A publication of Beta Publishing Group

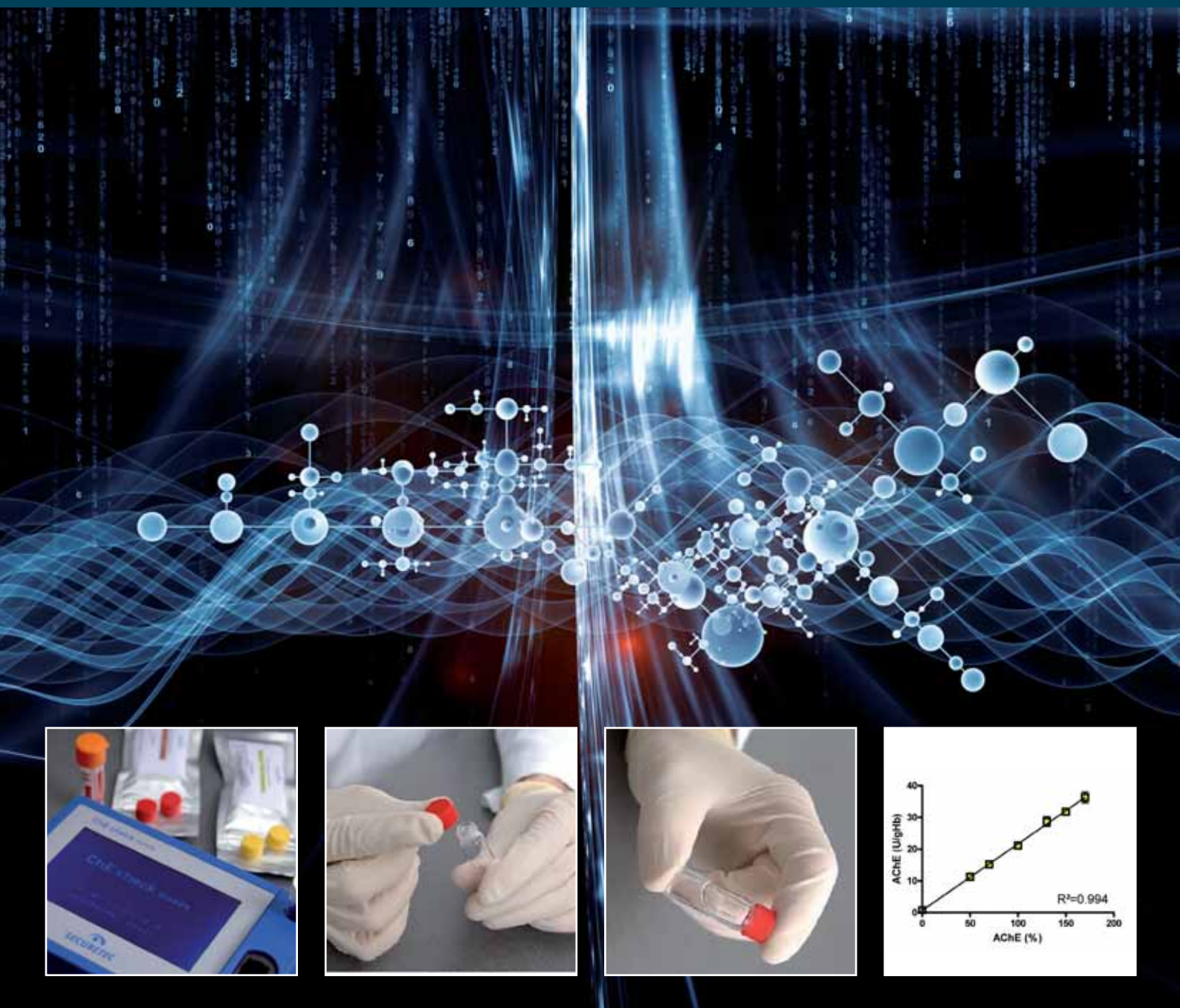
Special Print

CBRN Medical Defense

CHALLENGE

CBRN MEDICAL DEFENSE INTERNATIONAL

Cholinesterase Kit for Field Diagnosis of Organophosphate Exposure



F. WOREK, M. BAUMANN, B. PFEIFFER, N. AURBEK, F. BALSZUWEIT, H. THIERMANN (GERMANY)

Cholinesterase Kit for Field Diagnosis of Organophosphate Exposure

Objectives: Presently, the evaluation of clinical signs and symptoms is the only possibility for rapid diagnosis of poisoning by organophosphorus compounds (OP), i.e. nerve agents and pesticides. Up to now laboratory procedures, e. g. determination of erythrocyte acetylcholinesterase (AChE) activity in whole blood, are not available for on site diagnosis.
Methods: A Bundeswehr funded research project resulted in the development and CE certification of a mobile in vitro test system for the analysis of AChE and butyrylcholinesterase (BChE) activity in whole blood (ChE Rapid Test IVD).
Results: The handy test system enables the precise and reproducible determination of AChE and BChE activity within 4 minutes at ambient temperatures of 10 to 50°C. The simple test procedure requires few menu-driven operations and the interpretation of test results is supported by additional information and instruction thus enabling the test execution after short training.
Conclusions: The now available CE certified test system provides a laboratory tool for the rapid and precise determination of AChE and BChE activities in whole blood in medical installations and mobile forces and may broaden the diagnostic capabilities of the Bundeswehr medical service, especially during deployed field operations, in case of suspected exposure to OP.
Keywords: Organophosphorus compounds; nerve agents; acetylcholinesterase; diagnosis; test system

1. Introduction

Irreversible inhibition of the pivotal enzyme acetyl acetylcholinesterase (AChE) is the predominant toxic effect of organophosphorus compounds (OP). Many OP are still being used as pesticides whereas some have been abused as chemical warfare agents [1]. Inhibition of AChE by OP results in accumulation of acetylcholine (ACh) in the synaptic cleft of cholinergic innervated tissues, affecting many organ dysfunctions and ultimately resulting in death by central and peripheral respiratory failure. [2].

