Field Verification of Test-Mate ChE

Guarantor: CPT Patterson W. Taylor, USA
Contributors: CPT Patterson W. Taylor, USA*; LTC Brian J. Lukey, USA*; Connie R. Clark, BS*; Robyn B. Lee, MS†; MAJ Robert R. Roussel, MS USA‡

The objective was to evaluate the ability of the Test-mate ChE to determine acetylcholinesterase (AChE) activity under field conditions. To mimic nerve agent exposure, the U.S. Army Medical Research Institute of Chemical Defense spiked blood samples with variable amounts of soman. Blinded to the identity of the samples, the 520th Theater Army Medical Laboratory tested the samples during a field training exercise inside their environmentally controlled mobile facility. The technicians repeated measurements for 6 consecutive days, and on 1 of the days repeated the measurements six times. The technicians accurately identified all of the samples and quantified the AChE activity. The major trend was that the Test-mate ChE is more precise and reproducible for smaller doses of soman. The results were reliable over all temperatures during the field exercise. In conclusion, the Test-mate ChE is a reliable field instrument to determine blood AChE activity.

Introduction

Organophosphate (OP) chemical warfare agents remain a threat to the U.S. warfighter.[1] These toxic compounds cause symptoms ranging from headaches and light-headedness to coma and death and affect not only the individual warfighter's health, but also the operation tempo of the unit. Consequently, commanders must determine quickly whether their warfighters have been exposed for both timely medical treatment of the affected and risk management for future operations. Exposure can be determined by measuring acetylcholinesterase (AChE) activity. Because OP agents such as sarin or soman (GD) inhibit AChE, exposed personnel will show decreased AChE activity. One way of measuring AChE activity is by using the Test-mate ChE kit.

The Test-mate ChE kit is a portable and easy-to-use medical device that measures blood enzyme erythrocyte cholinesterase and plasma cholinesterase. All supplies and equipment for performing 96 tests fit in a rugged, waterproof case with dimensions of 11 × 7 × 10 inches and weighs 10 lbs. A photograph of the device and supplies needed for 96 tests are shown in Figure 1. The system requires only 10 μL of blood, which may easily be obtained from a finger prick. The entire assay can be completed in less than 4 minutes. It is therefore a promising device to use in the field for detecting exposure to OP nerve agents.

EQM Research Inc. (Cincinnati, OH), the manufacturer of the Test-mate ChE, conducted studies that verified their devices were effective for determining pesticide exposure.[2] However, a recent study by Wilson et al. (G.H.Oliveira, J.D. Henderson, B.W. Wilson. Cholinesterase Measurements with an Automated Kit, submitted for publication) determined that different models of the Test-mate ChE did not produce reliable results at different temperatures. The Wilson study also found that the different models of the Test-mate ChE were unreliable at low temperatures (less than 15°C). Both of these studies were not designed to evaluate this kit for our intended military use. First, the manufacturer used a pesticide as their OP compound, whereas the U.S. Army is interested in chemical warfare agents (i.e., GD). Second, the Wilson study compared the results of different models. Some models were older and did not use the same algorithms that the newer models use. However, the 520th Theater Army Medical Laboratory (TAML) procured only the newer model. In addition, the Wilson study used the instrument under a variety of temperatures and found that the instrument was unreliable at lower temperatures. The TAML performs all measurements in a portable laboratory that has an environmental control unit. Third, the Wilson study used fetal bovine serum instead of human blood. Nevertheless, the Wilson study highlighted potential concerns such that we determined the need to test the instrument under the likely field conditions that our units would use. Therefore, we evaluated the Test-mate ChE with human blood spiked with an OP nerve agent and measured AChE activity under monitored real field conditions during a normal 520th TAML operational exercise.

