Test-mate (Model 400) erythrocyte acetylcholinesterase (AChE) test

Version 2

H.K. Jeevan Dhanarisi[1], Indika B. Gawarammana[2], Fahim Mohamed[3], Michael Eddleston[4]

[1]South Asian Clinical Toxicology Research Collaboration, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka, [2]Department of Medicine, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka, [3]Faculty of Allied Health Sciences, University of Peradeniya, Peradeniya, Sri Lanka, [4]Pharmacology, Toxicology, & Therapeutics, University/BHF Centre for Cardiovascular Science, University of Edinburgh, UK

dx.doi.org/10.17504/protocols.io.wpyfdpw

Jeevan Dhanarisi

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0200133

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Dhanarisi HKJ, Gawarammana IB, Mohamed F, Eddleston M (2018) Relationship between alcohol co-ingestion and outcome in profenofos self-poisoning – A prospective case series. PLoS ONE 13(7): e0200133. doi: 10.1371/journal.pone.0200133

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

- For the quantitative determination of erythrocyte acetylcholinesterase (AChE) in blood to monitor pesticide exposure.
- For in vitro diagnostic use.
- For laboratory use by trained laboratory technicians only.

MATERIALS

NAME	CATALOG #	VENDOR
Buffer: 2mL per assay tube. Contains phosphate, surfactant, dye and EDTA preservative.		
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.		
Distilled water: 15mL in plastic dropper bottle.		
Powder 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		
Filter papers (capillary wipes)		
Transfer pipettes.		

NAME	CATALOG #	VENDOR
Test-mate ChE Photometric Analyzer		
Capillary tubes (10μL)		

SAFETY WARNINGS

Precautions must be taken to eliminate hazards either to the operator or the person being tested. The following rules must be followed during the testing procedure.

1. Do not reuse blood lancets or capillaries.
2. Put used lancets and capillaries in a hazard bag.
3. The operator must wear gloves and safety glasses.

Erythrocyte AChE activity analysis

1 Remove Test-MateTM components from the carrying case and lay them out, within easy reach, in front of you on a flat surface.

2 Press the “Power” key. The display screen indicates a 15 seconds warm up count down.
Write up the temperature on your sheet.

3 Press the “Mode” key until “AChE mode” is displayed.

4 Remove the plastic covering of one of the reagent wells of the microplate with the biopsy punch. Add three drops of reagent to the well and dissolve the reagent by aspirating it using the transfer pipette. Fill the transfer pipette with the dissolved reagent.

5 Press the “Test” key. The display reads “Add blood” followed by “Insert tube” and “Press Test”. Add three drops of buffer to the tub e, mix with the stirring paddle.

6 Insert the tube into the colorimeter. Press the “Test” key. A 10 second “Blanking” is displayed ending in a “beep”. “Remove tube” followed by “Press Test” is displayed.

7 Fill the capillary tube with blood (by sticking the end of the cleaned finger with the lancet) and dispense into the buffer filled tube after removing any excess blood from the outside of the capillary by rolling the end of the capillary over a filter paper. Shake the assay tube vigorously for 15 seconds and insert the tube into the sample analyzer.

8 Press the “Test” key. The display reads “Add blood” followed by “Insert tube” and “Press Test”

9 Press the “Test” key. A 30 second “Reading” is displayed followed by a “beep”. During this time Hgb has been determined. The display reads “Remove tube” followed by “Press test”.

10 Press the “Test” key. The display reads “AT BEEP” followed by “Add reagent”. At the “beef” add the reagent in the transfer pipette to the diluted blood-filled tube. Insert tube followed by “Press test”. The display reads “Shake”. Shake the tubes by inverting five times.

11 Press the “Test” key. The display shows pre-incubation countdown followed by a 50 seconds reading phase ending in a “beep”. The analysis of erythrocyte AChE is concluded.
The display reads “Remove tube” followed by “Press test”. Remove the tube and pour the solution into the waste bottle.

12 Press the “Test” key to initiate results recovery. After the first result is displayed, keep pressing the “Disp.” key to recover the rest of the data.