Currently, evaluation of the clinical symptoms is the only way to diagnose a manifest poisoning by OP on-site, which is the prerequisite for adequate therapy [3]. Confirmation by a clinical laboratory is needed, in particular when symptoms are weak and unspecific. There are complex analytical methods such as gas chromatography - mass spectroscopy (GC-MS) and liquid chromatography - mass spectroscopy (LC-MS) to detect OP in biological samples, requiring expensive analytical equipment along with considerable time, labour and expertise. However, there is also the option to determine erythrocyte AChE activity in whole blood for preliminary rapid confirmation of exposure to AChE inhibitors [4]. Extensive experimental and clinical data have confirmed the good correlation between erythrocyte and synaptic AChE. Thus, erythrocyte AChE activity is a reliable, valid surrogate parameter of synaptic AChE activity [5-7].

For clinical diagnosis of OP poisoning determination of butyrylcholinesterase (BChE) activity is routinely used, despite well-documented structural and functional differences between AChE and BChE [8]. A lack of commercially available ready-to-use kits has thus far precluded adequate determination of AChE activity. Only a few specialised laboratories, including the Bundeswehr Institute of Pharmacology and Toxicology (InstPharmToxBw) are capable to conduct this analysis.

Recently InstPharmToxBw had evaluated a mobile test system (Testmate ChE Cholinesterase Test System, EQM Research, Cincinatti, USA) with regard to its usability for AChE activity determination under field conditions. Following optimisation, the device was considered appropriate for use in the Bundeswehr Medical Service [9] and was tested in a clinical setting [10]. However, this device could not be introduced into the Bundeswehr as the manufacturer was not willing to conduct the CE certification required by the German Medical Devices Law and related European guidelines. Thus, the Bundeswehr commissioned the company Securetec Detektions-Systeme AG, Brunnthal, to develop a rapid test system for

Fig 1: Components of the ChE rapid test IVD (ChE check mobile); photo kindly provided by Securetec Detektions-Systeme AG

whole blood samples in close cooperation with InstPharmToxBw. Designed for rapid detection of exposure to AChE inhibitors, the device was considered an *in-vitro* diagnostic (IVD) and had to pass CE certification as a Class I Medical Device. From March 2009 to August 2010, a device fully compliant to the contract and the technical specification was developed, validated and certified. CE certification on August 30th, 2010 marked the successful completion of the project.

2. Methods

2.1 Cholinesterase Rapid Test IVD (ChE check mobile)

The Cholinesterase Rapid Test IVD was designed as an integrated system for the determination of AChE and BChE activity in whole blood. It was required to be usable under field conditions, easy to use and to provide reliable, accurate and reproducible data. The IVD consists of the following components, depicted in figure 1:

- Reagent kits for determination of AChE and BChE activity.