Methods

Principal of the Method

The Test-mate ChE is based on the Ellman method.[3] Briefly, AChE hydrolyzes acetylthiocholine, producing a carboxylic acid and thiocholine, which reacts with the Ellman reagent (dithionitrobenzoic acid) to form a yellow color, which is measured spectrophotometrically at 450 nm. The rate of color formation is proportional to the amount of AChE present. AChE activity is measured with the addition of a specific inhibitor for plasma cholinesterase, 10-(α-diethylaminopropionyl)-phenothiazine.[4] A phosphate buffer system maintains a constant pH throughout the reaction. The device also measures the concentration of hemoglobin in the sample and automatically calculates the quotient (Q) value, which is a measurement of the AChE adjusted by an internal reagent blank, normalized to 25°C and to the hemoglobin (Hgb) concentration. Therefore, it is a measure of the AChE activity per unit of Hgb. By assuming the average person has 4.71 U AChE/mL and 15.0 g Hgb/dL, the Q value for a given person can also be roughly compared with a theoretical population mean value of 31.4 U/g and is expressed as Q%.

Samples

The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) provided eight samples of human blood.

*U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground-EA, MD 21010-5400.
†Robyn B. Lee & Associates, LLC, P.O. Box 267, Fawn Grove, PA 17321.
‡520th TAML, 5158 Blackhawk Road, Aberdeen Proving Ground-EA, MD 21010.

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Fig. 1. The Test-mate ChE and supplies needed for 96 tests.

Blood from an informed and consenting volunteer in 1.5-mL aliquots were placed into eight separate 2.0-mL tubes. These tubes were labeled 1 through 8 and systematically assigned levels of GD. Six of the tubes were spiked with GD and two tubes were spiked with normal saline. All tubes received 62.0-μL aliquots and were gently mixed for 6 hours. The tube number, concentration of GD in the aliquot, and the final concentration of GD in the blood tube are in Table I and referred to as H for high (120 nM of GD), M for medium (53 nM of GD), L for low (24 nM of GD), and C for control (0.0 nM of GD). The 520th TAML technicians were blind to the level of agent in the samples. The samples were stored in a refrigerator at a temperature between 1°C and 5°C for a total time of approximately 2 weeks. During transportation, the samples were stored in a cooler with cold packs for a total time of approximately 30 hours.

Equipment

Three different Test-mate ChE kits were used in the study. Two of the kits (kits 1 and 2) were used in the field inside the 520th TAML's mobile facility. The third Test-mate ChE (kit 3) was used at the USAMRICD to measure the AChE activity under laboratory-controlled conditions. The 520th TAML's laboratory is a collapsible building that can be deployed via aircraft or ship. The facility was equipped with an environmental control unit. Temperature inside the facility was adjusted for the comfort of personnel. No special effort was made to keep the temperature inside the laboratory within a preset range, and all measurements were made under real field conditions within normal operations of the 520th TAML. Temperature and humidity were measured using a Fisher brand Thermo-Humidity meter. All tests were conducted using the company's lyophilized prepared mixtures.

TABLE I

CONCENTRATIONS OF GD IN ALIQUOTS AND BLOOD SAMPLES

Tube	Concentration of GD of 62.0-μL Aliquot in Normal Saline (μM)	Final Concentration of GD in Blood Sample (nM)
1	0.62	24
2	1.39	53
3	3.12	120
4	0.0	0.0
5	0.62	24
6	1.39	53
7	3.12	120
8	0.0	0.0

Experimental Design

Each vial of blood was gently rotated for several seconds before each measurement. Each measurement was done according to the manufacturer's instructions. In brief, vials obtained from the manufacturer were blanked in the device. A 10-μL portion of blood was added to the manufacturer's vial, vigorously shaken for 15 seconds, and then placed back in the device. Three drops of water were added to the reagent to prepare the reagent solution, which was added to the vial and gently shaken for 5 seconds. The vial was then placed back into the device. After the device had incubated and read the sample, the results were recorded.

