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AChE Erythrocyte Cholinesterase Assay Kit (Model 460)
&
PChE Plasma Cholinesterase Assay Kit (Model 470)

Package Insert

Intended Use
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. The Test-mate ChE Cholinesterase Test System is useful in the assessment of pesticide poisoning. Most organophosphate or carbamate pesticides inhibit the blood enzymes erythrocyte cholinesterase (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. After exposure to pesticides, recovery of AChE activity is usually slower than PChE due to its longer half-life (1 month for AChE vs. 2 weeks for PChE).[3,4] Pre-exposure (baseline) measurements of AChE and/or PChE should be obtained to reduce the effect of biological variability.[1]

Principle of the Method
The Test-mate ChE reagents are 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.

Cholinesterase
thiocholine ester (AcTC/BuTC) ==========> thiocholine

thiocholine + DTNB ==========> TNB-thiocholine + TNB (*yellow*)

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

Contents
Each 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 reagent plate, 100 capillary tubes (10µL volume), 100 filter papers (capillary wipes), a clear plastic dropper bottle filled with 18mL of reagent solvent, 2 transfer pipettes and a 9 Volt battery. The reagent plate in the AChE Assay Kit has a red "Erythrocyte" label and the PChE Assay Kit has a blue "Plasma" label. The transfer pipettes in the AChE Assay Kit have a red band 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.*

Instrumentation
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.

Reagents

1. ***Buffer*:** 2mL per assay tube. Contains phosphate, surfactant and EDTA preservative.
2. ***Reagent Solvent:*** 18mL of distilled water and EDTA preservative in a plastic dropper bottle.
3. ***Erythrocyte Cholinesterase Reagent:*** (AChE Erythrocyte Cholinesterase Assay Kit)
   Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°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.
   Plasma Cholinesterase Reagent: (PChE Plasma Cholinesterase Assay Kit)
   Lyophilized, 96 tests per plate. Store lyophilized regent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°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.

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]

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. Vigorously shake the assay tube 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 reagent solvent. Add the dissolved reagent to the assay tube using the transfer pipette. Immediately, press the TEST key to continue the assay.
7. Gently shake the assay tube by 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.

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.

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

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

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 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.

Limitations
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 therefore, AChE activity. PChE is depressed in liver failure and malnutrition. PChE is 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 artificially decrease values.[9] Washing the skin with quaternary ammonium-containing detergents, such as benzethonium chloride can also artificially decrease values; check the detergent label before using.

Accuracy
The Test-mate ChE was compared with the Boehringer Mannheim Cholinesterase Kit No. 450035 on the Hitachi 704 (BM/H).[10] The BM/H method is performed on plasma (PChE) or diluted whole blood (AChE) corrected by hematocrit; therefore, 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 RBCs (AChE) at 37°C.

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	0.322	±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 - 5U/mL: 3 - 5%CV. Between-run, N=40, 1 - 5U/mL: 5 - 7%CV.

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

Expected Values
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	.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

Between-Operator Variability
Ten operators each performed ten measurements on both a normal and an abnormal venipuncture sample (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

Interpretation of Results
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]

References

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