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Evaluation of the performance of an handheld device for measuring cholinesterase activity, in order to use in cases of organophosphate poisoning

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ABSTRACT

Cholinesterase activity monitoring during the organophosphorus pesticides poisoning is still a challenge in the remote areas. The objective of this study was to evaluate the performance of the Test-mate Model 400 EQM® for the determination of cholinesterase. This is a cross-sectional analytical study conducted with two different levels of quality control sera (QCs) and 40 sera of different cholinesterase concentrations from patients samples. The coefficient of variation obtained for repeatability was compared to the supplier's values. Spearman's coefficient and kappa coefficient (κ) were used to examine correlation and assess agreement between measurements obtained with the handheld device and the INDIKO analyzer, used as a reference test. For repeatability of EQM Test-mate ChE®, coefficients of variation was 5.5% and 6% for the normal and pathological control sera respectively, that is lower than 7.5% as recommended by the supplier. A Spearman correlation coefficient of r=0.7451 (p<0.0001) was obtained and a good agreement between the measurements (κ=0.7253 (95%CI: 0.655- 0.841)). This handheld device is repeatable and showed a good correlation and agreement with the INDIKO® analyzer. It would therefore be suitable for determining cholinesterase activity in remote areas in case of organophosphate poisoning.

Keywords : Cholinesterase, organophosphorus poisoning, Test mate model 400 EQM, Analyzer Indiko.

INTRODUCTION

Organophosphate (OP) pesticides are still used in Burkina Faso in the agriculture field (Sanou et al., 2020). Persons exposed to these chemicals can exhibit a spectrum of signs and symptoms ranging from mild anxiety to cardiovascular collapse and rarely death (Lessenger and Reese, 2001).

The toxic effects of OP pesticides are due to their ability to inhibit the catalytic activity of acetylcholinesterase in the nervous system. The complexes formed between these poisons and the enzyme are hydrolysed relatively slowly or not at all with some phosphorylated enzyme complexes, thereby prolonging the action of acetylcholine within the synapse (Lessenger and Reese, 2001).

Cholinesterase activity includes butrylcholinesterase, which inactivates butyrylcholine in plasma, and acetylcholinesterase, which inactivates acetylcholine in red blood cells (RBCs) (Maroni et al., 2000). The level of serum cholinesterase activity has been used as a confirmatory test of acute organophosphate insecticide poisoning. In mild poisoning the serum cholinesterase activity is only 20% to 50% of the normal level, whereas in severe cases the activity is less than 10% (Testud and Grillet, 2007). Cholinesterase especially acetycholinesterase and butyrylcholinesteras are useful biomarkers for monitoring exposure to organophosphate (OP) pesticides (Maroni et al., 2000; Lessenger, 2005; Worek et al., 2005). A portable device, the Test-mate ChE ® kit, manufactured by EQM Research, Inc (EQM Researcher, Cincinnati, USA) can be used for the monitoring of cholinesterase activity (Carmona-Fonseca, 2007). The test system is based on the Ellman method (Carmona-Fonseca, 2007). It is a portable field kit that can measure acetylcholinesterase and butyrylcholinesterase within 4 minutes and was designed for determining exposure to anticholinesterase pesticides in agricultural workers (EQM Research, 2003). Because of its ease of operation, the device has attracted much interest among public health workers. Several studies have been conducted with the instrument (Carmona-Fonseca, 2006; Carmona-Fonseca, 2007; Diatta et al., 2021). In a country with limited resources like Burkina Faso, Organophosphorus pesticides is still used by the farmers (Sanou et al., 2020; Traore et al., 2020). Cholinesterase monitoring during the organophosphorus pesticides poisoning is still a challenge in the remote areas because of an unavailability of analyzer and a lack of power supply. The Model 400 EQM Test-mate ChE ® device would be a solution as it works with just a battery. Before using this device it is important to evaluate his performance. It is for this reason that we initiated this study whose general purpose was to evaluate the performance of the Model 400 EQM Test-mate ChE ® kit for the cholinesterase activity measurement compare to the INDIKO® analyzer the reference–test before its use in the remote areas in Burkina Faso.