The temperature and humidity were monitored inside and outside the facility and were recorded before each run. Although it might be assumed that humidity will not change the measurements of the Test-mate ChE kit, this measurement allowed conclusions to be drawn about the effectiveness of the environmental control unit. Each run consisted of measuring the blood AChE activity for each of the eight samples with both Test-mate ChE kits 1 and 2. During the field exercise, kits 1 and 2 were used for runs on six consecutive days (days 1–6). On day 2, a total of six runs were done on the eight samples. All measurements in the field were taken during an actual exercise. Other activities of 520th TAML were not curtailed, and operators in the field participated in the other exercise activities. At the end of the field exercise the blood samples were returned to the USAMRICD in a cooler with cold packs and refrigerated at 1°C to 5°C until analysis. The blood AChE activity was measured for each sample using kit 3. A total of six AChE measurements were done for each sample with kit 3, and all measurements were performed on the same day.

Operators

Three operators performed the measurements. The same operator performed all measurements with the same kit. The operators in the field were personnel either assigned or attached to the 520th TAML and would have been the actual personnel deploying if the measurements had been part of a real-world deployment. The operator who used kit 2 (O2) had the least experience. Although inexperienced in using the Test-mate ChE kit, operator 1 (O1) had over 10 more years of laboratory experience than the operator of kit 2. The operator who used kit 3 (O3) at the USAMRICD was one of the most experienced, has had years of experience using various methods to measure AChE activity, and is recognized as an expert in this field.

Data Analysis

As with any analytical instrument, we are concerned about the precision and accuracy of the Test-mate ChE. However, without a standard measured amount in the blood, it is difficult to determine accuracy. Therefore, only precision was evaluated. Precision takes on two forms, namely repeatability and reproducibility. Repeatability is the precision of the measurements within a single run or day. Reproducibility is the precision of the measurements across runs or days.

Means, SD, and coefficients of variation (CV) were calculated for all parameters measured. AChE and Q parameters are presented here. Precision was measured by CV (CV = 100 × SD/mean) and was compared using a z test.[5] In addition, a repeated measures analysis of variance was used to compare AChE and Q between the kits for both repeatability and reproducibility. Statistically significant differences in replicates within a day or across days were considered a test of precision. The analysis of variance also compared the kits to determine whether they produced the same value for each parameter. Statistical significance was defined as $p < 0.05$.

Results

Temperature and Humidity

The temperature inside the facility during the exercise varied from a low of 19.8°C to a high of 26.8°C. The actual temperature extremes during measurements of AChE using the Test-mate ChE were 21.3°C and 26.8°C. The humidity inside the building varied from a low of 31% to a high of 58% relative humidity. The outside temperature during the exercise varied from a low of 10.5°C to a high of 23.3°C, and the outside humidity varied from a low of 38% relative humidity to a high of 98% relative humidity (Fig. 2).

AChE and AChE%

The mean, SD, and CV of measurements taken all in the same day for all three kits are reported in Table II. Significant differences in mean AChE and AChE% were observed among the three kits ($p < 0.01$). In addition, significant differences between replicates in mean AChE and AChE% were observed ($p < 0.01$). However, comparison of each kit's CV by dose resulted in no significant differences between kits.

The mean, SD, and CV for measurements taken on consecutive days for kits 1 and 2 are reported in Table III. Significant differences in mean AChE and AChE% were observed between kits 1 and kit 2 ($p < 0.01$). However, no significant day differences in mean AChE and AChE% were observed, implying that the kits had reproducible results. The major trend in the measurements is that the Test-mate ChE is more precise and reproducible for smaller doses of OP. This trend is consistent for all three kits.

