Cholinesterase Measurements With an Automated Kit

Georgino H. Oliveira, PhD,[1,2] John D. Henderson, BS,[1] and Barry W. Wilson, PhD[1]*

Background *The Test-mate kit determines acetylcholinesterase (AChE, EC 3.1.1.7) and hemoglobin content of a drop of blood, displaying enzyme activities normalized to 25°C. Previous models produced inconsistent results at different temperatures. This report focuses on the current model, ChE 400, and two instruments of a previous OP model.*
Methods *AChE activities were determined by the Ellman assay, using the three kits and a 96-well microplate reader. Temperatures ranged from 10 to 37°C. Fetal bovine serum was the source of AChE.*
Results *Normalized activities decreased below 20°C in the ChE model and below 25°C in the OP models. Activities of the same serum sample differed between the three Test-mate kits, ranging from 1.03 to 1.49 μmoles/min/ml. Percent errors were greater than with the microplate reader at all temperatures.*
Conclusions *Neither we nor the manufacturer recommend the current Test-mate model for fieldwork. Nevertheless, there have been field measurements with Test-Mate kits, and we recommend that an enzyme activity standard be run in parallel with their use.* Am. J. Ind. Med. Suppl. 2:49–53, 2002. © *2002 Wiley-Liss, Inc.*

KEY WORDS: Test-mate; acetylcholinesterase determinations; accuracy and reliability; portable cholinesterase assay device

INTRODUCTION

Cholinesterases (ChEs), such as acetylcholinesterase (AChE, EC 3.1.1.7) hydrolyze esters of choline. AChE itself hydrolyzes the neurotransmitter acetylcholine (ACh). ChEs, especially AChE, are useful biomarkers for monitoring exposure to organophosphate (OP) and carbamate (CB) ester pesticides [WHO, 1986a,b]. Monitoring blood ChEs is required of those using OPs and CBs in California [Wilson et al., 1997]. Common clinical assays for AChE use ACh and pH [Nabb and Whitfield, 1967] and acetylthiocholine (ACTh) with dithiobisnitrobenzoate (DTNB) substrate/colorimetric indicator combinations [Ellman et al., 1961].

A portable device, the Test-mate kit, manufactured by EQM Research, Inc. (Cincinnati, OH) [Magnotti and Eberly, 1996], measures AChE activity using the Ellman assay [Ellman et al., 1961] and hemoglobin (Hb) content to determine red blood cell and plasma ChEs on a drop of blood, normalizing the results to 25°C. Several studies have been conducted with the instrument [e.g., McConnell et al., 1992, 1999; Keifer et al., 1996; Simcox et al., 1999]. Because of its portability and ease of operation, the device has attracted much interest among public health workers not necessarily familiar with clinical biochemical procedures. Nevertheless, problems have been found in the temperature compensation of previous models of the instrument [London et al., 1995]. This article examines a late model of the Test-mate (Model 400) and a previous OP model, comparing their responses to temperature.

[1]Departments of Animal Science and Environmental Toxicology, University of California, Davis, California
[2]School of Pharmaceutical Sciences, UNESP, Arraquara, Brazil
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*Correspondence to: Barry W. Wilson, Animal Science Department, Meyer Hall, University of California, 1 Shields Ave., Davis, CA 95616. E-mail: bwwilson@ucdavis.edu

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TABLE I. Constituents of Cholinesterase Assay

Assay constituent	Stock concentration (mM)	Assay concentration (mM)	Test-mate volumes (μl)	Microplate volumes (μl)
Phosphate buffer, pH 8	100	—	1,820	270
DTNB	10.3	0.32	65	10
ATCh	10.7	1.00	195	30
FBS	—	—	10	10
Total volume	—	—	2,080	320

MATERIALS AND METHODS

A single instrument of the current Model 400 ChE, version B and two instruments of an earlier OP model of the Test-mate (EQM Research, Inc.) were tested at temperatures ranging from 10 to 37°C. ChE activities were compared to a standard laboratory assay run with a 96-well microplate reader (Model EL 340, Bio-Tek Instruments, Inc., Winooski, VT). The source of AChE was fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS). This serum has little, if any butyrylcholinesterase/non-specific cholinesterase activity [Hoffman et al., 1999]. Assay reagents (Table I) were prepared in the laboratory rather than with the company's lyophilized mixtures to reduce the number of variables investigated in the trials. Sodium phosphate, ATCh, and 5,5′-dithiobisnitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO).

