity when it actually was depressed to the point of severe intoxication (Magnotti et al., 1988). The authors also defined the optimal criteria for a field kit to measure ChE:

1. Ability to accurately and specifically measure erythrocyte and/or plasma cholinesterase.

2. Low cost.

3. Precision to determine a baseline pre-exposure value for erythrocyte and/or plasma cholinesterase for subsequent comparison during exposure.

4. Stability of reagents to varying temperature and humidity for at least several months.

5. Being operational without need for line voltage, a balance, or a centrifuge.

6. Portability.

7. Capacity to analyze, reasonably quickly, a large number of samples in the field. (Magnotti et al., 1988)

The earliest published study that specifically mentions a Test-mate™ kit was by McConnell et al. (1992). Recognizing the importance of the interindividual variability of ChE, the authors used an early model of Test-mate™ to characterize the AChE variability of workers at a pesticide formulation plant in Mexico. They discovered the AChE coefficient of variation (CV) was similar to that in previous studies, approximately 12%. However, when the researchers adjusted the AChE to the hemoglobin, the CV was reduced markedly to 7.4%. This underscores the importance of measuring the hemoglobin when using Test-mate™ to measure AChE activity. Although the authors stated that the Test-mate™ normalized the AChE activity to 25°C, they did not state the temperature conditions at which the assays were performed.

Karr et al. (1999) evaluated the Test-mate™ kit with respect to its feasibility and capability to detect ChE depression among orchard workers. Although this study was published in 1999, the actual project was conducted during the apple-growing season of Washington State in 1993. The specific version of the Test-mate™ used was the Test-mate™ OP kit, and several issues were experienced while using it during the study. The most significant issue was a recall of the Test-mate™ kits by the manufacturer for a faulty filter component that could alter the kit's accuracy over time. Anticipating potential problems, the authors measured ChEs from 26 unexposed employees prior to returning the kit and after receiving the modified kit. They discovered the mean baseline values for AChE and BChE activity decreased by 10.3% and 15.9%, respectively. Therefore, before the trend in ChE activity of the orchard workers could be analyzed, the authors had to lower the pre-recall ChE activity data by the mean difference values of the unexposed group.

Another technical issue experienced by Karr et al. (1999) with the Test-mate™ was related to the functional temperature range of the kit. Prior to initiation of the study, the authors conducted laboratory-based experimentation with the Test-mate™ and identified inaccuracies at low temperatures (<18°C). An attempt was made to conduct all ChE monitoring of orchard workers in situations where the temperature was at or above 18°C. During one month of the study, however, this was not possible, and the data obtained during that period was excluded from analysis.

Despite the issues encountered with the Test-mate™, the study by Karr et al. (1999) provided useful information for conducting field monitoring of ChE. One advantage of the Test-mate™ kit was the use of fingerprick collection of blood instead of venous puncture. The fingerprick collection was more agreeable to the orchard workers and reduced potential exposures to blood borne pathogens from needle sticks. However, a problem was identified with pesticide residues on the skin that can contaminate fingerprick specimens, resulting in erroneous ChE activity measurements. Another important observation was the effect of BChE depression of workers with hepatotoxicity

from isoniazid therapy. The authors also identified and confirmed significant levels of ChE depression among particular orchard workers. Furthermore, they observed a difference in the trends between AChE and BChE activities over the growing season, i.e., AChE was more indicative of cumulative exposures, while BChE was more responsive (suppression and recovery) to short-term exposures.

Using an early model of the Test-mate™ kit, London et al. (1995) encountered some problems with its sensitivity. The researchers measured the activity of both AChE and BChE under three conditions: (1) field application of the Test-mate™, (2) non-field application of the Test-mate™, and (3) in the laboratory using the Ellman method. The blood specimens were collected from agricultural workers during ambient temperature ranges from 20°C to 28°C. Compared with the laboratory-based Ellman method, the Test-mate™ demonstrated much less sensitivity to drops in both AChE and BChE activity. Nevertheless, the authors concluded that the AChE activity measurements were sufficiently repeatable for routine surveillance. On the other hand, the BChE activity measurements were deemed not sufficiently repeatable for routine surveillance. The authors also offered several possible explanations for the poor repeatability of the Test-mate™ kit. One suggested explanation was that the Test-mate™ has inherent problems with differences in the ambient temperatures, while other explanations involved the relative skill of the operators and the more precise and controlled preparatory steps used with the apparatus of permanent laboratories (London et al., 1995).

In response to the findings of poor performance with the Test-mate™ kit by London et al. (1995), a comment was later published by Amaya et al. (1996). The authors reiterated that the Test-mate™ performed with good reproducibility under similar circumstances in a previously reported study (McConnell et al., 1992). To better understand the conflicting views of performance with the Test-mate™ kit, Amaya et al. (1996) conducted additional experiments. Using blood samples split into six separate aliquots, the Test-mate™ was used to measure AChE and BChE activity at different ambient temperatures. Their data demonstrated that the temperature normalization adjustment by the Test-mate™ was the source of significant error for both AChE and BChE activity. In another experiment, they examined the rate of thermal equilibration of the Test-mate™ after moving it from a cool room (8°C) to warmer room (40°C). The Test-mate™'s internal thermometer registered only 30°C after 120 min in the room with an ambient temperature of 40°C. They suggested that rapid changes in temperature with the Test-mate™ immediately before measuring ChE activity would result in considerable error. The authors further suggested this problem might be solved if the Test-mate™ manufacturer were to modify the kit to measure the temperature of the reagent solution rather than the colorimeter.

Three additional studies were published using the Test-mate™ to measure ChE activity levels among workers and residents exposed to organophosphate (OP) pesticides. Ciesielski et al. (1994) used an early version of the Test-mate™ kit to identify significant differences in AChE activity between farm workers and non-farm workers. Among the farm workers, they determined that AChE activity was lower specifically with the pesticide applicators, and they found a strong association of illness symptoms (diarrhea) with lowered AChE activity levels. The Test-mate™ (Model 176 colorimeter) was also

used in a study that determined rural residents who are potentially exposed to pesticides had lowered AChE activity levels and a greater proportion of illness symptoms compared with a similar population without pesticide exposures (Keifer et al., 1996). Tinoco-Ojanguren and Halperin (1998) also used the Test-mate™ OP kit to compare the AChE activity rates of residents among different communities in Mexico; their results suggested that the poorest communities were at greater risk of health effects from pesticide exposures. While the Test-mate™ proved to be a useful tool for studying pesticide exposures and toxicity under field conditions, the analytical precision and accuracy of the Test-mate™ kits were not included in the design of these studies. Consequently, the validity of the data collected during these studies is questionable.

One of the most comprehensive field studies attempted with respect to exposure parameters was conducted by Simcox et al. (1999). This study followed orchard workers over an apple-thinning season and sampled a variety of exposure parameters to an OP pesticide, including environmental concentrations and biological monitoring. The latter parameters included urinary metabolites of the pesticides, and AChE and BChE activity using the Test-mate™ kit. The urinary metabolites were analyzed at a fixed laboratory, while the Test-mate™ determinations of ChE activity were performed on-site near the workers and control subjects. However, the results of the Test-mate™ were anomalous in some cases, and the authors were concerned about previous reports of erroneous temperature corrections by the Test-mate™. Since the temperature changes varied greatly during the seasonal study, the authors considered the performance of the Test-mate™ unreliable and excluded its results from their study.

After having gained experience with the strengths and limitations of the Test-mate™ kit, McConnell et al. (1999) studied the BChE activity of children potentially exposed to OP pesticides. Instead of using automated algorithms in the Test-mate™, the authors used a correction chart provided by the manufacturer to manually adjust BChE activity for ambient temperatures. After finding a significant difference in the BChE activity levels between exposed and unexposed children, the authors wished to verify the difference was not due to Test-mate™ temperature-correction errors. The authors used the corrected mean BChE activity data and dummy variables with multiple linear regression modeling; the results indicated a difference in BChE activity (albeit slightly less) still remained between the exposed and unexposed children. This quality control approach involved only statistical manipulation and not the use of analytical standards or verification by laboratory analysis.