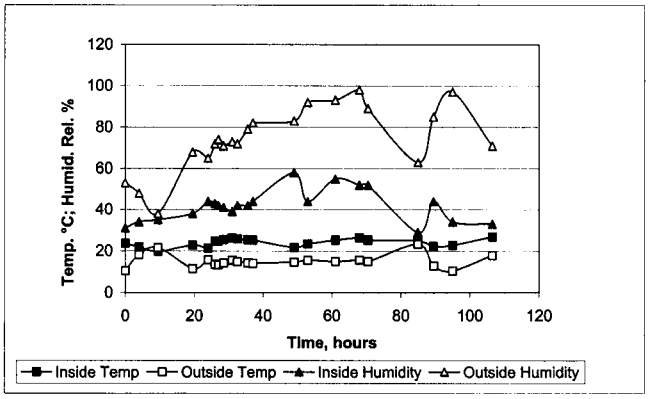

Fig. 2. Temperature and humidity vs. time.

Q and Q%

The mean, SD, and CV of measurements taken all in the same day for all three kits are reported in Table IV. Significant differences in mean Q and Q% were observed between the three kits ($p < 0.01$). In addition, no significant differences between replicates in mean Q and Q% were observed, and comparison of each kit's CV by dose resulted in no significant differences between kits.

The mean, SD, and CV for measurements taken on consecutive days for kits 1 and 2 are reported in Table V. Borderline significant differences in mean Q and Q% were observed between kits 1 and kit 2 ($p = 0.06$ and $p = 0.05$, respectively). In addition, no significant day differences in mean Q and Q% were observed, implying that the kits had reproducible results. The AChE% vs. dose for all measurements for all three kits are presented as box-and-whisker-plot format in Figure 3 and the Q% vs. dose for all measurements for all three kits are presented in the same format in Figure 4.

There are three trends in the measurements. The Test-mate ChE is more precise for smaller doses of OP and the Q and Q% are more precise than the AChE and AChE%. Both of these trends are consistent for all three kits. The third trend is that the more experienced operator tended to have the most precise results. The most experienced operator, O3, took a total of 48 measurements and did not have a single measurement of AChE% or Q% that was outside the 5th to 95th percentiles. The second most experienced operator, O1, took a total of 88 measurements of AChE% and had only two measurements outside the 5th to 95th percentiles (Fig. 3). O1 did not have a single measurement of Q% outside the 5th to 95th percentiles (Fig. 4). The least experienced operator, O2, took 88 measurements of AChE% and had three measurements outside the 5th to 95th percentiles (Fig. 3). Of the 88 measurements of Q%, O2 had three measurements outside the 5th to 95th percentiles and one measurement that was more than 3 times the length of the 25th to 75th percentiles box away from the mean (Fig. 4). This trend can also be seen in the SD and CV (Tables II-V). O3 had the best SD and CV values for every dose and O1 generally had better SD and CV values than O2.

Discussion

Because Wilson et al. (G.H. Oliveira, J.D. Henderson, B.W. Wilson. Cholinesterase Measurements with an Automated Kit, submitted for publication) found that different Test-mate ChE models were not reliable at 15°C, it is important to stay above this temperature. The temperature inside the facility was consistently maintained well above 15°C. Even when the temperature outside was 13.4°C, the temperature inside was 24.8°C. Because this study included one run taken at 20.3°C, a good lower limit for the temperature inside the facility is 20°C. This should be a lower limit temperature that can be easily maintained and will also allow reliable measurements with the Test-mate ChE kit. The difference between the inside and outside humidity is also a good indication that the environmental control of the facility is adequate.

The first trend that the Test-mate ChE is more precise for lower doses of OP was expected. For any device that approaches its limit of sensitivity, the relative variability increases as the values become smaller. Since the instrument measures the ac-

TABLE II

ACHE AND ACHE% REPEATABILITY: MULTIPLE RUNS WITHIN DAY (*N* = 12)