The Test-mate kits and the plate reader were kept overnight in a controlled temperature room (± 1°C) before each run. The temperature of the cuvette was measured with a Tele-Thermometer probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) and recorded after each run. The enzyme activity results displayed by the Test-mate kit were automatically normalized to 25°C by an internal program.

ChE activities were assayed in triplicate according to the standard Ellman assay [Ellman et al., 1961] and the constituents shown in Table I. Assay volumes of 320 μl consisted of 270 μl 0.1 M sodium phosphate buffer, pH 8.0, 10 μl of 322 μM DTNB, 10 μl of enzyme sample, and 30 μl of ACTh. ACTh final concentrations were routinely pegged to1.0 mM. Activities were read at 405 nM and 25–27°C.

RESULTS

The normalized AChE activity of a single sample of FBS was different for each Test-mate kit tested (Table II). Variances of each set were acceptable, averaging 8% or less, but the means were significantly different between the kits (*t*-test, $P \leq 0.05$).

The uncorrected AChE activity of the fetal calf serum sample increased with increasing temperature over the range tested (Fig. 1). The displayed AChE activities of the two models of Test-mate kits tested did not satisfactorily ''correct'' activities over the entire temperature range (unfortunately, the devices also did not display the uncorrected readings). The older OP model did not satisfactorily adjust ChE activities at ambient temperatures below 25°C (Fig. 2). The newer ChE kit model 400 ''adjusted'' activities at temperatures down to 20°C, but fell off at 15 and 10°C (Fig. 3). (Temperatures above 37°C were not tested due to limitations of the constant temperature room.)

Comparison of the performance of the CHE 400 kit and the plate reader using the same enzyme samples (Table III) show that the percent error of the Test-mate, although generally satisfactory, was higher than that of the plate reader at all temperatures investigated.

DISCUSSION

There were problems in the performance of the three Test-mate kits: First, they did not display the same enzyme activities at the same temperatures. Whether this is a matter of quality control, changes in the accuracy of the instruments over time or some design factor cannot be determined from the tests. Regardless, it means that values obtained from one instrument may not be comparable to another's without some external standardization. Recently, we have been testing a bovine ghost red blood cell standard that could be suitable. The activities of refrigerated and frozen samples of the standard were relatively stable over a period of 150 days [Wilson et al., 2000]. Second, and equally important, the Test-mate kits did not correctly normalize activities at temperatures below 25°C for the OP model and below 20°C for the ChE model, confirming reports of others [London

TABLE II. ChE Activity of Three Test-mate Kits

Test-mate model	OP no. 1	OP no. 2	ChE
Activity	1.27	1.49	1.03
S.D.	± 0.09	± 0.08	± 0.05
Displayed temperature	23.5	24.3	24.5

Activity is in μmoles/min/ml; n = 8 and temperature is in °C.