As part of a multifaceted study, Higgins et al. (2001) measured the AChE activity migrant workers and their children using the early model Test-Mate™ OP kit. During the study in 1998, the Test-mate™ OP kit was replaced by EQM Research, Inc. with the Model 400 Test-mate™ ChE kit, and supplies for the old model were no longer available. The authors then initiated a validation study to compare the performance of the Model 400 Test-mate™ ChE kit with the laboratory-based Ellman method. They also compared the performance of the old and new models of the Test-mate™ kits. Blood samples for the validation studies were taken from staff volunteers and diluted serially to yield varying degrees of AChE activity. All analyses using the Test-mate™ kits and the Ellman

method were conducted at temperatures of approximately 25°C. As an added measure of validation, the authors also evaluated the hemoglobin measurements of the Test-mate™ kits against the HemoCue system (HemoCue AB, Angelhom, Sweden). The following results and conclusions are summarized from their study:

1. Although the measurements of AChE activity of undiluted blood using the Test-mate™ were approximately 87% of the AChE activity compared with the benchmark Ellman method, a strong linear relationship existed between the Test-mate™ and Ellman methods using serially diluted blood to yield varying degrees of AChE activity. The correlation coefficient squared (r^2) between the methods was 0.98.

2. The measurements of hemoglobin by both the old and new Test-mate™ kits were significantly lower compared with the benchmark HemoCue kit. In addition, the hemoglobin measurements between the old and new Test-mate™ kits were significantly different, with the old kit providing consistently lower results.

3. The paired AChE activity levels of the old Test-mate™ OP kit were significantly and consistently lower compared with newer Model 400 Test-mate™ ChE kit. However, when the results of both kits were corrected for hemoglobin, the results were no longer significantly different, despite the fact that each kit yielded different results for hemoglobin.

4. The authors noted several improvements in the Model 400 Test-mate™ ChE kit over the old model. Among them was a better method for sample preparation and sample absorbance at 450 nm (vs. 470 nm) against a reagent blank.

5. The authors observed differences in reliability of the Test-mate™ kits based on the experience of the operator. Novice operators had more problems than seasoned operators did. The modifications to the new Model 400 Test-mate™ ChE kit improved the ease of use and eliminated some operator errors in sample preparation.

6. The authors reiterated that technique of choice for surveillance is the use of urinary metabolite levels and benchmark ChE activity levels (using the laboratory Ellman method) with established baseline data. Nonetheless, they concluded that when used properly, the Test-Mate™ kit provides useful data on OP exposures.

To evaluate the performance of the Test-mate™, Oliveira et al. (2002) tested three models or versions of the Test-mate™ kit. One of the kits was the latest Test-mate™ Model 400 ChE, while the other two kits were earlier versions. Using fetal bovine serum as a source of AChE, the authors tested the performance over a temperature range from 10° to 37°C. The results of the Test-mate™ kits were compared with the laboratory-based Ellman method using a 96-well microplate reader. As expected, the uncorrected AChE activity of the fetal calf serum sample increased with increasing temperatures using the laboratory-based microplate reader. On the other hand, the Test-mate™ kits automatically normalized the AChE activity to 25°C by an internal program; none of the

Test-mate™ kits displayed the uncorrected AChE activity, i.e., the operator relied entirely on the hardware and internal algorithms for temperature-corrected results.

The two old versions of the Test-mate™ kits did not satisfactorily normalize AChE activities at temperatures below 25°C. Most importantly, the values between the three Test-mate™ kits were not comparable to one another without some external standardization. The performance of the newer Test-mate™ ChE Model 400 kit was generally satisfactory only from 20–37°C. However, although the performance was satisfactory at these temperatures, the Test-mate™ ChE Model 400 kit yielded higher percent error rates at all temperatures compared with the laboratory-based microplate reader. The authors recommended that the Test-mate™ be used with a laboratory standard and under carefully controlled temperature conditions. Furthermore, the authors expressed concern about the validity of ChE data collected in the field using the Test-mate™ without the aforementioned controls. Despite these shortcomings, the authors stressed the need for a portable and inexpensive device to rapidly measure ChE activity in the blood (Oliveira et al., 2002).

Shortly after the Oliveiri et al. (2002) study, the Test-mate™ kit was used in a study of human blood samples spiked with variable amounts of the nerve agent soman (GD). Unlike the previous study, which used several versions of the Test-mate™, this study used three of the latest Model 400 Test-mate™ ChE kits, and the assays were run under normal temperature-controlled conditions in both a mobile laboratory (520th Theatre Army Medical Laboratory) and a permanent-structure laboratory (Taylor et al., 2003). Along with the control samples, the study included blood samples spiked with GD at levels designated as high, medium, and low. The blood samples were blinded to the Test-mate™ operators and used to examine the precision of the three kits in terms of repeatability (measurements in a single run or day) and reproducibility (measurements across runs or days). The following summary of observations and conclusions regarding the performance of the Test-Mate kits came from the study:

1. Using analysis of variance, significant differences in repeatability of AChE activity were observed between the three Test-mate™ kits.

2. Comparison of each kit's CV by GD dose revealed no significant differences in repeatability between the three Test-mate™ kits.

3. No significant differences were observed in daily mean levels of AChE activity among the Test-mate™ kits, implying each kit had reproducible results across the days.

4. The Test-mate™ kit had greater precision with the smaller doses of GD, i.e., less inhibition and thus greater AChE activity.

5. The AChE activity normalized to hemoglobin was more precise compared with using only blood volume.

6. The more experienced kit operator had the most precise results.

7. The lower limit for ambient temperatures when using the Test-mate™ kit should be 20°C.

8. Even though some variability in repeatability exists within GD doses among the Test-mate™ kits, the control samples were easily distinguished from all GD-spiked samples.

9. The CVs of all control samples were 3% or less, which lead the authors to conclude that the precision of the Test-mate™ kit is excellent and its performance is acceptable.

Under a contract by the U.S. Army Medical Research and Materiel Command (USAMRMC), the performance of the Test-mate™ Model 400 kit was compared with the laboratory Delta pH and Ellman methods (Wilson, 2006). Along with evaluating the performance of these methods, the goal was to derive conversion equations for AChE activity between the different methods for the direct comparisons. After obtaining blood samples from human volunteers, the red blood cells were separated and spiked with different concentrations of ChE-inhibitors to yield a range of AChE activity, which was then measured by the different methods. Initially, Wilson was to receive a customized Test-mate™ kit from the manufacturer that would provide direct read-out of AChE activity without correction or normalization for temperature. However, the manufacturer (EQM Research, Inc.) did not provide the customized Test-mate™, and the study was conducted with the standard Model 400 Test-mate™ ChE kit. Although not specifically stated in the contract report, it is assumed that the Test-mate™ was operated in the laboratory under normal and controlled temperature conditions.

As measured by linear regression, the overall agreement of the Test-mate™ and the Delta pH methods was excellent, resulting in $r^2 = 0.99$. The agreement of the Test-mate™ and Ellman Methods resulted in $r^2 = 0.91$. Similarly, the agreement between the Delta pH and Ellman methods resulted in $r^2 = 0.92$. One explanation for the slightly lower r^2 with the Ellman method appears to be the greater sensitivity of the Ellman method at higher levels of AChE activity compared with both the Test-mate™ and Delta pH methods. The inferred conclusion is that the AChE activity must be more depressed for detection by the Test-mate™ or Delta pH methods, whereas the Ellman method is more sensitive at detecting lower levels of exposure to ChE inhibitors (Wilson, 2006).

2.4 Conclusions

Despite its longevity, the tintometric field kit is limited to semi-quantitative estimation of ChE activity. This kit is not reliable for establishing baselines of ChE activity and may not detect significant depression of ChE activity. Hence, its utility is very limited in terms of biological monitoring and medical surveillance.

