Test-mate ChE Cholinesterase Test System (Model 400)

Instruction Manual

For the quantitative determination of cholinesterase in whole blood to monitor pesticide exposure. For in vitro diagnostic use. For laboratory use by trained laboratory technicians only.

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Manual-E (27-APR-03)

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Intended Use

The Test-mate ChE is intended for use in the assessment and diagnosis of asymptomatic pesticide poisoning. Most organophosphate or carbamate pesticides inhibit the blood enzymes erythrocyte acetylcholinesterase (AChE) and/or plasma cholinesterase (PChE).[1,2] The degree of enzyme inhibition is proportional to the extent of exposure. AChE is generally preferred because of its lower biological variability and lack of interferences relative to PChE. Pre-exposure (baseline) measurements of AChE and/or PChE should be obtained to reduce the effect of biological variability.[1] The short-term method of treatment is to simply remove the patient from exposure to pesticides. The long-term method of treatment is to promote safe handling procedures for the pesticides in order to avoid future exposure. *The emergency treatment of a severely pesticide poisoned patient should always be based primarily upon the patient's physical symptoms, rather than delaying treatment pending cholinesterase test results.*

The Test-mate ChE is intended to be used in a clinical laboratory by a medical technician under the supervision of a laboratory director. Before the operator begins to use this system on patient blood samples, it will be necessary to practice the entire assay procedure several times in order to produce consistent and reliable results. Measurements taken with the Test-mate ChE test system should be evaluated by a qualified health official, such as a doctor, nurse, or trained public health worker.

Although cholinesterase testing may be valuable in the diagnosis of succinylcholine sensitivity and in the diagnosis of liver dysfunction, this system is <u>not</u> intended to be used for these purposes.

Explanation of the Test-mate ChE

The Test-mate ChE is useful in monitoring occupational exposure to pesticides. By routinely measuring the blood cholinesterase levels of workers handling pesticides, these workers may be protected from over exposure to pesticides before showing symptoms. Also, pesticide handling safety programs may be assessed for effectiveness and compliance, leading to the improved long-term protection of workers.

The Test-mate ChE is a complete cholinesterase testing system. All of the equipment and reagents necessary for performing 96 tests fit conveniently within the storage case. The system requires only 10µL for each blood test, which may be easily obtained from a fingerstick sample. The entire assay may be completed in under 4 minutes, facilitating the rapid evaluation of poisoning status.

The main component of the Test-mate ChE system is the photometric analyzer. The photometric analyzer is a compact dedicated application, fixed wavelength absorption photometer, powered from either a 9-volt battery or an external power adapter. This analytic instrument guides the operator in a step-by-step fashion through the assay procedure. The photometric analyzer measures absorbances during the assay procedure, calculates the final analyte concentrations, temperature compensates these concentrations using a built-in temperature sensor, and then displays the final test results.

Principles of the Test-mate ChE

The Test-mate ChE Cholinesterase Test System is based on the Ellman method.[5] Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or 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 450nm. The rate of color formation is proportional to the amount of either AChE or PChE.

$$\text{thiocholine ester (AcTC/BuTC)} \xrightarrow{\textit{Cholinesterase}} \text{thiocholine}$$

$$\text{thiocholine} + \text{DTNB} \longrightarrow \text{TNB-thiocholine} + \text{TNB (}\textit{yellow}\text{)}$$

The measured cholinesterase activity is calculated by the photometric analyzer using the following equation:

$$\text{U/mL blood} = \frac{\text{(A/min) (mL assay volume)}}{(\varepsilon, \text{mM}^{-1}\text{cm}^{-1})\ \text{(cm light path) (mL blood)}}$$

The measured cholinesterase activity is further refined by the following adjustments to derive the final displayed cholinesterase value:

Reagent Blank Adjustment: A small (approximately 15%) nonspecific blank reaction is subtracted from the measured cholinesterase activity.

Temperature Adjustment: Using the temperature sensor in the photometric analyzer, both the measured cholinesterase activity and the reagent blank activity are normalized to 25°C.

Hemoglobin Adjustment: For AChE, hemoglobin normalizes varying sample size and iron status; therefore AChE is most accurately expressed as U/g Hgb.

Setting Up the Test-mate ChE (Installation)

The watertight storage case features an automatic pressure equalization valve to correct for changes in atmospheric pressure that may be caused by altitude changes during transportation. To open the case, with the lid of the case facing upward, press down on the lid with one hand while prying free each of the two latches with the other hand. Considerable force may be necessary to move the latches.