Dose	Kit 1 (Mean ± SD, CV)	Kit 2 (Mean ± SD, CV)	Kit 3 (Mean ± SD, CV)
AChE			
C	3.09 ± 0.08, 2.7%	2.62 ± 0.08, 3.0%	2.94 ± 0.06, 2.1%
L	1.85 ± 0.13, 7.1%	1.53 ± 0.13, 8.8%	1.77 ± 0.11, 6.1%
M	0.48 ± 0.05, 9.9%	0.31 ± 0.03, 11.2%	0.46 ± 0.04, 7.6%
H	0.00 ± 0.00, —	0.00 ± 0.00, —	0.00 ± 0.00, —
AChE %			
C	65.67 ± 1.87, 2.8%	55.83 ± 1.64, 2.9%	62.33 ± 1.30, 2.1%
L	39.25 ± 2.80, 7.1%	32.25 ± 2.93, 9.1%	37.67 ± 2.42, 6.4%
M	10.17 ± 1.19, 11.7%	6.42 ± 0.67, 10.4%	9.58 ± 0.79, 8.2%
H	0.00 ± 0.00, —	0.00 ± 0.00, —	0.00 ± 0.00, —

TABLE III

ACHE AND ACHE% REPRODUCIBILITY: MULTIPLE RUNS ACROSS DAYS (*N* = 5)

Dose	Kit 1 (Mean ± SD, CV)	Kit 2 (Mean ± SD, CV)
AChE		
C	3.07 ± 0.07, 2.2%	2.59 ± 0.07, 2.8%
L	1.83 ± 0.11, 6.2%	1.54 ± 0.18, 11.5%
M	0.47 ± 0.04, 8.3%	0.30 ± 0.05, 16.6%
H	0.00 ± 0.00, —	0.00 ± 0.00, —
AChE %		
C	65.08 ± 1.56, 2.4%	55.00 ± 1.54, 2.8%
L	38.92 ± 2.27, 5.8%	32.25 ± 2.93, 9.1%
M	10.17 ± 0.94, 9.2%	6.42 ± 0.67, 10.4%
H	0.00 ± 0.00, —	0.00 ± 0.00, —

tivity of the AChE, the control doses yield the most precise results because they had more AChE that was not inhibited. The low doses should yield more precise results than the medium doses. Nevertheless, the absolute values at the medium GD dose are still fairly close to each other, indicating good reproducibility in this relatively low AChE activity range. The high GD dose samples showed no AChE activity, and no statistics were determined. However, every operator properly identified all the high GD samples as the highest inhibition levels with no activity.

It was also expected that the Q and Q% values should be more precise than the AChE and AChE%. Q values are less influenced by errors. For a given patient, a deviation in the desired sample volume (10 μL of blood) would be corrected by the same deviation in Hgb added to the vial. Also, assuming the operator is fairly efficient in obtaining repeatable sample volumes, patients who have erythrocyte levels lowered by fluid replacement would have their erythrocyte AChE levels correctly adjusted by Hgb.

This study reveals the case in point with the normal control AChE% value being approximately 55% to 65% with no chemical warfare agent added as compared with the expected value of 100%. We believe this single blood donor was extraordinarily low in AChE levels and most likely an outlier in the normal population. The Q% was a better indicator of inhibition than AChE%.

It is unclear what causes the third trend. It could be argued that the most experienced operator would produce the most precise results, but that operator also performed all measurements in the controlled laboratory facilities of the USAMRICD. Also, the two operators in the field, O1 and O2, had ancillary duties and worked shifts that were longer than 12 hours. Fatigue could easily explain higher variability in their results.

The precision required for an acceptable analytical method depends upon the criteria necessary to make an appropriate clinical decision. Generally a CV of 10% or less is desired for an analytical method in the range of interest. Since treatment of OP poisoning is based on symptoms and not level of inhibition, the Test-mate ChE is only needed to confirm exposure, and the range of interest is only the control samples. The control samples were distinguished from all other doses. Even though the Test-mate ChE is not needed to distinguish low dose from me-

TABLE IV

Q AND Q% REPEATABILITY: MULTIPLE RUNS WITHIN DAY (*N* = 12)