Each kit contains 100 sample vials (filled with 2 ml assay buffer and covered with a white cap, 100 reagent caps for AChE (labelled red) or BChE (labelled yellow) determination, 100 capillaries for blood sampling (10 µl, containing EDTA) and 100 pieces of adsorbent tissue.

- Analysis unit for photometric determination of haemoglobin and of AChE/BChE activity in diluted blood samples at 470 nm, temperature sensor, large touchpad for data entry, menu, instructions for use and display of results, power supply via (rechargeable) battery or mains adaptor, internal data storage, accessible via USB interface.

The following materials required for measurements were not included in the IVD: protective gloves, waste container, disinfectant, lancets and swabs.

2.2 Measurement

Determination of AChE or BChE activity is carried out using the following menu-driven steps, which are indicated on the screen (Fig 2):

- Positioning of the sample vial into the analysis unit, measurement of baseline absorption (blank value).
- Addition of 10 µl blood sample, using a capillary, shaking and measurement of haemoglobin content in the diluted sample.
- Addition of reagents by positioning the desired, colour-coded cap onto the vial, mixing.
- Determination of AChE or BChE activity by photometric measurement of their coloured product.
- Display of results, normalised to the haemoglobin content, in units per g haemoglobin, activity normalized to 37 °C.
- Display of error messages (if applicable), additional information and instructions in case of slightly or severely decreased AChE/BChE activity.

The entire analysis, from sampling to results display, takes approx. 4 minutes.

2.3 Specifications

Device operational at temperatures from 10 to 50 °C, data normalized to 37°C.

Range: AChE (BChE) activities from 3 - 200 % of average norm values, haemoglobin from 5–20 g/dl.

Precision: data correlate to those obtained with a reference method, R^2>0.9. Reference method is the photometric determination of AChE/BChE in whole blood samples, developed, and validated by InstPharmToxBw, using automated pipetting system in a fixed laboratory [9, 11].

Reproducibility: intra-day and inter-day variability below 5 %, inter-operator variability below 7 %.

3. Results and Discussion

Aim of this project, funded by the Bundeswehr, was the development and CE certification of a mobile *in-vitro* diagnostic (IVD) for rapid determination of AChE in whole blood, to be used by the Bundeswehr Medical Service, in particular under the conditions of a deployed field operation. This IVD was to provide the capability for rapid confirmation in case of an assumed clinical diagnosis of OP poisoning. Especially in case of weak or unspecific symptoms, this capability was