Once the case is opened, two boxes will be visible. The box on the left hand side contains a Model 460 AChE Assay Kit. The box on the right hand side contains the reagent-opening tool and the assay tube rack. The photometric analyzer is stored underneath the box on the right hand side facing downward into the protective foam insert.

Do not discard the zip-lock bubble bags for the reagent-opening tool, the assay tube rack or the photometric analyzer. These items should always be returned to their reusable bags for storage. The photometric analyzer should always be stored within the supplied bag with the front panel facing into the protective foam insert.

The Model 460 AChE Assay Kit consists of three boxes along with a detailed package insert. Box one and two of the assay kit each contain 48 bottles filled with precisely measured amounts of buffer solution. Box three contains a 96 well erythrocyte reagent plate, 100 capillary tubes (10µL volume), 100 filter papers (capillary wipes), a 30mL clear plastic dropper bottle filled with 18mL of distilled water and 2 transfer pipettes.

The Model 460 AChE Assay Kit is used for the measurement of erythrocyte cholinesterase and the Model 470 PChE Assay Kit is used for measuring plasma cholinesterase. The type of cholinesterase inhibitor to which the patients have been exposed determines which assay kit to use. Often monitoring both types of cholinesterase is useful. The Test-mate ChE system is initially supplied with the AChE assay kit.

The Test-mate ChE Photometric Analyzer

The Test-mate ChE photometric analyzer is a very easy-to-use microprocessor based instrument. Using patented LED (light emitting diode) light source technology, no user maintenance or recalibration is ever required. A temperature sensor inside the analyzer accurately compensates the final results for ambient temperature. A 16 character LCD (liquid crystal display) guides the operator through the test procedure in a step-by-step fashion. The instrument is powered by either the self-contained 9-volt battery or an external power adapter (not furnished with system) connected to the jack on the right hand side of the unit.

To begin using the analyzer, install the 9-volt battery from box three of the assay kit into the battery compartment located on the bottom of the instrument. A fresh battery is shipped with every replacement assay kit and should always be used even though the current battery is still in good condition. Turn the analyzer on by sliding the power switch on the right hand side of the instrument forward. A short beep will sound and the version number and revision of the internal software system will be displayed for approximately three seconds. During this time period, the microprocessor will perform internal diagnostics to assure that the instrument is in proper operating condition. The battery saver feature of the analyzer will turn the instrument off should it be left unattended for more than 30 minutes.

After the internal self-testing period, the system prompt is displayed. This prompt shows the current testing mode, the current instrument temperature in degrees Celsius, and the current voltage of the battery or external power adapter. AChE indicates that the analyzer is ready to test for erythrocyte cholinesterase while PChE indicates that the instrument is ready to test for plasma cholinesterase. When in PChE mode, the P in PChE will flash on and off. The temperature of the instrument is displayed in one-tenth degree Celsius increments. Should the temperature be outside of the recommended operating range (less than 15 or greater than 30 degrees Celsius), the temperature section of the prompt will flash on and off. The battery voltage is displayed in one-tenth volt increments. Should the voltage drop below 6.2 volts, the voltage section of the prompt will flash. The instrument still provides accurate results when the voltage section of the prompt is flashing. Once the voltage becomes too low for reliable operation, the instrument will display a battery error message.

The Test-mate analyzer is controlled using three keys. The mode key is used to select between the AChE and PChE testing functions. Pressing the mode key from the system prompt will cause the testing mode to change. The analyzer will always be in AChE mode upon power up. The done key is used to return to the system prompt, once the results from a testing procedure have been recorded. The done key may also be used to abort a testing procedure, should a mistake occur in performing the assay. The test key is used to initiate and step through a testing procedure.

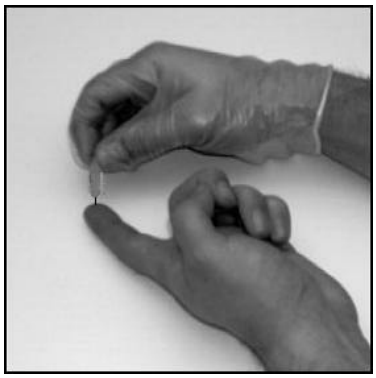
Performing the Assay Procedure

After unpacking the test system, arrange all of the equipment in the following easily accessible fashion. To the left of the operator place the assay tubes and the sampling supplies (see Appendix D - Additional Materials Needed for Testing). In front of the operator place a biohazard container, the reagent plate, the reagent-opening tool, the 30ml clear dropper bottle filled with distilled water, and the transfer pipettes. To the right of the operator place the photometric analyzer and the assay tube rack.

