LETTER to EDITOR

Clarifying an Electrometric Method for Determining Blood Cholinesterase Activity: A Scientific Letter

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Abstract

In this scientific letter, a modified electrometric method is clarified for rapid and accurate determination of blood cholinesterase (ChE) activity in man and various animal species. Our electrometric method for ChE determination is a modified one that we refined and developed after several years of research and validations in various animal species as well as in man. The developed method has been applied in many research projects on poisoning with organophosphate and carbamate insecticides. Given the importance of the subject of ChE biomonitoring, and to further clarify the assay technique of the modified electrometric method, a brief and a concise description of the procedure would be beneficial for researchers of limited resources. The enzymatic (ChE) reaction mixture consisted of 3 ml of distilled water, 0.2 ml of plasma, erythrocytes or whole blood, and 3 ml of barbital-phosphate buffer (pH 8.1). The initial pH (pH1) of the mixture is measured with a pH meter, and thereafter 0.1 ml of the substrate acetylcholine iodide (7.1%) or acetylthiocholine iodide (7.5%) is added. The mixture is incubated at 37 ºC for 30 min in most animal species or for 20 min in man; the pH2 of the reaction mixture is measured again. The activity of blood ChE is calculated as follows:
ChE activity (Δ pH/20 min-in man) = (pH1 – pH2) - Δ pH of blank (no blood sample).

Keywords: Cholinesterase Method, Organophosphates, Carbamates, Insecticide poisoning

How to cite this article: Mohammad FK. Clarifying an Electrometric Method for Determining Blood Cholinesterase Activity: A Scientific Letter. **Asia Pac J Med Toxicol 2022; 11(1):30-32.**

Monitoring blood (whole blood, plasma, serum or erythrocytes) cholinesterase (ChE) activity is used to assess the exposure of man and animals to organophosphate and carbamate insecticides [1-3]. Usually a reduction of blood ChE activity by 20-30% from baseline values is an indication of exposure to ChE inhibiting pesticides [2,3]. Enzyme inhibition by > 50% calls for immediate healthcare measures (1-3). Several methods are available to measure ChE activity in the blood and nervous tissues [3,4]. A modified electrometric method is available for measuring blood and brain ChE activity that is a simple procedure which does not need elaborate equipment and was adopted by many researchers [5-27]. However, a published article erroneously referenced this modified electrometric method mentioned above [28]. This prompted us to clarify and elucidate few key points about the method. The original electrometric Michel method has two incubation periods for a total of more than one hour at 25 ºC [29], whereas our electrometric method is mainly characterized by a single incubation period of 20 min in man (5) and 30 min or even more in different animal species at 37 ºC [6-27].

Our electrometric method of ChE determination is a modified one that we refined and developed after several years of research and validations in various animal species [6-27] as well as in man [5,25]. We also applied the developed method on many research projects on poisoning with organophosphate and carbamate insecticides [6-26]. For review of differences in animal species and man, see published references [5,18,23-26,30].

Because of the importance of the subject of ChE biomonitoring [1-4] and to further clarify the assay technique of the modified electrometric method, here is a brief but concise description of the procedure. It is hoped that it would be beneficial for researchers of limited resources.

Chemicals:

7.5% aqueous solution of acetylthiocholine iodide (or 7.1% acetylcholine iodide), sodium barbital, potassium dihydrogen phosphate, sodium chloride, and double distilled water.

Barbital-Phosphate buffer:

1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride/ L distilled water, pH 8.1.

Equipment:

Centrifuge, Water bath, pH meter

Procedure:

1. Collect venous blood samples in heparinized test tubes.

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2. Separate the plasma from erythrocytes by centrifugation.

3. To a 10-ml beaker add 3 ml distilled water, 0.2 ml plasma, erythrocytes or even whole blood.

4. Add 3 ml of barbital-phosphate buffer solution.

5. Measure the pH of the mixture (pH1).

6. Add 0.10 ml of 7.5% acetylthiocholine iodide (or 7.1% acetylcholine iodide) to the mixture.

7. Incubated at 37 ºC for 30 min for most animal species; 20 min in man.

8. Measure the pH of the mixture (pH2).

9. Calculate cholinesterase activity:

ChE activity (ΔpH/30 min or 20 min) = (pH1 – pH2) - Δ pH of blank

The blank is without sample. The unit of activity is Δ pH/30 or 20 min.

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