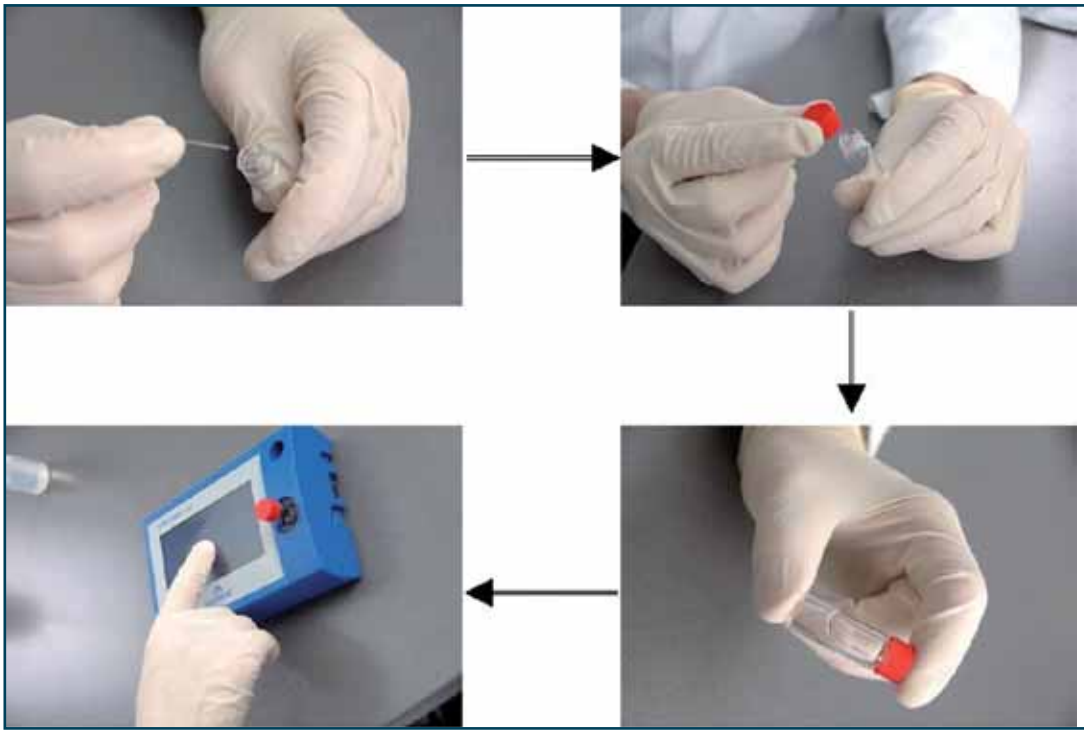


Fig 2: Steps for AChE determination using the ChE rapid test IVD. Addition of blood sample (upper left), positioning of the reagent cup (upper right), mixing of the solution (lower right) and initiation of measurement (lower left); photos kindly provided by Securetec Detektions-Systeme AG

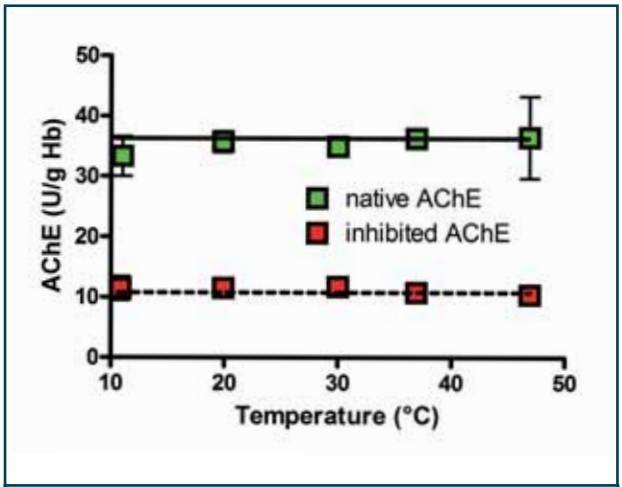


Fig 3: Determination of ruggedness of ChE rapid test IVD results against temperature effects,Measurement of native and inhibited AChE activities in whole blood samples (n=4, means ± SD)

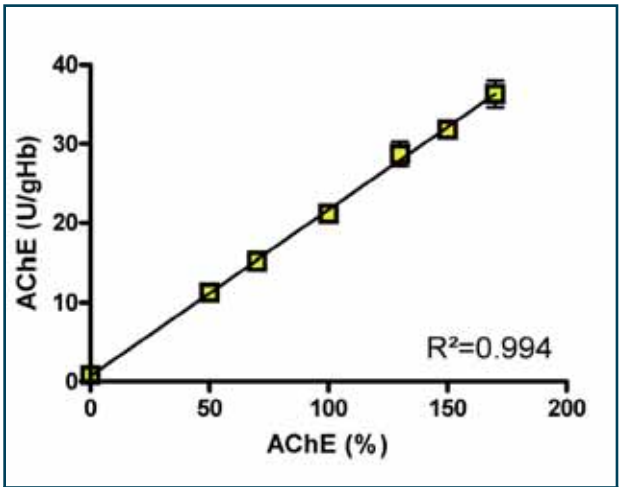


Fig 4: Linear correlation of AChE values determined by ChE rapid test IVD in blood samples with different (known) AChE activities (n = 4, Means ± SD).

meant to enable the medical officer in charge to establish a rapid and correct diagnosis and to provide timely and adequate antidote therapy.

An important prerequisite for the use of the cholinesterase rapid test IVD under field conditions, including a variety of climatic conditions, is the ability to function in a wide range of temperatures and to normalise values to 37 °C. Thus, a sensor determines the temperature next to the vial in the photometer. The AChE/BChE activity, determined under actual temperature conditions is normalized to its equivalent at 37°C. Figure 3 shows examples of AChE activities determined in native and OP-inhibited samples at different temperatures and the validity of the algorithm to compensate for temperature effects. Thus, it is possible to determine AChE and BChE activity at temperatures from 10 to 50 °C and to compensate temperature-related effects.