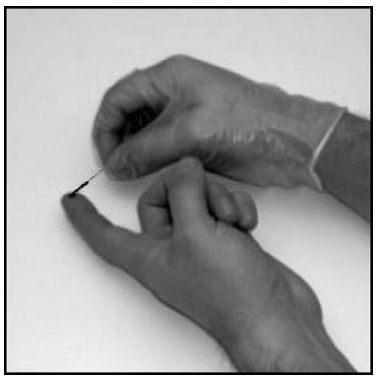
Turn the Test-mate analyzer on by sliding the power switch forward. Select the correct testing mode (AChE for erythrocyte cholinesterase or PChE for plasma cholinesterase) using the mode key. Enter the date and
time along with the patient's name in the test record. Also, enter the current temperature as reported in the center section of the system prompt.

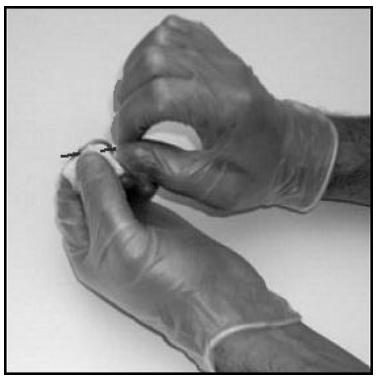
Press the test key and the analyzer will display a message to insert a new tube. Grasp an assay tube by the white screw top and gently slide it into the analyzer. *Never touch the bottom half of the glass vial, it must be kept clean in order for the photometric analyzer to operate properly!* Press the test key and the blanking message will appear for 10 seconds followed by a message to remove the tube.

Slide the assay tube out of the analyzer and while holding the tube by the upper half of the glass vial, unscrew the white cap. Set the glass vial into the assay tube rack and set the cap aside. Press the test key and the analyzer will display a message to add blood.



Always wear powder free gloves when handling blood samples! To prevent damaging the optical system inside the analyzer, avoid using powdered gloves with the Test-mate ChE system. Instruct the patient to thoroughly wash their hands using soap and water. Rest the patient's hand palm up with the index finger extended. Prepare to draw the blood sample by wiping the fingertip with an alcohol swab and then allowing it to air-dry for about 30 seconds. Expose the tip of the sterile blood lancet by twisting off its protective cover. Then solidly stick the end of the cleaned finger with the lancet.





Allow a drop of blood to form on the fingertip and then wipe it away with a sterile gauze pad. Wait several seconds to allow a second drop of blood to form on the fingertip for sampling. *Never squeeze the finger to obtain blood! If a second drop of blood does not readily form, it is necessary to lance the patient's fingertip again using a new lancet.*

Dispense a single capillary tube by inverting and gently shaking the container of capillaries (before the first capillary in a new assay kit may be obtained, the white shipping plug under the silver screw cap must be removed and discarded). Hold the capillary horizontal between the thumb and first finger and then insert the end of the capillary into the drop of blood on the patient's finger. Slowly the blood will fill the capillary. Once the capillary is completely full, withdraw the capillary. Carefully wipe any blood from the outside of the capillary by rolling the end of the capillary over a filter paper. Place the sterile gauze pad on the patient's finger and have the patient hold it in place until the lanced finger stops bleeding. Apply a small round bandage to the puncture site. Insert the capillary into the vial of the assay tube and then screw the cap back on tightly. Hold the assay tube by its white cap and shake it vigorously for 15 seconds to disperse the blood.

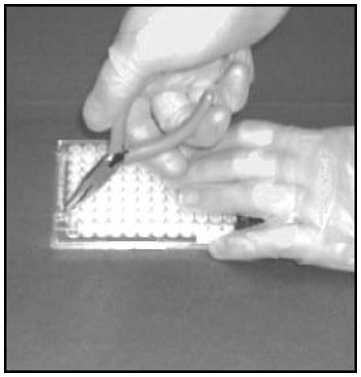
The capillary must be correctly oriented before the assay tube is inserted back into the analyzer. Hold the assay tube by the cap so that it is horizontal and let the capillary settle to the side of the vial. Gently turn the assay tube upright in such a fashion that the capillary stuck on

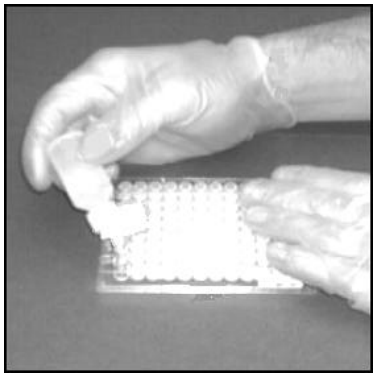
the side of the vial is now facing the operator. Insert the tube into the analyzer so that the capillary is toward the small black dot on the front panel of the analyzer. Press the test key and the reading message will appear for 10 seconds, followed by a message to remove the tube.