Dose	Kit 1 (Mean ± SD, CV)	Kit 2 (Mean ± SD, CV)	Kit 3 (Mean ± SD, CV)
Q			
C	30.07 ± 0.64, 2.1%	29.07 ± 0.60, 2.1%	28.85 ± 0.39, 1.4%
L	17.83 ± 1.38, 7.7%	16.93 ± 1.47, 8.7%	17.46 ± 1.02, 5.9%
M	4.62 ± 0.44, 9.6%	3.40 ± 0.38, 11.1%	4.37 ± 0.29, 6.7%
H	0.00 ± 0.00, —	0.00 ± 0.00, —	0.00 ± 0.00, —
Q%			
C	95.75 ± 2.09, 2.2%	92.50 ± 2.02, 2.9%	91.83 ± 1.34, 1.4%
L	56.92 ± 4.25, 7.5%	53.92 ± 4.64, 8.6%	55.58 ± 3.15, 5.7%
M	14.83 ± 1.34, 9.0%	10.83 ± 1.27, 11.7%	13.83 ± 1.03, 7.4%
H	0.00 ± 0.00, —	0.00 ± 0.00, —	0.00 ± 0.00, —

TABLE V

Q AND Q% REPRODUCIBILITY: MULTIPLE RUNS ACROSS DAYS
(N = 5)

Dose	Kit 1 (Mean ± SD, CV)	Kit 2 (Mean ± SD, CV)
Q		
C	29.41 ± 0.76, 2.2%	28.51 ± 0.54, 1.9%
L	17.43 ± 1.06, 6.0%	16.91 ± 2.31, 13.7%
M	4.53 ± 0.37, 8.2%	3.31 ± 0.49, 14.9%
H	0.00 ± 0.00, —	0.00 ± 0.00, —
Q%		
C	93.75 ± 2.22, 2.4%	90.67 ± 1.67, 1.8%
L	55.50 ± 3.18, 5.7%	53.92 ± 7.27, 13.5%
M	14.42 ± 1.24, 8.6%	10.42 ± 1.73, 16.6%
H	0.00 ± 0.00, —	0.00 ± 0.00, —

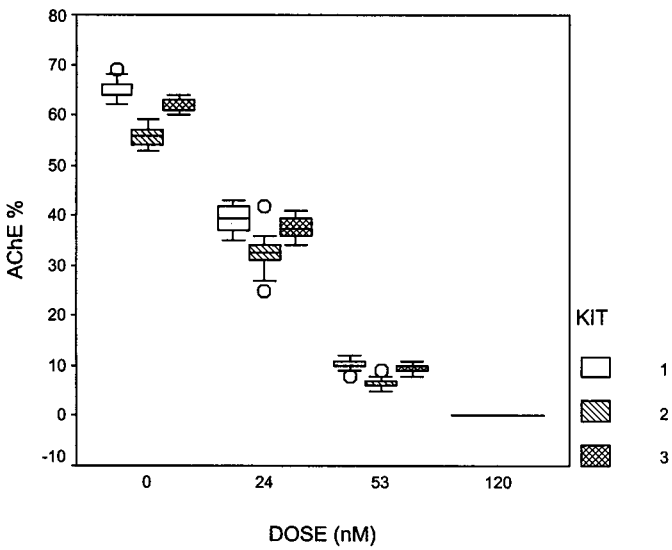

Fig. 3. AChe%. The interquartile range (25th–75th percentiles) is represented by boxes. Whiskers represent the 5th to 95th percentiles. Circles denote values outside of the 5th to 95th percentiles and these outliers were also more than 1.5 times the interquartile range away from the mean. O1 had a total of 2 outliers of 88 measurements. O2 had a total of 3 outliers of 88 measurements. O3 had no outliers of 48 measurements.

dium doses of OP poisoning, the results for these doses were distinguished, and the performance was acceptable. Because the CVs of all control samples were 3% or less, the precision of the Test-mate ChE is excellent, and the performance of the instrument is acceptable.