The Test-mate™ system has 20 years of published history on its use, including several validation studies. Despite the Test-mate™'s shortcomings, it represents the best commercially available field test kit for ChE activity. The manufacturer has also endeavored to improve the Test-mate™ system when problems were identified through user feedback and publications. Based on the publications reviewed in this report, the following conclusions are offered:

1. The Model 400 Test-mate™ ChE kit appears to provide valid results for AChE activity under steady temperature conditions above 20°C and when used by an experienced operator.

2. The normalization of AChE with hemoglobin is preferable in terms of reduced interindividual variability and the comparison of results.

3. The interpretation of BChE activity measured with the Model 400 Test-mate™ ChE kit is less definitive, but the instrument probably provides sufficient accuracy and precision to detect significant depression levels of BChE activity, i.e., 20-40% depression from baseline (Wilson et al., 2005).

4. The compactness of the Test-mate™ system, along with method of blood collection (fingerprick) and the time to obtain results are definite advantages over laboratory-based determinations of ChE activity.

5. Precautions are necessary when collecting blood specimens to prevent unintentional contamination with pesticide residues.

6. Desirable modifications to the Model 400 Test-mate™ ChE kit would include an additional display/recording of AChE activity uncorrected for temperature, and the use of analytical standards for calibration or quality assurance purposes.

7. Earlier models of the Test-mate™ are not reliable for routine biological monitoring of either AChE or BChE activity.

3. Survey of Handheld and Portable Devices for Measuring Biological Markers

3.1 Introduction

This survey is not an exhaustive inventory of all handheld or portable devices that measure biological markers. An all-inclusive list of such devices – particularly those in the research and development (R&D) stages – would be expensive, time-consuming, and difficult to compile. The identification of private industry projects is difficult due to the intellectual property issues and because publications and advertisements about them are rare during the R&D stages. Federally funded R&D projects (including military Research, Development, Testing and Evaluation; or RDT&E projects) are often

fragmented and span many departments and agencies across the government. While this fragmentation can foster competition and innovation, it also results in a lack of communication, cooperation, and coordination – making discovery very difficult. Therefore, the discussion within this report is limited to handheld or portable devices that are readily discoverable and/or commercially available for measuring biological markers.

3.2 Currently Funded Projects

An inquiry was made to the Research Area Directorates of the U.S. Army Medical Research and Materiel Command (USAMRMC) for a list of all intramural projects to develop handheld or field-portable devices that can measure biological markers of interest, e.g., antibodies to infectious agents, chemical or biological warfare agents, toxic industrial chemicals, etc. Only five projects were identified, and these are listed in Table 3-1 along with two other devices that are discussed later.

The first three projects in Table 3-1 were sponsored by the Defense Threat Reduction Agency (DTRA). This agency centrally funds many research projects related to defense against weapons of mass destruction, including biological and chemical warfare agents (BWAs and CWAs). While some information about the DTRA's projects is available, many of the projects and their details are classified, and information about them is difficult to obtain. Multiple projects related to detecting BWAs and CWAs are likely being funded by the DTRA and may be in various stages of R&D. However, before any device that involves medical surveillance or clinical decision making can be fielded, it requires a 510(k) review by the U.S. Food and Drug Administration (FDA), and the USAMRMC is usually the lead agency for fielding such military medical devices.

Table 3-1: U.S. Army Medical Research Projects to Develop Handheld or Portable Devices for Measuring Biological Markers.		
Research Project Title	**Command and Company**	**Funding Sponsor**
Integrated immunological and nucleic acid detection technologies for the simultaneous detection of multiple biological warfare agents.	U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and Akubio Ltd. (Cambridge, UK)	Defense Threat Reduction Agency (DTRA)
Diagnosis of exposure to biological threat agents using host immune cell molecular responses.	Walter Reed Army Institute of Research (WRAIR) and Nanogen, Inc. (San Diego, CA)	DTRA
Advancement of the WRAIR whole blood cholinesterase assay.	WRAIR Kumetrix, Inc.	DTRA
Chemical Warfare Agent (CWA) Lightweight Field-Portable (Hand-Held) Medical Diagnostic Tool	U.S. Army Medical Research Institute for Chemical Defense (USAMRICD) and Kumetrix, Inc.	U.S. Army Medical Research and Materiel Command's (USAMRMC's) Small Business Innovation Research (SBIR) Program
A Handheld Monitoring System for Biomarkers of Response from Blood.	U.S. Army Center for Environmental Health Research (USACEHR) and Siloam Biosciences, LLC (Cincinnati, OH)	SBIR
The GlucoScope Monitor	Telemedicine and Advanced Technology Research Center (TATRC) and Visual Pathways, Inc. (Prescott, AZ)	Congressional Funding Sources
Binax Now® Malaria Test - K061542	U.S. Army Medical Materiel Development Activity (USAMMDA) and Binax, Inc. (Scarborough, ME) and Iverness Medical Innovations, Inc. (Waltham, MA)	USAMMDA

Of the three DTRA-sponsored projects listed in Table 3-1, the most relevant one to this survey is the blood cholinesterase (ChE) assay by Dr. Richard Gordon of the Walter Reed Army Institute of Research (WRAIR). Dr. Gordon has developed a novel method of measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Gordon et al., 2004). The primary objective of this DTRA-sponsored work is to develop a sensitive and high throughput laboratory method for measuring ChEs. Current funding of the laboratory ChE device at the WRAIR is aimed at developing Good Laboratory Practice standards. Dr. Gordon also initiated and managed a project with Kumetrix, Inc. (Union City, CA) to develop a handheld device for measuring ChEs. Unfortunately, the DTRA funding for the handheld device was discontinued, and the project is currently inactive, awaiting additional funding (telephone conversation with Richard Gordon on November 8, 2007). In a telephone conversation with Brian Sullivan of Kumetrix, Inc. on February 1, 2008, it was learned the company is also being funded by DTRA to detect a protein biomarker for BWA exposures/effects; this project is receiving oversight from the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD). In addition, Kumetrix, Inc. is working with USAMRICD on a handheld device to detect specific CWAs as part of a Small Business Innovation Research (SBIR) award, now in Phase II funding.

The fifth project in Table 3-1, also funded by an SBIR award, involves the U.S. Army Center for Environmental Health Research (USACEHR) and Siloam Biosciences, LLC (Cincinnati, OH). The objective of this project is to develop one handheld device with interchangeable "biochips" capable of measuring several biological markers or chemical residues: AChE and BChE, metallothionein, and blood lead. The biochips are fabricated on the basis of Bio-MicroElectroMechanical System concepts and employ an immunoassay detection technology. The project was initiated in 2005, and funding is expected to be terminated in February 2008, at which time a functional handheld device with prototype biochips are expected to be demonstrated.

A request was made in December 2007 to the Telemedicine and Advanced Technology Research Center (TATRC) of the USAMRMC for a list of handheld or portable devices under development. The TATRC database of funded projects was searched using the terms "portable," "handheld," and "point of care." Twenty-four projects were identified in the search, but twenty-three of them were solely involved with information management/information technology – not the actual measurement of biological markers (Eva Lai, December 26, 2007, email message to author). Only one portable device actually measured a biological marker: The GlucoScope Monitor by Visual Pathways, Inc. (Prescott, AZ), designed for use by diabetics to non-invasively measure their blood glucose (Table 3-1).

Within the DoD, the USAMRMC is responsible for advanced development and fielding of military medical equipment, including handheld or field-portable medical devices, for all the branches of service. This responsibility is accomplished through a subordinate command called the U.S. Army Medical Materiel Development Activity (USAMMDA). To date, only one portable or point of care device has been developed and made ready for fielding: The Binax Now® Malaria Test. This device (Table 3-1) provides rapid

diagnosis of malaria and is also the only device in 3-1 to have received 510(k) clearance for marketing by the U.S. FDA (2007). Several other clinical tests for diagnosing infectious diseases are in the early research phases, but none of them are ready for advanced development by USAMMDA (telephone conversation with Scott Doughty of USAMMDA on February 1, 2008).