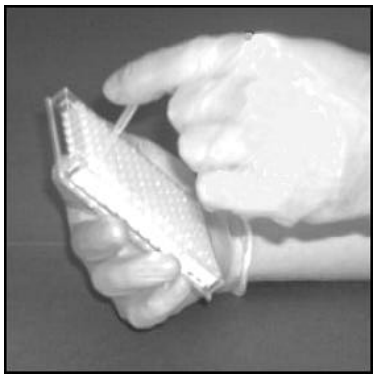
Slide the assay tube out of the analyzer and while holding the tube by the upper half of the glass vial, unscrew the white cap. Set the glass vial into the assay tube rack and set the cap aside. Press the test key and the analyzer will display a message to add the reagent.

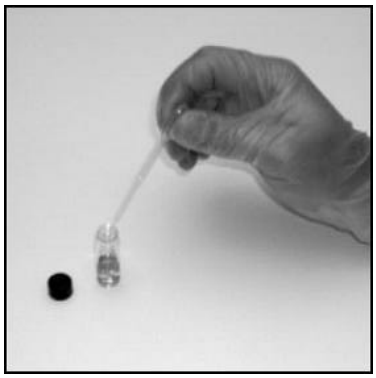
Unplug a single well of the reagent plate by holding the plate down with one hand while grasping the plug with the reagent-opening tool using the other hand. Pull the plug back at an angle and discard it into the biohazard bag.

Add three drops of distilled water to the reagent well from the 30ml clear dropper bottle. Using a transfer pipette, stir the contents of the well until the reagent is completely dissolved. Note that the powdered reagent may change from a white to a yellowish color with age. This









color change has no effect on the assay.

While holding the plate slightly tilted in one hand, load the dissolved reagent into the transfer pipette. Expel the dissolved reagent into the assay tube and immediately press the test key. After the test key has been pressed, the analyzer displays a message to shake the assay tube.

Screw the cap back onto the vial tightly and shake by gentle inversion for 5 seconds in order to mix the solutions together. *The capillary must be correctly oriented before the assay tube is inserted back into the analyzer*. Hold the assay tube by the cap so that it is horizontal and let the capillary settle to the side of the vial. Gently turn the assay tube upright in such a fashion that the capillary stuck on the side of the vial is now facing the operator. Insert the tube into the analyzer so that the capillary is toward the small black dot on the front panel of the analyzer. Press the test key and the incubation message will appear for about a minute (80 seconds maximum from the time the test key was pressed after adding the reagent). Next, the reading message will be displayed for 50 seconds followed by a message to remove the tube. Remove the

tube from the analyzer and discard it into the biohazard bag. Press the test key and the first test result message will be displayed.

The Test-mate analyzer provides six test result messages in AChE mode and four test result messages in PChE mode. Enter the first result into the patient's test record and then press the test key. Repeat this process until all of the results have been entered into the test record. When the last result message is displayed, pressing the test key will return the analyzer to the first result message. After all of the results have been entered into the record book, press the done key to return to the system prompt. The photometric analyzer is now ready to begin the next assay.

Calibration and Quality Control

Calibration: The Test-mate ChE photometric analyzer is factory-calibrated. No additional calibration is required.

Quality Control: The use of an unexposed operator is best; the intraindividual variability of both erythrocyte and plasma cholinesterase is less than 5% per week and less than 10% per month.[8] Alternatively, refrigerated venipuncture blood (anticoagulated with EDTA) is stable for at least one month. Controls should be run on each day of testing.

Expected Values and Interferences with the Test-mate ChE

These were determined using normal male and female blood bank donors, between 20 and 60 years of age, located in the Midwestern United States.

	N	Mean	SD	Range
AChE, U/mL	40	3.68	0.47	2.77 - 5.57
AChE, U/g Hgb	40	27.1	2.9	21.9 - 37.3
PChE, U/mL	40	2.03	0.40	1.35 - 3.23

***Physiological Interferences*:** AChE is depressed in paroxysmal nocturnal hemoglobinuria (PNH).[4] In severe macrocytic or microcytic anemia, the ratio of hemoglobin/cholinesterase may interfere with hemoglobin correction and hence, AChE activity. PChE is depressed in liver failure, malnutrition; increased in alcoholic/viral hepatitis and infection.[3]

***Analytical Interferences*:** Drugs which inhibit cholinesterase, such as pyridostigmine, will decrease cholinesterase. Pesticide residues adsorbed to the skin can artifactually decrease values.[9] Washing the skin with quaternary ammonium-containing soaps and detergents, such as benzethonium chloride can also artifactually decrease values; carefully check the label when selecting a cleansing product.