A plausible scenario for Q measurement would be a soldier who appears to be confused and disoriented. This warfighter might be a nerve agent casualty who has not developed all of the nerve agent poisoning symptoms, or the warfighter might be suffering from a heat injury or some other etiology. The Q measurement is needed to quickly distinguish the OP casualty from a heat injury or injury with similar symptoms with a different etiology. For pesticides, possible exposure is indicated if the AChE activity is depressed more than 50%.[6] If a baseline is available, possible exposure to pesticides is indicated if the AChE activity is depressed more than 70% of the baseline.[7]

If a commander suspects that a selected group of personnel, such as a chemical/biological recon team, will likely be exposed

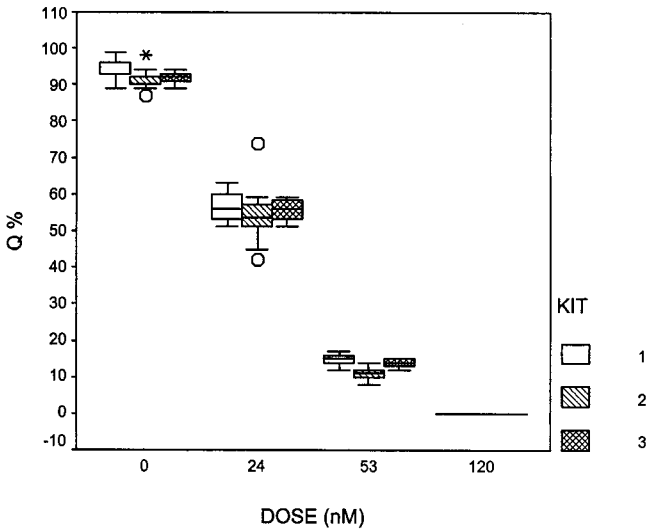

Fig. 4. Q%. The interquartile range (25th–75th percentiles) is represented by boxes. Whiskers represent the 5th to 95th percentiles. Circles denote values outside of the 5th to 95th percentiles and these outliers were also more than 1.5 times the interquartile range away from the mean. O1 had no outliers of 88 measurements. O2 had a total of 3 outliers of 88 measurements. O3 had no outliers of 48 measurements. Asterisk denotes extremes with a value more than 3 times the length of the 25th to 75th percentiles box away from the mean. O2 had one extreme. O1 and 3 had no extremes.

to a nerve agent, the best practice would be to obtain a pre-exposure baseline Q for those personnel. In this case, a decrease in the Q of 70% of any individual from their baseline Q would suggest exposure to OP. For most warfighters, a baseline Q will not be available, and a Q% value of 50% or less would indicate exposure. It is possible that a warfighter could be exposed to OP with Q% value over 50%. Warfighters exhibiting symptoms of mild OP poisoning with Q% greater than 50% should still be monitored closely, and medical personnel should have antidotes available in case the casualty develops more serious symptoms. A complete history of this patient should also be obtained to determine possible sources of OP exposure. Appropriate decontamination actions should be conducted for this patient and for personnel who have come into contact with the casualty.

Although this study has shown the Test-mate ChE to be successful with these doses, future studies should use smaller doses of nerve agent. Currently, it is not clear what is considered a low dose. Ongoing research is attempting to determine what constitutes a low dose of nerve agent as defined as the lowest dose that does not produce any outward sign or symptom.[8]

Conclusions

The Test-mate ChE is a reliable and useful device for the measurement of AChE activity in the field environment. The Q value was found to be the most useful indicator of AChE activity. If the temperature inside the laboratory is less than 20°C, the environmental control unit should be adjusted to bring the laboratory above 20°C. With this incorporated into the 520th TAML's standing operating procedure, the Test-mate ChE will provide the laboratory with a rapid and reliable method to help identify OP poisoning from nerve agents. If current research successfully determines the threshold dose of OP nerve agent poisoning that does not produce any signs or symptoms, these

concentrations should be used in future field measurements of AChE activity with the Test-mate ChE.

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