A request was also made to the National Institute for Occupational Safety and Health (NIOSH) for an inventory of handheld or portable devices being developed to measure biological markers of exposure to agents. The NIOSH has several areas of interest in technology for biological monitoring of occupational exposures, including carbon monoxide in blood, volatile organic compounds in breath, etc. (John Snawder of NIOSH, November 27, 2007, email message to author). These interests are broad and limited mostly by budget constraints and the maturity of technologies. Except for commercially-available technologies such as the Test-mate™ ChE test kit, most current efforts appear to involve research with laboratory-based technologies. One novel, portable and mature technology was identified to measure anti-anthrax protective antigen immunoglobulin G in serum and whole blood (Biagini et al., 2006).

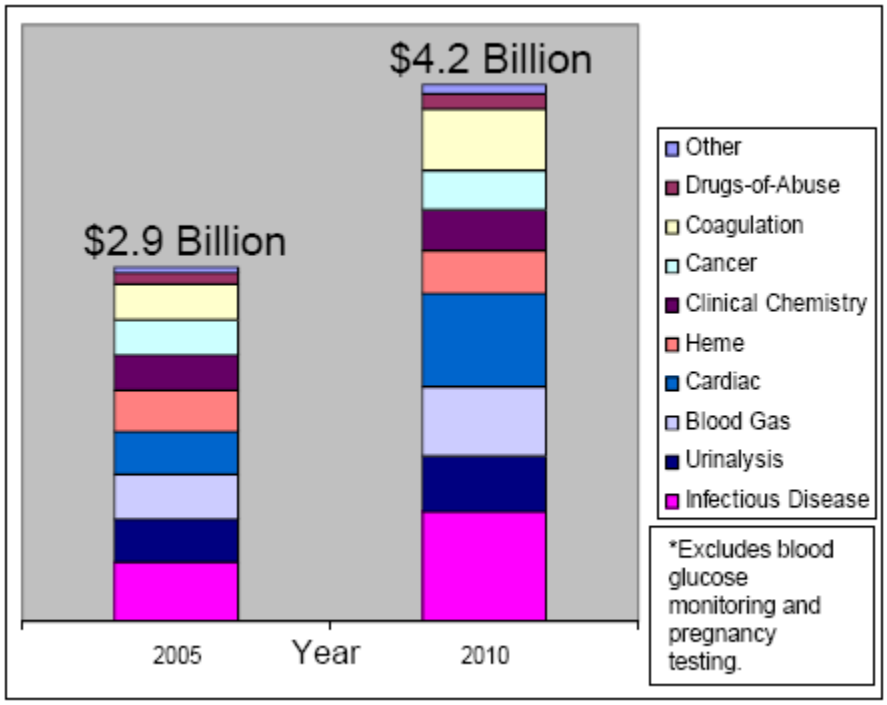
With the exception of DoD and a few other federal agencies, most funding by the federal government for biomedical technology development is limited to the basic and applied research phases of the R&D process. The final engineering development, production, and marketing of medical devices are usually accomplished through the private sector. However, two federal programs called the SBIR Program and the Small Business Technology Transfer (STTR) Program are intended to spur the advanced development and commercialization of technologies. For most federal agencies, the SBIR/STTR programs provide the sole opportunity to sponsor advanced development of a technology or device.

A database of SBIR/STTR project awards is maintained by the Small Business Administration (http://www.sba.gov/SBIR/indexsbir-sttr.html). Several searches (non-Boolean) of this database were conducted using the following terms: biological monitoring, biomonitoring, biomarkers, and point of care. Only a few projects were identified using the terms biological monitoring and biomonitoring, and among them only two were relevant to this survey. In contrast, a great number of projects were identified using the terms biomarkers (190 total projects) and point of care (133 total projects). This greater number of projects reflects a growing trend in biomarkers discovery research and point of care devices, which are discussed more fully in the next section of the report. The success rates of SBIR/STTR projects are not readily available from federal agencies or tracked in accessible databases. This may stem from a number of reasons, including the licensing or selling of technologies by small businesses to larger corporations that can produce and market the new technologies. Many of the technologies discussed in the next section of the report may have been initially funded from federal research budgets such as the SBIR/STTR programs.

3.3 Leveraging Opportunities with Commercially-Ready and -Available POC Devices

While the number of portable or hand-held devices for biological monitoring of toxic chemicals is limited, a growing number of portable devices are being marketed for clinical healthcare. These devices are referred to as either POC, near the patient, bedside, or extra laboratory testing (Price, 2001). In recent years, the trend in the development and commercialization of these devices has created a new segment in the medical market called POC Diagnostics. More precisely, the following definition has been proffered: "POC Diagnostics are composed of tests that are performed near the patient and over the counter/patient self testing that require a quick turnaround time and do not require permanent dedicated space in a clinical laboratory" (Scientia Advisors, 2007).

The advent of POC diagnostics is made possible from technological advances in materials science, chemistry, molecular biology, and electronics. These enabling technologies have achieved greater sensitivity and permitted the miniaturization of laboratory testing, along with the identification of new biological markers for clinical applications. The majority of growth in the market for POC diagnostics has been in the testing of diabetics, but the market for other testing and diseases is expected to increase from $2.8 billion in 2005 to $4.2 billion in 2010 (Scientia Advisors, 2007). Figure 3-1 illustrates the projected growth in the market by the specific application or disease; this figure excludes diabetes and pregnancy testing, which constitute the majority of the POC diagnostics market segment. On the other hand, the POC market for public health services (e.g., biological monitoring) is not even acknowledged in most economic reports or market projections.



(Redrawn from Scientia Advisors, 2007)

Figure 3-1: Point of Care Diagnostics market by application and disease

A review of U.S. disaster planning documents and the public health literature revealed little or no mention of POC devices (Kost, 2006). The primary reason that POC devices have been overlooked is uncertain, but it may be related to the high cost of product development/approval relative to the small market size of public health services. In addition, expensive or advanced technologies are often unaffordable by many state and local health departments. The expansion of the healthcare market for POC diagnostics will eventually result in economies of scale and competition, possibly making such devices more available and affordable for public health services. This process may also be accelerated by generous donations from private entities such as the Bill and Melinda Gates Foundation. Under its initiative titled "Grand Challenges in Global Health," the Foundation has awarded millions of dollars to develop POC devices for diagnosing diseases in the developing world (Yager et al., 2006).

After an extensive review, compendia of POC tests and devices were compiled by Tran and Kost (2006) for mobile, emergency, critical and primary care; and infectious diseases. Approximately 150 different instruments or platforms were identified for POC diagnostics and testing. The medical care instruments were categorized as handheld, portable, transportable, or for personal monitoring (*ex vivo* or *in vivo* placement). Only one commercial device was identified in the personal monitoring category, and that device is now defunct. The infectious disease tests were categorized as chip, disposable reagent, disposable reagent kit, and nucleic acid testing. The authors concluded that POC testing is becoming "smaller, smarter, faster and cheaper" in all areas of application (Tran and Kost, 2006). Tables 3-2 and 3-3 provide an overview of the compendia for clinical care and infectious diseases, respectively.