Accuracy, Precision and Linearity of the Test-mate ChE

Accuracy: The Test-mate ChE was compared with the Boehringer Mannheim Cholinesterase Kit No. 450035 on the Hitachi 704 Analyzer (BM/H).[10] The BM/H method is performed on plasma (PChE) or diluted whole blood (AChE) corrected by hematocrit; hence, in contrast to the Test-mate ChE units of U/mL whole blood at 25°C or U/g Hgb at 25°C, the BM/H results are expressed as U/L plasma (PChE) at 37°C or U/L

Normal Donors: (X,BM/H,Venipuncture) vs. (Y,Test-mate,Fingerstick)

	N	*r*	Slope	Intercept	Range†
AChE, U/L RBCs vs. U/g Hgb	44	0.78	0.000894	10.8	± 25% CV
PChE, U/L plasma vs. U/L blood	44	0.96	0.253	440	± 50% CV

Pesticide-Dosed & Normal Donors: (X,BM/H,Venipuncture) vs. (Y,Test-mate,Venipuncture)

	N	*r*	Slope	Intercept	Range†
AChE, U/L RBCs vs. U/g Hgb	86	0.98	0.00158	.322	± 100% CV
PChE, U/L plasma vs. U/L plasma	87	0.98	0.457	-210	± 100% CV

†Note: *r*, the correlation coefficient, is extremely range-sensitive (increases with %CV range).

Precision:

Within-run, N=40, 1 - 5 U/mL: 3 - 5% CV. Between-run, N=40, 1 - 5 U/mL: 5 - 7% CV.

Linearity:

Erythrocyte AChE: 0 - 7 U/mL; 0 - 50 U/g Hgb. Plasma PChE: 0 - 7 U/mL.

Between-Operator Variability of the Test-mate ChE

Ten operators each performed ten measurements on both a normal and abnormal venipuncture samples (N=100). The abnormal sample was prepared by dosing with pesticide (paraoxon).

	Normal				Abnormal			
	AChE U/mL	AChE U/g	PChE U/mL	Hgb g/dL	AChE U/mL	AChE U/g	PChE U/mL	Hgb g/dL
Mean	5.63	33.8	1.72	16.8	1.38	9.7	1.03	14.3
SD	0.21	0.8	0.15	0.5	0.12	0.8	0.08	0.3
%CV	3.7	2.4	8.5	2.7	9.0	7.9	7.5	2.2

This between-operator variability of the Test-mate ChE is well within CAP (College of American Pathologists) user-group proficiency criteria of ±20% of mean for all enzymatic analyses (including cholinesterase), and compares favorably with 1996 CAP Survey data for cholinesterase testing (Chemistry Survey p.84, specimen C7-02) among all instruments. The World Health Organization, as well as the states of California and Texas, have made the specific recommendation of testing for erythrocyte cholinesterase (hemoglobin-corrected AChE) when testing for pesticide poisoning. The between-operator precision of the hemoglobin-corrected AChE, reported in this study (N = 100, CV = 2.4%), is superior to the mean of any of the 1996 CAP cholinesterase instrument/reagent system user groups.

Interpretation of Results

In AChE mode the photometric analyzer displays the following results:

AChE (erythrocyte cholinesterase) in U/mL(units per milliliter),
AChE in %N (percent normal) relative to 4.71 U/mL,
Hgb (hemoglobin) in g/dL (grams per deciliter),
Hgb in %N relative to 15.0 g/dL,
Q (quotient) in U/g (units per gram),
Q in %N relative to 31.4 U/g.

Q is a hemoglobin corrected value of erythrocyte cholinesterase. Q is computed by dividing the AChE result by the Hgb result. Should the hemoglobin level be below 5 g/dL, the Q is not calculated.

In PChE mode the photometric analyzer displays the following results:

PChE (plasma cholinesterase) in U/mL,
PChE in %N relative to 2.55 U/mL,
Hgb in g/dL,
Hgb in %N relative to 15.0 g/dL.

Depression of cholinesterase to <50% normal indicates possible pesticide poisoning requiring removal from exposure and/or treatment with anticholinergics such as atropine and pralidoxime.[1] Suspected cases of poisoning can be confirmed by cholinesterase monitoring for a subsequent rise and plateau of activity 1 - 3 months after exposure. If baseline values are obtained, depression of cholinesterase to <70% of baseline can be taken to indicate possible pesticide poisoning.[11]

Operational Precautions and Limitations

The Test-mate ChE Cholinesterase Test System is for in vitro diagnostic use only. The system is for laboratory use by trained laboratory technicians only.

