Cholinesterase Kit for Field Diagnosis of Organophosphate Exposure
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**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
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, R²>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
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.

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.

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.

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:


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<tr>
<th>Parameter</th>
<th>Coefficient of variation (CV) (%)</th>
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<tbody>
<tr>
<td>Intra-Day variability (n = 91)</td>
<td>AChE 1.3</td>
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<td></td>
<td>BChE 4.5</td>
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<tr>
<td>Inter-Day variability (n = 45; 5 days)</td>
<td>AChE 1.3</td>
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<tr>
<td></td>
<td>BChE 3.3</td>
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<tr>
<td>Inter-Operator variability (n = 30; measurement of 10 samples by 3 persons)</td>
<td>AChE 1.7</td>
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<td>BChE 5.9</td>
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Tab 2: Reproducibility of AChE and BChE activity determination in whole blood samples, using ChE rapid test IVD

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