Table 3-2: Commercially-Available Point of Care (POC) Device Manufacturers and Biomarkers/Tests for Mobile, Emergency, Critical, and Primary Care Applications.*

Manufacturers of POC Devices

Abraxis	Cholestech Corp.	Life Scan	Radiometer
Abbott	Dade Behring	Maritech	Respironics
Acon Labs	Dainippon Pharmaceuticals	Metracor Technologies	Response Biomedical
Adeza Biomedical	Diametrics Medical	Metrika	Roche Diagnostics
Animas Technologies	Draeger Medical	NOVA Biomedical	Spectral Diagnostics
APEL	HemoCue AB	Orion Diagnostica	StanBio
Axis-Shield	Instrumentation Laboratory	Osmetech	Sysmex
Bayer Diagnostics	i-STAT	Ostex	Wako Diagnostics
Biosafe International	ITC	Polymer Technology Systems	
Biosite Diagnostics	Khon Kaen University	PTH Testing	

Biomarkers Measured or Tests Performed

Acetoaminophen	CRP, C-reactive protein;	HDL, high density lipoprotein;	PDAO, protamine dose assay;
ACT; activated clotting time	cTnI, cardiac troponin I;	H-FABP, human-type fatty acid	pH;
ALP; alkaline phosphatase	cTnT, cardiac troponin T;	binding protein;	pO2, partial pressure of oxygen;
ALT, alanine aminotransferase	differential leukocyte count;	HHb, deoxyhemoglobin;	PSA, prostate specific antigen;
APTT, activated partial	d-dimer;	HNTT, heparin-neutralized thrombin	PT, prothrombin time;
thromboplastin time; ascorbic acid;	FCOHb, fraction of	time; HR-ACT, high range activated	RACT, recalcified activated
AST aspartate aminotransferase;	carboxyhemoglobin; FHbF, fraction	clotting time; K^+;	clotting time;
A-hCG; beta-human chorionic	of fetal hemoglobin;	Lac, lactate;	sO2, oxygen saturation;
gonadotropin; BNP; B-type	FHHb, fraction of	LR-ACT, low range activated clotting	SO2%, percent oxygen saturation;
natriuretic peptide;	hemodeoxyhemoglobin; FmetHb,	time; microalbumin;	tBil, total bilirubin;
BUN, blood urea nitrogen;	fraction of methemoglobin;	MetHb, met-hemoglobin;	transcutaneous bilirubin;
CBC, complete blood count;	FO2Hb, fraction of oxyhemoglobin;	myoglobin;	TCO2, total carbon dioxide;
CGMS, continuous glucose	GGT, gamma glutamyl transferase;	Na^+; NMP22 protein (bladder cancer	tHb, total hemoglobin;
monitoring system; cholesterol;	glucose; GOT, glutamic oxaloacetic	marker);	triglycerides;
creatinine;	transaminase;	O2Cap, oxygen capacity;	TSH, thyroid stimulating
CK, creatine kinase;	GPT, Glutamic pyruvic	O2Ct, oxygen content; pancreatic	hormone;
CK-MB, creatine kinase-	transaminase;	amylase;	TT, thrombin time;
myoglobin;	Hb, hemoglobin;	pCO2, partial pressure of carbon	urea;
COHb, carboxy hemoglobin;	HbA1c, hemoglobin A1c;	dioxide;	uric acid.
Creat, creatinine;	Hct, hematocrit;		

*Several devices may be available from different manufacturers for each test listed above. Every device in the original report was categorized as handheld, portable, transportable, or for personal monitoring. The patient specimen used for the test is usually the same for the traditional laboratory test.

From Tran and Kost (2006).

Table 3-3: Commercially-Available Point of Care (POC) Tests for Infectious Diseases.

Diseases or Pathogens	
Adenovirus, Avian Flu (H5N1, H7N3)	Influenza A/B
Bacillus anthracis	*Leptospira*
Bacterial Endotoxin	*Listeria monocytogenes*,Malaria
Biological Weapons	*Mycobacterium tuberculosis*
Candida species	*Mycoplasm pneumoniae*
Clostridium difficile	*Neisseria gonorrhea*
Chlamydia trachomatis	Parvovirus
Cholera (*Vibrio cholerae*)	*Pseudomonas aeruginosa*
Cryptosporidium parvum	Respiratory Syncytial Virus
Dengue Fever Virus	Rotavirus
Enterococcus faecalis/faecium	Rubella
E. coli 0157	*Salmonella typhi*
Hepatitis B virus	SARS virus
Hepatitis C virus	*Staphylococcus* (including MRSA)
Helicobacter pylori	*Streptococcus pyogenes*
HIV-1 or 2	Syphilis (*Treponema pallidum*)
Herpes Simplex Virus	*Trichomonas vaginalis*
Human papillomavirus	West Nile Virus.
Manufacturers of Tests	
Abbott Labs	LifeSign;
AccuDx, Inc.	MBDr;
Acon Labs	Medical Services International;
Akers Laboratories	Meridian Biosciences;
AmeriTek	Omega Diagnostics;
Beckman Coulter	Orasure Technologies;
Binax	PanBio;
Bio-Rad;	PBM;
Biostar	Polymedco;
Cardinal Health	Quidel Corp;
Cepheid	Remel;
Core Diagnostics;	Roche Diagnostics;
Fisher Healthcare;	Sandia National Labs;
GeneLabs Diagnostic;	Smiths Detection;
Genzyme;	Spectral Diagnostics;
Germaine Labs;	Stanbio;
Hema Diagnostics;	Teco Diagnostics;
Henry Schein, Inc.;	Trinity Biotech;
Home Access;	University of Colorado;
IDI;	Wampole Laboratories;
Immunostics;	ZymeTx.
Jant Pharmacal;	
Formats/Methods of Tests	
Benchtop Polymerase Chain Reaction (PCR) and Multiplexed PCR; Disposable Reagent Kits; Disposable Reagent Cards.	
Patients Specimens Tested	
Serum, plasma, whole blood, nasal swab/aspirate/wash, vaginal/rectal swab, throat swab, stool.	

From Tran and Kost (2006).

Many of the system components for POC devices conceivably could be modified or re-engineered for other applications in public health, including biological monitoring for deployment health surveillance. For example, the POC applications for drug-of-abuse screening, emergency diagnosis of a drug overdoses, and the monitoring of medication dosage (Melanson, 2006 and 2007) are technically similar with the biological monitoring of exposure to industrial and agricultural chemicals. The characteristics of POC devices for drug-of-abuse testing have been reviewed by Melanson (2005), and these characteristics are very similar to hand-held or portable devices that may be used for biological monitoring. An important point, however, is that no single underlying technology can accommodate all types of desired testing. Some devices could be multiplexed or have interchangeable chips that can perform multiple types of testing, but several POC devices would likely be needed to perform biological monitoring because of the diversity of analytes and measurement technologies.

4. Conclusions and Recommendations

4.1 Leveraging the Growing Market in POC Diagnostics

As discussed in chapter three of this report, the POC diagnostic kits and devices represent a growing business market. The demand for POC devices is expected to dramatically increase, and private investors, along with nonprofit foundations, will likely continue investing in advanced development of these technologies. The types of tests performed or biomarkers measured will be driven by the demands of patients and the healthcare industry. Unfortunately, without government support, the development of POC devices specifically for public health applications (including ChE testing) will lag far behind development for the healthcare industry.

For biological monitoring applications such as ChE testing, an opportunity exists to leverage the development trend of POC devices by the healthcare industry. A good leveraging strategy would begin by identifying POC devices that have already reached technological maturity to receive FDA clearance/approval for patient testing. A systems analysis of selected devices could then be undertaken to determine if a component or subsystem could be modified to measure specific biomarkers (e.g., ChEs) for public health applications or military deployment health surveillance. Assuming that intellectual property issues can be resolved, this strategy would minimize government investment and reduce the risk of failure.

An important shortcoming of most POC devices is that they are not suitable for the rigors of field conditions, e.g., the tailgate of a truck. Most POC devices for the healthcare industry are designed for permanent building structures and countertops such as found in a physician's office or examination room. Such environments are usually protected from the weather and have normal/stable relative humidity and temperature. Therefore, in addition to re-engineering components of a POC system, additional investment and engineering may be necessary to "harden" a POC device for field use.

4.2 Suggested Ideal Performance Characteristics of a Field Cholinesterase Testing Device

For civilian public health applications, the performance characteristics of a field ChE test device for biological monitoring of pesticide workers have been articulated by Magnotti et al. (1988) and discussed in chapter two of this report. For military health surveillance applications, some general guidance about ChE testing exists within various military documents (discussed in chapter one), but the desired performance characteristics of a military field ChE test device have not been formalized into criteria. The process of developing performance criteria usually involves the combat developer/doctrine centers and the materiel developer. The first step should involve developing a concept of operations (CONOPS) for ChE field testing devices.