The Test-mate ChE Cholinesterase Test System is for the quantitative determination of cholinesterase in whole blood to monitor pesticide exposure. Although cholinesterase testing may be valuable in the diagnosis of succinylcholine sensitivity and in the diagnosis of liver dysfunction, this system is not intended to be used for these purposes.

The photometric analyzer supplied as part of the Test-mate ChE Cholinesterase Test System is for use only with the AChE Erythrocyte Cholinesterase Assay Kit and the PChE Plasma Cholinesterase Assay Kit and are not intended for use with any other reagent kit.

The AChE Erythrocyte Cholinesterase Assay Kit and the PChE Plasma Cholinesterase Assay Kit are for use only with the photometric analyzer supplied as part of the Test-mate ChE Cholinesterase Test System and are not intended for use with any other manual or automated test method or equipment.

Never touch the bottom half of the glass vial when handling the assay tubes, it must be kept clean in order for the photometric analyzer to operate properly. To prevent damaging the optical system inside the photometric analyzer, use only powder free gloves and avoid using powdered gloves with the Test-mate ChE system.

Hazards

The Test-mate ChE is safe for both the operator and the patient, as long as safety procedures are carefully followed, especially with regard to using gloves and disposal of waste. *Always exercise universal precautions. Blood samples can transmit infectious diseases such as hepatitis and AIDS, and therefore such samples should be handled with appropriate care.* To prevent damaging the optical system inside the photometric analyzer, use only powder free gloves and avoid using powdered gloves with the Test-mate ChE system.

The emergency treatment of a severely poisoned patient should be based primarily upon the patient's physical symptoms, rather than cholinesterase test results.

> The therapeutic index of the 1995 Physicians' Desk Reference for Generics lists only two treatments for pesticide poisoning: atropine (pp.248-249) and pralidoxime (pp.2303-2306). In the latter reference (p.2304) it is stated "Treatment of organophosphate poisoning should be instituted without waiting for the results of laboratory tests. Red blood cell, plasma cholinesterase and ... [other tests] may be helpful in confirming the diagnosis and following the course of the illness."
>
> In Chapter 4 of Drug Evaluations 1995, published by the American Medical Association, "Drugs Used in the Management of Poisoning" pp. 66-67, "Diagnosis of Poisoning. Known exposure associated with compatible symptoms is sufficient to initiate atropine therapy. If nicotinic effects persist, pralidoxime should be administered (Murphy, 1986; Minton and Murray, 1988). Therapy should not be delayed pending the results of laboratory tests; red blood cell and plasma cholinesterase ... help to confirm the diagnosis." Even if inappropriately used, neither atropine nor pralidoxime are significantly toxic at recommended dosages.

Maintenance and Service Information

The Test-mate ChE Cholinesterase Test System does not require any routine maintenance. Unlike other photometric instruments, the Test-mate ChE photometric analyzer employs a solid-state light emitting diode (LED) as an analytic light source. This LED does not burn out and is not subject to breakage.

Should the front panel of the photometric analyzer become smudged with fingerprints, simply wipe the panel with a lightly dampened paper towel. Do not use harsh cleansers or cleaning solutions on the instrument panel.

Should any component of the Test-mate ChE Cholinesterase Test System not function properly, please contact EQM Research for assistance before returning the system. If the malfunctioning component requires factory service, a Return Merchandise Authorization (RMA) number will be issued. Send the Test-mate ChE with a description of the difficulty, postage and insurance prepaid, to EQM Research. Please clearly mark the RMA number on the outside of the shipping carton. EQM Research cannot assume responsibility for damage in transit. Warranty repairs are free of charge. Non-warranty service is billed on a time and materials basis. The Test-mate ChE will be returned to you, postage and insurance prepaid.

Appendix A - Outline of the Test Procedure

1. Turn on the photometric analyzer. Press the mode key to select either the AChE assay procedure or the PChE assay procedure. Press the test key to begin the assay.

2. Insert the new assay tube into the analyzer. Press the test key to continue the assay.

3. When prompted by the analyzer, remove the assay tube. Press the test key to continue the assay.

4. Fill the 10µL capillary with blood (wipe excess with filter paper) and place it into the assay tube. Shake the assay tube vigorously for 15 seconds. Align the capillary and then insert the assay tube into the analyzer. Press the test key to continue the assay.