MATERIALS AND METHODS
Study design and setting

An analytical observational cross-sectional study was carried out from September 1 to 30, 2022.

Study population and sample collection

Samples of marketed controls were used, as well as those of patients received in the sampling room of the medical biology laboratory who had given their consents were included in this study. The controls sera were used to evaluate the repeatability of the Test-mate ChE® at two-levels: Normal sera control (Reference 981044) and pathological sera control (Reference 981043) both from Thermo Fisher Scientific (Thermo Fisher Scientific, Fremont, USA).

The patients samples were used for assessing agreement between the Test-mate ChE® and the INDIKO® analyzer used as a reference method (Comité Français d'Accréditation, 2023). Regardless to the SH GTA 04 recommendations, a total of 40 patients samples were selected at random, as they arrived at the laboratory (Comité Français d'Accréditation, 2023).

Test mate ChE®

The Test-mate ChE® Cholinesterase Test System is based on the Ellman method. Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by acetylcholinesterase (AChE) or plasma cholinesterase (PChE) respectively, producing carboxylic acid and thiocholine which reacts with the Ellman reagent (DTNB,

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 either AChE or PChE. The Test-mate ChE photometric analyzer is factory-calibrated and no additional calibration is required (EQM Research, 2003).

Cholinesterase INDIKO®

Cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate (II). Absorbance is measured at 405 nm (Thermo Fisher Scientific, 2017). Calibration of the INDIKO analyzer® were performed during the study with a standard or a calibrator. Quality Control (CQ) were used before testing samples (Thermo Fisher Scientific, 2017). In contrast to the Test-mate ChE® units of U/mL of whole blood at 25°C, the INDIKO® analyzer results were expressed as U/L of plasma at 37°C.

Determination of the performance of Test-mate ChE®

The evaluation of the performance of the Test-mate ChE® was carried out following the SH GTA 04, a guide recommended by the French Accreditation Committee (COFRAC) (Comité Français d'Accréditation, 2023).

Repeatability (or accuracy) of Test mate CHE®

The repeatability of the Test-mate ChE® was assessed using commercially available control sera (normal and pathological control). The assays were performed on these two controls sera measured twice a day for 15 days. The results were interpreted by comparing the coefficient of variation (CV) with the manufacturer allowable CV limit which is between 5-7% for the both level of control.

Correlation and agreement between Test-mate ChE® and INDIKO® analyzer

Cholinesterase activity was measured using both Test-mate ChE® kit and the reference laboratory automate INDIKO® analyzer for each sample. The data from the two devices were classified into three categorical variable: high, normal and low using references values provided by manufacturers.

Statistical analysis

Data recorded into Excel 2016 were analyzed using STATA version 16.0 software. The coefficient of variation (CV), was calculated to evaluate the repeatability of the Test-mate ChE®. The Spearman's correlation coefficient was performed to evaluate correlation between measurements from the Test-mate ChE® and the INDIKO® analyzer. The kappa (κ) test was performed to evaluate the agreement between the two devices.

RESULTS
Repeatability (accuracy) of the Test-mate ChE®

The Test Mate ChE® kit demonstrated a CV of 5.5% for normal QCs and a CV of 6.02% for pathological QCs. Both CV were within the CV reference range provided by the manufacturer which is between 5-7% for the normal and pathological QCs (Table 1). Then the repeatability of Test Mate ChE® kit is in line with those announced by the supplier.

Correlation and agreement between TEST Mate ChE® and INDIKO® analyzer

The Spearman's correlation coefficient was r = 0,7451 (p < 0.001) between the two devices, showing a strong correlation between the two devices, and the result is statistically significant (Schober et al., 2018).

For the agreement assessing, the kappa agreement test showed a coefficient of κ = 0,7253 (CI95%: 0.655 -0.841) (Table 2). The values of the kappa coefficient between 0,6 and 0,8 show a substantial agreement between the measurements of the two devices (Hallgren, 2012). Indeed according to the guidelines of Landis and Koch, a coefficient, k < 0 show a poor agreement, k between 0 and 0,2 , a slight agreement, k between 0,2 and 0,4 a fair agreement, k between 0,6 and 0,8 a substantial agreement and k between 0,8 and 1 an almost perfect agreement (Hallgren, 2012).