Parameter	Coefficient of determination (R^2)
AChE (n = 10)	0.93
BChE (n = 10)	0.98

Tab 1: Correlation of results of AChE and BChE activity determination in whole blood samples, using ChE rapid test IVD and reference method.

To use the ChE rapid test IVD for laboratory diagnosis of exposure to AChE inhibitors, the IVD needs to provide precise and accurate data on AChE and BChE activity over a wide range. Determination of AChE and BChE activity in whole blood samples with very different enzyme activities in fact confirmed a linear correlation in a range from minimal to above-average physiological values, as shown in Figure 4.

Another requirement for the evaluation of the ChE rapid test IVD was the ability to provide data similar to those of a reference method, in this case, a modified Ellmann method established at the InstPharmToxBw [4, 11, 12] and accredited under DIN EN ISO 15189. Determination of AChE and BChE activities with both methods demonstrated correlations (Tab 1) that, with regard to biological samples can be considered excellent. Investigations of additional criteria, including intra-day, inter-day and inter-operator variability also demonstrated compliance with specifications.

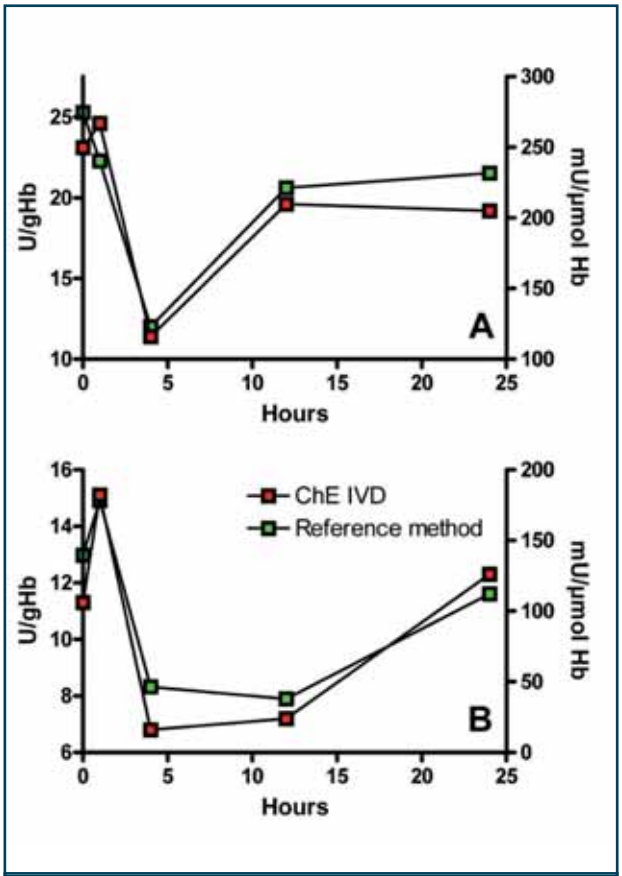


Fig 5: Determination of AChE activity in whole blood samples of patients with dimethoate (A) and chlorpyrifos poisoning (B) using the ChE rapid test IVD and the reference method. Dr Andrew Dawson, University of Peradeniya, Sri Lanka, kindly provided samples.

Subsequent investigations demonstrated a good correlation between the ChE rapid test IVD and the reference method in actual samples from OP pesticide-poisoned patients (Fig 5).

The small, lightweight ChE rapid test IVD is also easy and comfortably to use. Only a few working steps are required, the menu dialogue provides the specific instructions, facilitates the interpretation of results and provides immediate advice in case of inhibited AChE activity. Thus, even inexperienced personnel can use the device after minimal training.

Parameter	Coefficient of variation (CV) (%)	
	AChE	BChE
Intra-Day variability (n = 91)	1.3	4.5
Inter-Day variability (n = 45; 5 days)	1.3	3.3
Inter-Operator variability (n = 30; measurement of 10 samples by 3 persons)	1.7	5.9

Tab 2: Reproducibility of AChE and BChE activity determination in whole blood samples, using ChE rapid test IVD

4. Conclusions

The newly available ChE rapid test IVD provides an important laboratory diagnostic capability, allowing rapid and accurate determination of AChE and BChE activity in whole blood, both in permanent medical treatment facilities and under field conditions, in particular during deployed field operations. Moreover, if basic values of the individual AChE activity are available confirmation of exposure to AChE inhibitors at presence or even absence of slight acute clinical symptoms should be possible. Thus, the diagnostic capabilities of the Bundeswehr Medical Service with regard to suspected exposure to OP were significantly improved.