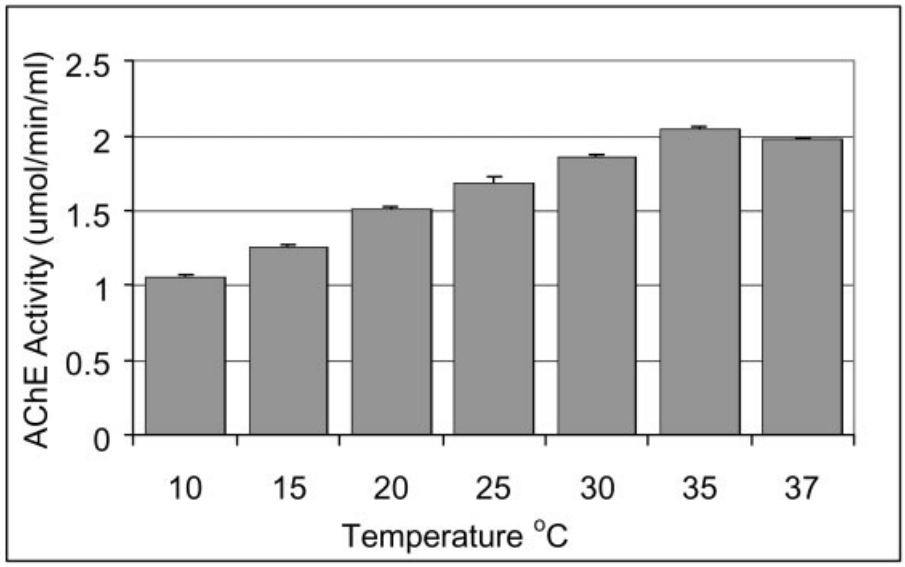

FIGURE 1. AChE activity of FBS varies with temperature. Activity determined with a microplate reader. Mean ± SD, n = 8.

et al., 1995] for the earlier OP model. Unfortunately, the ''raw values'' are not displayed by the kits; they are hidden from the operator because the devices adjust the results to ''25°C'' with internal algorithms. The manufacturer told us that the placement of the temperature sensor in the OP model had been reoriented in the newer 400 ChE model. Perhaps, the algorithm itself also needs to be re-examined.

Considering the results reported here, we recommend that the instrument be standardized against a laboratory standard, such as glutathione absorbance or a stable cholinesterase preparation. In addition, the assays are better run under controlled temperature conditions rather than under the more variable conditions found in the field. Indeed, the instructions for the ChE model recommend the instrument is

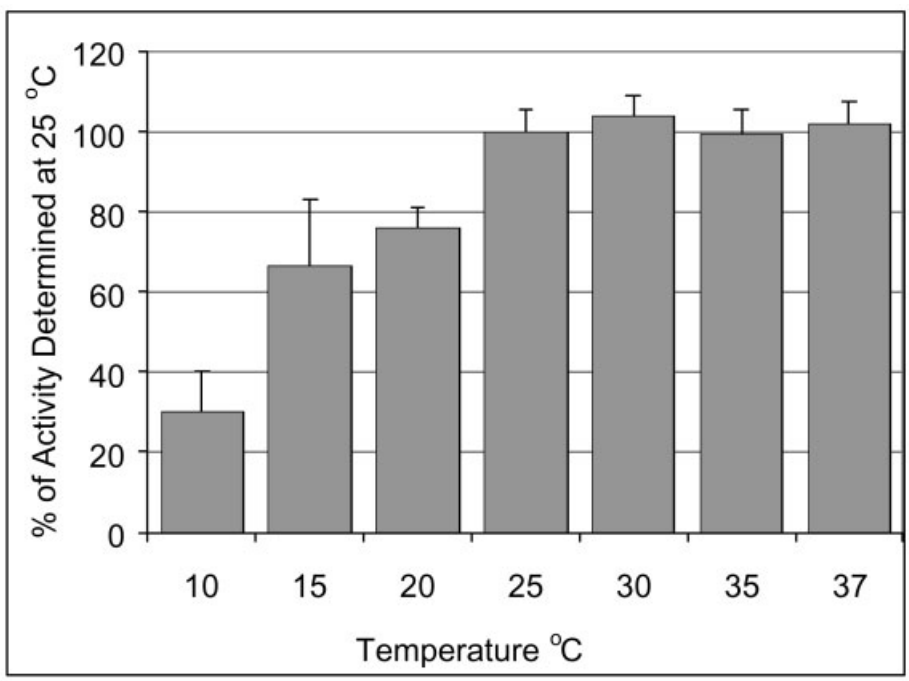

FIGURE 2. Normalized AChE activities determined at various temperatures with the OP model Test-mate kit. Reported activity (normalized to 25°C) is shown as a percentage of the activity determined at 25°C. Mean ± SD, n = 6.