Table 1: Repeatability of Test mate ChE®.

	Samples (N)	Mean ± SD U/mL	CV %	CV Reference range (supplier) %
Normal Quality control	30	2.56 ± 0,14	5.50	5 - 7
Pathological Quality Control	30	1.39 ± 0,08	6.02	5 – 7

Table 2: Agreement test between Test Mate ChE® and INDIKO® analyzer.

Kappa (κ) coefficient	95% confidence Interval	p-value
0,725	0,655 – 0,841	0,0000

Interpretation of coefficient kappa: < 0 Poor, 0 - 0.2 Slight, 0.2 - 0.4 Fair, 0.4 - 0.6 Moderate, 0.6 - 0.8 Substantial, 0.8 - 1 Almost Perfect.

DISCUSSION

Evaluation of the Model 400 EQM Test mate ChE® is the demonstration on site that the device works correctly under the operating conditions and that it gives accurate and reliable results for the patients.

Test-mate ChE ® measurements of cholinesterase compare to our reference test (INDIKO® analyzer) exhibited a good Spearman's correlation coefficient (r=0,7451, p < 0.001). Correlation analysis assesses the linear correlation between two measures but does not automatically imply good agreement between measurements (Nikiema-Ndong et al., 2020; Ndour et al., 2021). For this reason, the Bland-Altman method had to be used to study bias and precision, in other words, to evaluate the agreement between the results of the Test-mate ChE ® and the INDIKO® analyzer since they generate quantitative variables. But since the two methods operate at different temperatures to determine the enzymatic activity of cholinesterase, while temperature is a physicochemical factor that influences the enzymatic activity, a systematic bias does exist between the two sets of measurements, making the Bland Altman method unsuitable to compare the two techniques. Therefore, we chose to determine the accuracy by determining the Test-mate ChE ® repeatability and the agreement by performing the Kappa test between the handheld device and the reference test. The repeatability was determined from the quantitative variables generated from the Quality Control sera (QCs). To perform the Kappa agreement test, it was necessary to transform the quantitative variables (the measurements of the two series of tests) into categorical variables (low, normal or high activities) taking into account the reference range provided by the manufacturers.

This methodological approach allowed us to observe, using both normal and pathological QCs, a repeatability that was within the expected limits proposed by the manufacturer of the Test mate ChE® suggesting a good accuracy of the method (Table 1). Hofmann et al. (2008) study also demonstrated high accuracy of the Model 400 EQM Test-mate® kit. They got 3.3% as a coefficient of variation (Hofmann et al., 2008).

Furthermore, the comparison of the results of the Test-mate ChE® and the INDIKO® analyzer allowed to obtain a very good correlation with a Spearman's correlation coefficient (r) between 0,7 -1 (r = 0,7451; p< 0.001). Moreover, the kappa test led to a kappa (κ)-coefficient of 0.725 (CI95%: 0.655 -0.841) which is within the range 0.60 - 0.80 showing thus a good agreement between the measurements of the two devices. Previous studies supported the findings of good correlation, indeed Rajapakse et al. (2011) in Sri Lanka evaluate the Test-mate ChE®

(Cholinesterase) field kit in Acute Organophosphorus Poisoning and the measurement of PChE also showed good Spearman's correlation coefficient of 0.76 (95% CI 0.66 to 0.84).

Conclusion

The Test-mate ChE® kit appears to provide a reliable method to rapidly measure plasma cholinesterase in organophosphorus-poisoned patients in remote areas in a poor country. We can recommend the measurement of PCHE with the Test-mate ChE® in the resource-limited country to deal with the lack of equipment for the organophosphorus poisoning monitoring specially in remote areas.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CT, AH, HH, coordinates, wrote and revised the paper. EMD, AO, OD analyze, interpreted the data, and revised the paper. KN, PT, KA, MM collected, analyzed literature and revised the paper. All authors read and approved the manuscript.

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