5. When prompted by the analyzer, remove the assay tube. Press the test key to continue the assay.

6. Dissolve the reagent with 3 drops of distilled water. Add the dissolved reagent to the assay tube using the transfer pipette. Immediately, press the test key to continue the assay.

7. Shake the assay tube by gentle inversion for 5 seconds. Align the capillary and then insert the assay tube into the analyzer. Press the test key to continue the assay.

8. When prompted by analyzer, remove and discard the assay tube. Press the test key to continue the assay.

9. Record the analyzer readings using the test key to advance the display. Press the done key to finish the assay.

Appendix B - Specimen Collection and Reagent Specifications

Specimen Collection: Either fresh fingerstick blood or venipuncture blood (anticoagulated with EDTA) can be used. The puncture site should be thoroughly washed before sampling in order to minimize possible sample contamination from pesticide residue adsorbed to the skin. To avoid clotting, the capillary should be placed into the assay tube within 10 seconds. Cholinesterase can reactivate, especially from carbamate pesticide inhibition during prolonged storage. Such reactivation can produce a "false negative".[7]

Reagent Specifications: For in vitro diagnostic use. For the quantitative determination of cholinesterase in whole blood to monitor pesticide exposure. For laboratory use by trained laboratory technicians only.

1. ***Buffer*****:** 2mL per assay tube. Contains phosphate, surfactant, dye and EDTA preservative.
2. ***Distilled water*****:** 15mL in plastic dropper bottle.
3. ***AChE Erythrocyte Cholinesterase Reagent:*** Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of distilled water. Stable 72 hours at 15 - 35°C after reconstitution. Final assay includes: 1mM AcTC, 0.3mM DTNB, 20µM As1397, 50mM potassium phosphate and **0.03% Triton X-100** (white cap), pH 7.6.
4. ***PChE Plasma Cholinesterase Reagent:*** Lyophilized, 96 tests per plate. Store lyophilized regent at 15 - 30°C, protected from light. Reconstitute with 3 drops of distilled water. Stable 72 hours at 15 - 35°C after reconstitution. Final assay includes: 2mM ButC, 0.3mM DTNB, 50mM potassium phosphate and **0.03% Triton X-100** (white cap), pH 7.6.

The AChE reagent is >95% specific due to the addition of the specific PChE inhibitor As1397 (10-(α-diethylaminopropionyl)-phenothiazine**)**.[6]

The PChE reagent is >95% specific due to the specificity of ButC.

Appendix C - Components of the Test System

The Model 400 Test-mate ChE Cholinesterase Test System contains the following components: an instruction manual, a hard-shell storage case with foam insert, a photometric analyzer, one Model 460 AChE Assay Kit, one reagent-opening tool and one assay tube rack. The reagent-opening tool and the assay tube rack are shipped in a reusable cardboard storage box.

The Model 460 AChE Assay Kit contains three boxes and a package insert. Box one contains 48 assay buffer tubes. Box two contains 48 assay buffer tubes. Box three contains a 96 well erythrocyte reagent plate, 100 capillary tubes (10µL volume), 100 filter papers (capillary wipes), a 30mL clear plastic dropper bottle filled with 18mL of distilled water and 2 transfer pipettes. The reagent plate in the AChE Assay Kit has a red “Erythrocyte - AChE” label and the transfer pipettes in the AChE Assay Kit have a red band.

The Model 470 PChE Assay Kit contains three boxes and a package insert. Box one contains 48 assay buffer tubes. Box two contains 48 assay buffer tubes. Box three contains a 96 well plasma reagent plate, 100 capillary tubes (10µL volume), 100 filter papers (capillary wipes), a 30mL clear plastic dropper bottle filled with 18mL of distilled water and 2 transfer pipettes. The reagent plate in the PChE Assay Kit has a blue “Plasma - PChE” label and the transfer pipettes in the PChE Assay Kit have a blue band.

Note: Never interchange the reagent plate or the transfer pipettes when switching between AChE and PChE testing.