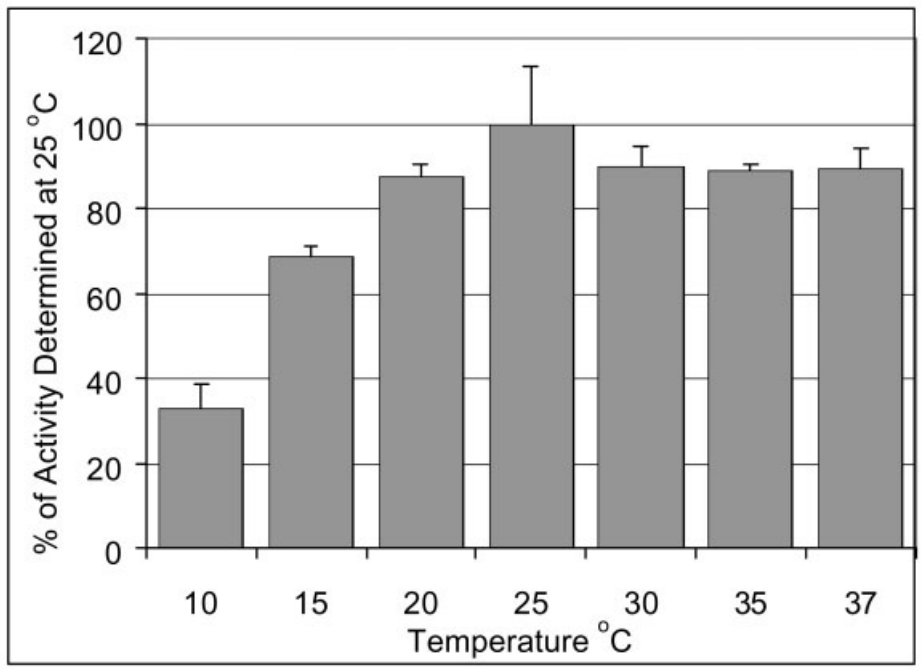

FIGURE 3. Normalized AChE activities determined at various temperatures with the model 400 ChE Test-mate kit. Reported activity (normalized to 25°C) is shown as a percentage of the activity determined at 25°C. Mean ± SD, n = 5.

"for laboratory use by trained laboratory technicians only". Nevertheless, the needs of field exposure studies may lead to ChE surveillance under inappropriate conditions. For example, a recent study from Sri Lanka [Peiris-John et al., 2002] of farmers spraying OPs and fishermen controls used an unspecified Test-mate kit model at unspecified temperatures and report that "The farmers had higher erythrocyte AChE levels than the controls... during and between cultivation seasons" and that during the cultivation season there was a "significant reduction in ... AChE activity in both groups." It is to be hoped that the temperature range satisfactory for AChE normalization was not exceeded.

Determination of blood ChE activities is sometimes the only practical way to establish whether exposure to anticholinesterase agents has occurred. Previous models of the Test-mate kit have been used by experienced workers to provide internally-consistent cholinesterase levels under conditions where a full clinical laboratory was not available [Keifer et al., 1996; McConnell et al., 1999].

In general, there has been much difficulty comparing ChE findings from different laboratories. This is partly due to the inadequacy of the chemical kits used by the clinical laboratories [Wilson et al., 1997; Wilson, 2001], and partly because of a lack of widely accepted methods of sampling, shipping, storage, and execution of assays [Hoffman et al., 1999].

There is need of a portable, inexpensive device capable of rapidly determining blood values for enzymes, such as

TABLE III. Comparison of Plate Reader and Test-Mate Serum Cholinesterase Activities

	10°C	15°C	20°C	25°C	30°C	35°C	37°C
Plate reader							
Mean	1.03	1.27	1.52	1.56	1.84	2.15	2.11
SD	0.06	0.02	0.03	0.03	0.03	0.04	0.01
% error	5.6	1.5	1.8	1.6	1.3	2.0	0.6
Test-Mate							
Mean	0.46	1.01	1.53	1.52	1.58	1.51	1.55
SD	0.15	0.25	0.08	0.08	0.07	0.09	0.09
% error	32	24.8	6.5	5.2	4.5	6.0	5.8

Values are expressed in μmoles/min/ml; mean ± SD, and percent error, n = 6.

ChEs, creatine phosphokinase, glutamic-oxaloacetic transaminase, and lactic dehydrogenase. We acknowledge the efforts of the designers of the Test-mate kit and look forward to a model that will correct its temperature problems, and display both adjusted and raw values. Until then, we are concerned that such instruments may be used uncritically in the field, generating unreliable data.

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