Since a CONOPS specifically for field ChE test devices has not been developed, the performance characteristics described below were derived from documents reviewed for this report. These performance characteristics should not be considered formal or final. Nevertheless, they represent a reasonable starting point for discussion and eventual incorporation into performance criteria.

1. Monitored Parameters, Sensitivity, and Accuracy.

a. The field ChE test device should accurately and specifically measure blood acetyl cholinesterase (AChE) and butrylcholinesterase (BChE) activities.

b. The benchmark performance standard for most organizations is the Ellman method performed by a credible laboratory. Comparisons between different methods requires conversion formulae, and the agreement between methods must have at least a 0.9 correlation coefficient squared (r^2) (DoD, 2007).

c. To attain FDA's 510(k) clearance as "substantially equivalent" to existing ChE test methods, the appropriate performance analysis must be performed. Guidance on performance analysis in terms of reliability, validity, and add-on value is provided elsewhere (Schwenke, 2007). The suggested analysis is the Bland-Altman statistical approach for the comparison of methods (Nizamettin Gul, January 10, 2008, email message to William van der Schalie).

d. Hemoglobin should be accurately measured and used to normalize AChE activity.

e. A reference or ghost standard should be used with the device to ensure performance reliability.

2. Precision.

a. Precision must be sufficient to determine baseline pre-exposure values for AChE and BChE activities and for subsequent comparisons during exposure, thus permitting the detection of "significant" exposures.

b. The definition of "significant" exposure may vary, but generally a reduction of 80 percent below baseline activity of either AChE or BChE is necessary for retesting and/or investigation of exposures (Cal/EPA, 2002).

c. Acceptable precision in terms of repeatability (measurements in a single run or day) and reproducibility (measurements across runs or days) as previously described are also necessary (Taylor et al., 2003).

3. Cost.

a. To be cost-effective, the device and reagents should be affordable to both the military and civilian public health agencies.

b. The benchmark of cost is the Model 400 Test-mate™ ChE kit, which currently costs less than $3,000. Reagent kits for 96 tests generally cost a few hundred dollars.

4. Stability of Reagents and Consumable Supplies.

a. As specified by Magnotti et al. (1988), the reagents and consumable supplies should be stable to varying temperature and humidity for at least several months.

b. More specific requirements for the stability of reagents and supplies may be needed for military logistical support, depending upon the CONOPS.

5. Environmental Conditions.

a. For military medical applications, the device's performance under various environmental conditions is greatly dependent upon the CONOPS. At a minimum, the device should exceed the operational limitations of the Model 400 Test-mate™ ChE kit determined by the literature review:

i. Operational Temperatures. The device must perform reliably in less than an hour after common temperature fluctuations (e.g., removal from a vehicle to an outdoor test site) and at ambient temperatures lower than 20°C, and ideally at a range from 8°C to 40°C.

ii. Humidity Conditions. With respect to relative humidity, the device's operational performance should be consistent in a wide range of climates, from deserts to jungle environments.

iii. Ruggedness or Shock/Vibration Tolerance. The device should be shock resistant and made with hardened materials to withstand transport and use under normal field conditions. Specific criteria for field military medical devices may be available and should be used.

6. Portability: Size, Weight, Cube, and Logistics.

a. The desired portability is a handheld device about the size of a Personal Digital Assistant. However, this level of portability may be difficult to achieve without compromising other performance characteristics.

b. To have added value, the portability of any new device should be better than the Model 400 Test-mate™ ChE kit:

i. Weight: 10 pounds.

ii. Kit size in case (cube): 11" x 7" x 10".

iii. Size of fixed-wavelength absorption photometer: 3 ½" x 5 ¾" x 2".

iv. Transportable in a hard-shell case.

7. Power Requirements.

a. The field device should be operational without need for line voltage, a balance, or a centrifuge.

b. The minimum performance benchmark is the Model 400 Test-mate™ ChE kit, which operates using a 9-volt battery.

8. Speed, Sample Size, and Throughput.

a. The device should have the capacity to analyze a large number of samples in the field in reasonably quick time (Magnotti et al., 1988).

b. The minimum performance benchmark is the Model 400 Test-mate™ ChE kit:

i. The sampling method should consist of a finger prick and sample size approximately of 10 microliters (µL) of blood.

ii. The entire test for one sample should be performed in less than 4 minutes.

9. Operator Training and Device Complexity/Reliability.

a. Ideally, a device for field ChE testing should be automated, requiring little operator training and allowing minimal opportunity for human error/variability in the results. This ideal is difficult to achieve and is complicated by the Clinical Laboratory Improvement Amendments (CLIA) passed by Congress in 1988.

b. CLIA regulates all laboratory testing, including POC testing and the Test-mate™ ChE kit. Consequently, all POC testing sites are subject to CLIA regulations, which may include compliance with a number of quality control practices, depending upon the complexity of the tests performed. Ideally, a field device should be CLIA-waived, but in any case it should not exceed "moderate" complexity. Specific military medical guidelines should be followed regarding compliance with CLIA.

c. The device should also be capable of providing the data behind "black box" results – i.e., the device should provide raw instrument signal data as well as providing test results that have been normalized or corrected for temperature and other variables such as hemoglobin.

d. As mentioned above, a reference or ghost standard should be available and used with the device.

4.3 Conclusions on the Status of Handheld or Portable Devices for Measuring Blood Cholinesterase

Based on the literature review and interviews conducted for this report, only one "device" has been validated for cholinesterase (ChE) testing in the field: the Model 400 Test-mate™ ChE kit by EQM Research, Inc. (Cincinnati, OH). This test kit has been developed and improved over the course of twenty years and is the only field kit or device that has been reviewed by the U.S. Food and Drug Administration (FDA) and judged "substantially equivalent" to commercially-available ChE test systems used by clinical laboratories. Despite its development and application history, the Model 400 Test-mate™ ChE kit is not an ideal system for field use, and several issues discussed in chapter two of this report remain unresolved.

In recent years, a few companies with the support of federal funding have attempted to develop handheld or portable devices for ChE testing. However, the challenges of engineering a microfluidic "chip" that incorporates ChE test chemistry is daunting and must pass several hurdles before "substantial equivalence" is granted by the FDA. New handheld or portable devices must be subjected to rigorous paired testing and compared with the Ellman test method performed by a credible laboratory. Furthermore, before new devices can be used in the field, they must perform satisfactorily and consistently under a diverse set of environmental conditions. To date, no handheld or portable device has reached sufficient maturity to meet all the desired performance characteristics – including the Model 400 Test-mate™ ChE kit.

References

Amaya A, Keifer M, McConnell R. 1996. Comment on EQM testmate OP cholinesterase kit. Occup Environ Med 53(5):358.

AR 50-6, Chemical Surety, June 26, 2001.

Biagini RE, Sammons DL, Smith JP, MacKenzie BA, Striley CA, Snawter JE, Robertson SA, Quinn CP. 2006. Rapid, sensitive, and specific lateral-flow immunochromatographic device to measure anti-anthrax protective antigen immunoglobulin G in serum and whole blood. Clin Vaccine Immuno 13(5): 541-546.

Calderon-Margalit R, Adler B, Abramson JH, Gofin J, Kark JD. 2006. Butrylcholinesterase activity, cardiovascular risk factors, and mortality in middle-aged and elderly men and women in Jerusalem. Clin Chem 52 (5): 845-852.

California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. 2002. Guidelines for physicians who supervise workers exposed to cholinesterase-inhibiting pesticides, 4th edition. http://www.oehha.ca.gov/

Costa LG. 2006. Current issues in organophosphate toxicology. Clin Chim Acta 366: 1-13.

Ciesielski S, Loomis DP, Mims SR, Auer A. 1994. Pesticide exposures, cholinesterase depression, and symptoms among North Carolina migrant farmworkers. Am J Public Health 84(3):446-51.

Clinical Laboratory Improvement Amendments of 1988, Public Law 100-578, 100th Congress (October 31, 1988): U.S. Code 42 (201).

Carmona-Fonesca J. 2007. Cholinesterases in total blood measure with a semiquantitative technique, and plasma or erythrocyte cholinesterases measured with quantitative techniques [Article in Spanish]. Biomedica 27(2):244-56.