Appendix D - Additional Materials Required for Testing

In addition to the equipment and supplies provide with the Model 400 Test-mate ChE Cholinesterase Test System, the Model 460 AChE Assay Kit and the Model 470 PChE Assay Kit, the following readily available materials must be obtained:

- Power free gloves (2 gloves per patient)
- Finger stick lancets (1 lancet per patient)
- Alcohol wipes (1 wipe per patient)
- Sterile gauze pads (1 pad per patient)
- Small round bandages (1 bandage per patient)
- Biohazard disposal container

Appendix E - Photometric Analyzer Specifications

Size	5¾" x 3½" x 1¾"
Weight	10 oz.
Power Requirement	9 VDC, 0.025 Amp. (Battery or Line Adapter)
Light Source	Blue Light Emitting Diode
Light Detector	Silicon Photodiode
Monochromator	Interference Filter
Wavelength	450nm ±2.0nm
Bandwidth	10.0nm ±2.0nm
Lightpath	13mm
Optical Linearity	to 3.000A
Optical Resolution	0.001A
Optical Stability	<0.005A/assay
Temperature Accuracy	0.3°C @ 22.5°C
Temperature Resolution	0.1°C
Temperature Linearity	0.1°C

Appendix F - Photometric Analyzer Warning Messages

The Test-mate ChE photometric analyzer contains a sophisticated micro-processor. This computer performs several checks on the environmental conditions and assay reactions. Should an abnormal condition be detected, three short beeps will sound and a warning message will be displayed. To cancel the warning message, the test key should be pressed. Investigate the source of the warning and then repeat the assay procedure.

BLOOD DELAY: Over 120 seconds elapsed without the operator completing the blood sampling. Complete the blood sampling within this time period.

HGB < 5.0: The hemoglobin level was below a plausible level. Check the blood sampling technique.

REAGENT DELAY: Over 60 seconds elapsed without the operator adding the reagent. Add the reagent within this time period.

SHAKE DELAY: Over 20 seconds elapsed without the operator having finished shaking the assay tube after adding the reagent.

TEMP < 15.0C: The recommended ambient temperature range for the assay procedure is between 15.0°C and 30.0°C. Move to a warmer location.

TEMP > 30.0C: The recommended ambient temperature range for the assay procedure is between 15.0°C and 30.0°C. Move to a cooler location.

TEMP CHANGE: The temperature of the assay tube changed more than 0.7°C over the course of the assay procedure. Move to a temperature stable environment or allow the assay tubes additional time to equilibrate.

NO REACTION: The change in absorbance caused by the reagent was below a plausible level. Make sure to add the reagent when instructed by the photometric analyzer.

Appendix G - Photometric Analyzer Error Messages

The Test-mate ChE photometric analyzer contains a sophisticated micro-processor. This computer performs several checks on the electronics, optics, and the environmental conditions. Should a problem be detected, an error message will be displayed. To clear the error message the analyzer must be turned off and then back on.

ADC ERROR: The analog-to-digital converter is not functioning properly. Contact EQM Research, Inc. for assistance.

FULL SCALE ERROR: Not enough light was detected during blanking. Make sure that a fresh assay tube is in place during blanking.

LO BATTERY ERROR: The 9-volt battery that powers the analyzer is in need of replacement. Substitute a fresh battery for the old one.

HI BATTERY ERROR: The voltage of the external power supply is greater than 10.2 volts. Obtain a power supply with the correct output voltage.

RAM ERROR: The internal data storage of the microprocessor has been damaged. Contact EQM Research, Inc. for assistance.

ROM ERROR: The internal program storage of the microprocessor has been damaged. Contact EQM Research, Inc. for assistance.

TOO COLD ERROR: The test temperature was less than 10.0°C and the analyzer cannot temperature compensate the reaction. Move to a warmer environment.

TOO HOT ERROR: The test temperature was greater than 40.0°C and the analyzer cannot temperature compensate the reaction. Move to a cooler environment.

ZERO SCALE ERROR: Too much light was detected during blanking. Make sure that a fresh assay tube is in place during blanking.

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Warranty

EQM Research, Inc. (EQM) warrants the Test-mate ChE Cholinesterase Test System (Test-mate) to be free from defects in material and workmanship under normal use and service for one year from the date of purchase. This warranty extends to the original purchaser and does not apply to any Test-mate, which in EQM's sole opinion, has been subject to misuse, alteration, or abnormal conditions of operation or handling.

To obtain warranty service, please contact EQM to obtain a Return Merchandise Authorization (RMA) number. Send the Test-mate with a description of the difficulty, postage and insurance prepaid, to EQM. Please clearly mark the RMA number on the outside of the shipping carton. EQM assumes no risk for damage in transit.

EQM will, at its option, repair or replace the defective Test-mate free of charge. However, if we determine that the failure was caused by misuse, alteration, of abnormal conditions of operation or handling, you will be billed for the repair. The Test-mate will be returned to you, postage and insurance prepaid.

This warranty is exclusive and is in lieu of all other warranties, expressed or implied, including but not limited to any implied warranty of merchantability or fitness for a particular purpose of use. EQM will not be liable for any special, indirect, incidental or consequential damages or loss, whether in contract, tort or otherwise.