References:

1. *Holmstedt B:* Pharmacology of organophosphorus cholinesterase inhibitors. Pharmacol Rev 1959; 11: 567-688.
2. *Ballantyne B, Marrs TC*: Overview of the biological and clinical aspects of organophosphates and carbamates. In: Ballantyne B and Marrs TC [eds.]: Clinical and experimental toxicology of organophosphates and carbamates. Oxford: Butterworth & Heinemann 1992, 3-14.
3. *Baker DJ:* Organophosphate poisoning: aspects of emergency and critical care management. Crit Care&Shock 1999; 2: 72-78.
4. *Worek F, Koller M, Thiermann H, et al.:* Diagnostic aspects of organophosphate poisoning. Toxicology 2005; 214: 182-189.
5. *Eckert S, Eyer P, Herkert N, et al.:* Comparison of the oxime-induced reactivation of erythrocyte and muscle acetylcholinesterase following inhibition by sarin and paraoxon, using a perfusion model for the real-time determination of membrane-bound acetylcholinesterase activity. Biochem Pharmacol 2008; 75: 698-703.
6. *Thiermann H, Zilker T, Eyer F, et al.:* Monitoring of neuromuscular transmission in organophosphate pesticide-poisoned patients. Toxicol Lett 2009; 191: 297-304.
7. *Thiermann H, Worek F, Eyer F, et al.:* Obidoxime in acute organophosphate poisoning: 2 – PK/PD relationships. Clin Toxicol 2009; 47: 807-813.
8. *Aurbek N, Thiermann H, Eyer F, et al.:* Suitability of human butyrylcholinesterase as therapeutic marker and pseudo catalytic scavenger in organophosphate poisoning: A kinetic analysis. Toxicology 2009; 259: 133-139.
9. *Worek F, Pfeiffer B, Eyer P, et al.:* Optimierung labormedizinischer Verfahren zur Bestimmung der Acetylcholinesterase-Aktivität im Blut für die Diagnostik und Therapiesteuerung von Nervenkampfstoffvergiftungen. Teil 1: Feldverwendungsfähige Verfahren. Wehrmed Mschr 2005; 49: 298-302.
10. *Rajapakse BN, Thiermann H, Eyer P, Worek F, Bowe SJ, Dawson AH, Buckley NA:* Evaluation of the Test-mate ChE (cholinesterase) field kit in acute organophosphorus poisoning. Ann Emerg Med 2011, 58: 559-564.
11. *Worek F, Mast U, Kiderlen D, et al.:* Improved determination of acetylcholinesterase activity in human whole blood. Clin Chim Acta 1999; 288: 73-90.
12. *Worek F, Pfeiffer B, Eyer P, et al.:* Optimierung labormedizinischer Verfahren zur Bestimmung der Acetylcholinesterase-Aktivität im Blut für die Diagnostik und Therapiesteuerung von Nervenkampfstoffvergiftungen. Teil 2: Automatisierte Verfahren. Wehrmed Mschr 2005; 49: 302-305.

Corresponding Author

Prof Dr Franz Worek, Colonel (MC)

Deputy Commander Bundeswehr Institute of Pharmacology and Toxicology

Address for the authors:
Neuherbergstr. 11
80937 München, Germany
E-Mail: FranzWorek@Bundeswehr.org

Customers & Partners worldwide:

Australia • Austria • Belgium • Brazil • Canada • Czechia • Chile • Croatia • Denmark • Estonia
Finland • France • Germany • Hong Kong • Iceland • Indonesia • Ireland • Italy • New Zealand
The Netherlands • Poland • Portugal • Russia • Slovakia • South Africa • Spain • South Korea
Sweden • Switzerland • Taiwan • Thailand • United Kingdom • USA

SECURETEC
DETEKTIONS-SYSTEME AG

Lilienthalstr. 7
85579 Neubiberg
Germany

Tel.: +49 (0)89/203080-1651
Fax: +49 (0)89/203080-1652

E-mail: info@securetec.net
www.securetec.net

we detect to protect

EN 70523