Darvesh S, Hopkins DA, Geula C. 2003. Neurobiology of butyrylcholinesterase. Nature Reviews: Neuroscience 4: 131-138.

Da Silva ES, Midio AF, Garcia EG. 1994. A field method for the determination of whole blood cholinesterase. Med Lav May-Jun; 85(3): 294-254.

DA PAM 40-8, Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Nerve Agent GA, GB, GD, and VX. http://www.apd.army.mil/

DoD 6055.05-M, Occupational Medical Examinations and Surveillance Manual, May 2, 2007.

Edson EF. 1950. Blood tests for users of OP insecticides. World Crops 10:49-51.

Ellman GL, Courtney KD, Andres Jr,V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95.

EQM Research, Inc. Instruction Manual for the Test-mate™ ChE Cholinesterase Test System (Model 400). Manual-E (27-APR-03). Cincinnati, Ohio. http://www.eqmresearch.com.

Furlong CE, Li WF, Richter RJ, Shih DM, Lusis AJ, Alleva E, Costa LG. 2000. Genetic and temporal determinants of pesticide sensitivity: role of paraoxonase (PON1). Neurotoxicology 21(1-2):91-100.

Giles K. Cholinesterases. Introduction from D.Phil Thesis. http://www.weizmann.ac.il/sb/faculty_pages/Sussman/projects/che.html (accessed on August 8, 2007).

Glynn P. 2006. A mechanism for organophosphate induced delayed neuropathy. Toxicology Letters 164; Supplement 1, 20 September 2006: Page S9.

Gordon RK, Haigh JR, Garcia GE, Feaster SR, Doctor BP, Riel MA, Lefkowitz LJ, Lenz DE, Aisen PS, Smart W. 2004. Whole Blood Robotic Cholinesterase Assay for Organophosphate Exposure – Testing Soldiers, First Responders, and Civilians in the Field and Laboratory. Conference paper, 29 Nov-2 Dec 2004. DTIC ADA432861.

Grisaru D, Sternfeld M, Amiram E, Glick D, Soreq H. 1999. Structural roles of acetylcholinesterase variants in biology and pathology. Eur J Biochem 264, 672-686.

Haigh JR, Gordon RK, Chen G, Ficco1 MD, Herron PC, Lefkowitz LJ, Doctor BP, Capacio BR, Clark CR, Ambrosio AD, Gul N. 2006. Comparative AChE and BChE Methods: The Walter Reed Whole Blood Cholinesterase Assay, Test-mate™ OP, Michel (ΔpH), and MicroEllman Method. Poster Session. Society of Toxicology 45th Annual Meeting, March 5-9, 2006.

Higgins GM, Muniz JF, McCauley LA. 2001. Monitoring acetylcholinesterase levels in migrant agricultural workers and their children using a portable test kit. J Agric Saf Health 7(1):35-49.

Jaga K, Dharmani C. 2003. Sources of exposure to and public health implications of organophosphate pesticides. Rev Panam Salud Publica/Pan Am J Public Health 14(3):171-185.

Jeyaratnam J. 1990. Acute pesticide poisoning: A major global health problem. World Health Stat Q 43(3):133-144.

Johnson CD, Russell RI. 1975. Rapid simple radiometric assay for cholinesterase, suitable for multiple determinations, Anal Biochem 64:229–238.

Karr CJ, Keifer MC, Miller ME. 1999. Field-based monitoring of agricultural workers for overexposure to cholinesterase inhibiting pesticides: Evaluation of a trial program. Journal of Agromedicine 5(4):35-47.

Keifer M, Rivas F, Moon JD, Checkoway H. 1996. Symptoms and cholinesterase activity among rural residents living near cotton fields in Nicaragua. Occup Environ Med 53(11):726-9.

Kost GJ. 2006. Newdemics, public health, small-world networks, and point-of-care testing. Point of Care 5(4):138-44.

Kwong TC. 2002. Organophosphate pesticides: biochemistry and clinical toxicology. Therapeutic Drug Monitoring 24:144-149.

Lefkowitz LJ, Kupina JM, Hirth NL, Henry RM, Noland GY, Barbee JY, Zhou JY, Weese CB. 2007. Intraindividual stability of human erythrocyte cholinesterase activity. Clin Chem 53(7):1358-1363.

Lessenger JE, Reese BE. 1999. Rational use of cholinesterase activity testing in pesticide poisoning. J Am Board Fam Pract 12(4):307-314.

London L, Thompson ML, Sacks S, Fuller B, Bachmann OM, Myers JE. 1995. Repeatability and validity of a field kit for estimation of cholinesterase in whole blood. Occup Environ Med 52(1):57-64.

Lotti M. 2001. Clinical Toxicology of Anticholinesterase Agents in Humans. In: Krieger RI, editor, Handbook of Pesticide Toxicology. San Diego; Academic Press; 2001. p. 1043-1085.

Magnotti RA, Eberly JP, Quarm DEA, McConnell RS. 1987. Measurement of acetylcholinesterase in erythrocytes in the field. Clin Chem 33(10):1731-5.

Magnotti, Jr, RA, Dowling K, Eberly JP, McConnell RS. 1988. Field measurement of plasma and erythrocyte cholinesterases. Clin Chim Acta 176(3):315-32.

Mason HJ, Lewis PJ. 1989. Intra-individual variation in plasma and erythrocyte cholinesterase activities and monitoring of uptake of organo-phosphate pesticides. J Soc Occup Med 39:121-124.

McConnell R, Cedillo L, Keifer M, Palomo MR. 1992. Monitoring organophosphate insecticide-exposed workers for cholinesterase depression. New technology for office or field use. J Occup Med 34(1):34-7.

McConnell R, Magnotti R. 1994. Screening for insecticide overexposure under field conditions: A reevaluation of the tintometric cholinesterase kit. Am J Public Health 84(3):479-81.

McConnell R, Pacheco F, Wahlberg K, Klein W, Malespin O, Magnotti R, Akerblom M, Murray D. 1999. Subclinical health effects of environmental pesticide contamination in a developing country: Cholinesterase depression in children. Environ Res 81(2):87-91.

Melanson SEF. 2005. Implementing drug-of-abuse testing at the point-of-care: Device characteristics and decision criteria with selected emphasis on the biosite triage system. Point of Care 4(3):123-6.

Melanson SEF. 2006. What's new in point-of-care testing in 2005? Point of Care 5(2):74-6.

Melanson SEF. 2007. What Is New in Point-of-Care Testing? Point of Care 6(2):144-6.

Memorandum, OTSG, DASG-PPM-NC, 10 Sep 04. Occupational Cholinesterase Baseline Establishment for Personnel at High Risk for Chemical Warfare (CW) Nerve Agent Exposure Outside of Storage, Demilitarization, and Research Settings.

Memorandum OTSG, DASG-PPM-NC, 10 Sep 04. Medical Evaluation, Follow-Up, and Recording of Chemical Warfare (CW) Nerve Agent Casualties Outside of Storage, Demilitarization, and Research Settings.

Michel HO. 1949. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. J Lab Clin Med 34:1564–1568.

Milby T. 1971. Prevention and Management of organophosphate poisoning. JAMA 216:2131-33.

Miller S, Shah MA. 1982. Cholinesterase activities of workers exposed to organophosphorus insecticides in Pakistan and Haiti and an evaluation of the tintometric method. J Environ Sci Health B 17(2):125-42.

National Institute for Occupational Safety and Health (NIOSH). 1976. Criteria for a Recommended Standard: Occupational Exposure to Parathion. 76-190. Centers for Disease Control. Public Health Service. U.S. Department of Health, Education, and Welfare. http://www.cdc.gov/niosh/76-190.html.

Navy Environmental Health Center (NEHC). Medical Surveillance Procedures and Medical Matrix (Edition 9). NEHC-TM OM 6260, August 2007.

Oliveira GH, Henderson JD, Wilson BW. 2002. Cholinesterase measurements with an automated kit. Am J Ind Med Suppl 2:49-53.

Price PP. 2001. Regular Review: Point of care testing. BMJ 322:1285-8.

Quest Diagnostics. 2007. Test Menu: Cholinesterase RBC & Plasma. Test Code 7740N. http://cas2.questdiagnostics.com/scripts/dos.wls?wlapp=DOS (accessed September 7, 2007).

Schwarz M, Loewenstein-Lichtenstein Y, Glick D, Liao J, Norgaard-Pedersen B, Soreq H. 1995. Successive organophosphate inhibition and oxime reactivation reveals distinct responses of recombinant human cholinesterase variants. Brain Res Mol Brain Res 31: 101-110.

Schwenke C. 2007. Diagnostic Studies. In: Wiley Encyclopedia of Clinical Trials, Chow S-C, Liu J-P, Editors-in-Chief, John Wiley & Sons, New York, New York, in press. Available from http://mrw.interscience.wiley.com/emrw/9780471462422/wect/article/eoct019/current/html

Science Applications International Corporation. 1998. Description and Chronology of Acquisition of the Test-mate™ ChE Test Kit. Report compiled for contract DAMD17-93-C-3141, October 1998. DTIC ADA362285. Distribution limited to U.S. Government and Contractors.

Scientia Advisors, LLC. 2007. Strategic Review of Point-of-Care Diagnostics. Boston, MA.

Section 862.3240, Part 862, Title 21, Code of Federal Regulations, http://www.gpaccess.gov/cfr/index.html.

Senanayake N, Keralliedde L. 1987. Neurotoxic effects of organophosphate insecticides. An intermediate syndrome. N Engl J Med 316: 761-763.

Silver A. 1974. The Biology of Cholinesterases. North-Holland publishing Co. Ltd., Amsterdam.

Simcox NJ, Camp J, Kalman D, Stebbins A, Bellamy G, Lee IC, Fenske R. 1999. Farmworker exposure to organophosphorus pesticide residues during apple thinning in central Washington state. Am Ind Hyg Assoc J 60(6):752-61.

Stedman E, Stedman E, Easson LH 1932. Choline-esterase. An enzyme present in the blood-serum of the horse. Biochem J 26: 2056-2066.

Sun J, Lynn BC. 2007. Development of a MALDI-TOF-MS method to identify and quantify butyrylcholinesterase inhibition resulting from exposure to organophosphate and carbamate pesticides. J Am Soc Mass Spectrom 18: 698-706.

Taylor PW, Lukey BJ, Clark CR, Lee RB, Roussel RR. 2003. Field verification of Test-mate™ ChE. Military Medicine 168(4): 314-319.

TB MED 296, Assay Techniques for Detection of Exposure to Sulfur Mustard, Cholinesterase Inhibitors, Sarin, Soman, GF, and Cyanide, http://usamricd.apgea.army.mil/TBMED296.aspx (accessed on August 8, 2007).

TB MED 590, 30 November 2001. Occupational and Environmental Health: Red Blood Cell-Cholinesterase Testing and Quality Assurance. http://chppm-www.apgea.army.mil/tbm.htm.

Tinoco-Ojanguren R, Halperin DC. 1998. Poverty, production, and health: Inhibition of erythrocyte cholinesterase via occupational exposure to organophosphate insecticides in Chipas, Mexico. Archives of Environmental Health 53(1):29-35.

Tran NK, Kost GJ. 2006. Worldwide point-of-care testing: Compendiums of POCT for mobile, emergency, critical, and primary care and of infectious diseases tests. Point of Care 5(2): 84-92.

U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Cholinesterase Reference Laboratory. 2005. Standing Operating Procedure for the Seventeen-Minute Red Blood Cell Acetylcholinesterase Procedure. SOP NO: CRL40-2.9.

U.S. Environmental Protection Agency (USEPA), Office of Pesticide Programs. 2000. The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides. Washington, DC.

U.S. Food and Drug Administration (FDA). 2007. Decision Summary and Labeling Letter to Binax, Inc. for the Binax Now® Malaria Test. Dated June 13, 2007. http://www.fda.gov/cdrh/pdf6/k061542.pdf.

Vandekar M. 1980. Minimizing occupational exposure to pesticides: cholinesterase determination and organophosphorus poisoning. Residue Rev 75:67-80.

Weese CB. 2005. Physiologic Basis Used for the Determination of Significant Red Blood Cell Cholinesterase Depression – and the Source of the 70% Inhibition of Baseline as the Level of Significance. Unpublished review compiled in 2005.

Wessels D, Barr DB, Mendola P. 2003. Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health. Environmental Health Perspectives. 111(16):1939-1946.

Wilson B. 2005. Monitoring cholinesterases to detect pesticide exposure. Chemico-Biological Interactions. 157-158; 253-256.

Wilson B. 2006. Improving blood monitoring of enzymes as biomarkers of risk from anticholinergic pesticides and chemical warfare agents. Annual Report, Contract DAMD17-01-1-0772, 1 October 2006, DTIC.

Wilson BW, Arrieta DE, Henderson JD. 2005. Monitoring cholinesterases to detect pesticide exposure. Chemico-Biological Interactions 157-158: 253-256.

Worek F, Koller M, Thiermann H, Szinicz L. 2005. Diagnostic aspects of organophosphate poisoning. Toxicology 214: 182-189.

World Health Organization (WHO). 1967. Safe Use of Pesticides in Public Health. (WHO Tech. Rep. Ser. No 356). Geneva, Switzerland, 1967.2-2

Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH. 2006. Microfluidic diagnostic technologies for global public health. Nature 442: 412-8.

Abbreviations and Acronyms

µL; microliter

µmol; micromole

ACh; Acetylcholine

AChE; Acetylcholinesterase

AChE-S; Acetylcholinesterase – synaptic isoform

AChE-S; Acetylcholinesterase – erythrocytic isoform

AFRL; Air Force Research Laboratory

AR; Army Regulation

BChE; Butyrylcholinesterase

BWA; Biological Warfare Agent

C; Centigrade

Cal/EPA; California Environmental Protection Agency

ChE; Cholinesterase

CLIA; Clinical Laboratory Improvement Amendments

Cm; Centimeter

CNS/PNS; Central and Peripheral Nervous Systems

CONOPS; Concept of Operations

CONUS; Continental United States

CRL; Cholinesterase Reference Laboratory

CV; Coefficient of Variation

CWA; Chemical Warfare Agent

DoD; Department of Defense

DTRA; Defense Threat Reduction Agency

ECU; East Carolina University

FDA; U.S. Food and Drug Administration

GC-MS; Gas Chromatography-Mass Spectrometry

Hgb; Hemoglobin

hr; hour

LC-MS/MS; Liquid chromatography coupled to tandem mass spectrometry

MALDI-TOF-MS; Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

min; minute

mL; milliliter

NIOSH; National Institute for Occupational Safety and Health

OCONUS; Outside Continental United States

OP; Organophosphate

OPIDP; Organophosphate-induced delayed polyneuropathy

OTSG; Office of the Surgeon General

POC; Point of Care

PST; Patient Self Testing

R&D; Research & Development

r^2; correlation coefficient squared, or coefficient of determination

RAD; Research Area Directorate

RBC; Red Blood Cell

RDT&E; Research, Development, Testing & Evaluation

SBIR; Small Business Innovation Research

STTR; Small Business Technology Transfer

TATRC; Telemedicine and Advanced Technology Research Center

TB; Technical Bulletin

U; International Units of Activity

UC Davis; University of California at Davis

USACEHR; U.S. Army Center for Environmental Health Research

USACHPPM; U.S. Army Center for Health Promotion and Preventive Medicine

USAMMDA; U.S. Army Medical Materiel Development Activity

USAMRICD; U.S. Army Medical Research Institute of Chemical Defense

USAMRIID; U.S. Army Medical Research Institute of Infectious Diseases

USAMRMC; U.S. Army Medical Research and Materiel Command

USEPA; U.S. Environmental Protection Agency

WRAIR; Walter Reed Army Institute of Research