

Cholinesterase (ChE) Test Using Ellman's Photometric Method

Y. Kusumo Adi Arji Atmanto^{1*}, Mutmainnah^{2,3}, Ibrahim Abd. Samad^{2,4}

¹Clinical Pathologist Education Program, Faculty of Medicine,
Hasanuddin University, Makassar, South Sulawesi, Indonesia

²Department of Clinical Pathology, Faculty of Medicine,
Hasanuddin University, Makassar, South Sulawesi, Indonesia

³Clinical Pathology Laboratory Installation of dr. Wahidin Sudirohusodo Hospital,
Makassar, South Sulawesi, Indonesia

⁴Clinical Pathology Laboratory Installation of Ibnu Sina Hospital,
Makassar, South Sulawesi, Indonesia

*Corresponding author details: Y. Kusumo Adi Arji Atmanto; kusumoadi.pk@gmail.com

ABSTRACT

Cholinesterase (ChE) is a group of enzymes that hydrolyze cholinester. There are two ChE isoenzymes in the blood, namely Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE). Both ChE isoenzymes are required in the process of sending nerve signals. The activity of these two ChE isoenzymes will be decreased by exposure to toxic chemical agents, insecticides such as organophosphates or carbamates, anesthetic agents, and drug therapy for Alzheimer's disease (Donepezil or Rivastigmine). Therefore, AChE and BChE are potential biomarkers of suppression and increased activity of the central and peripheral nervous systems. In addition, measurement of serum ChE enzyme activity helps assess liver function, among other parameters. ChE enzyme activity can be measured by decreasing substrate concentration or increasing product concentration. Many methods have been developed to measure the activity of ChE as an enzyme. However, of these methods, the most popular and the gold standard is the photometric method based on increasing the concentration of Thiocholine, which is also known as Ellman's photometric method. ChE enzyme activity was measured according to the increase in yellow color production using a photometer using endpoint methods at a wavelength (λ) of 412 nm. ChE is slightly increased in diseases: thyrotoxicosis, schizophrenia, hypertension, acute emotional disturbances, and increases 2-3 times the normal value in nephrotic syndrome. Serum ChE is decreased in impaired hepatic synthesis, chronic liver disease, hypoalbumin, and after the use of anticholinesterase drugs. In acute and chronic hepatitis, ChE decreases by about 30%-50%. A decrease in ChE of 50%-70% can be seen in cirrhosis and carcinoma that metastasizes to the liver.

Keywords: cholinesterase; biomarker; Ellman's photometric

INTRODUCTION

Cholinesterase (ChE) is a group of enzymes that hydrolyze cholinester. There are two ChE isoenzymes in the blood, namely Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE). Acetylcholinesterase (AChE) is known as True ChE, Specific ChE, Red Blood Cells (RBC) ChE, or Cholinesterase I which is not present in serum but is present in RBC and is found mostly in nervous tissue, lungs, and spleen. Butyrylcholinesterase (BChE) is known as Pseudo ChE, Non-Specific ChE, Serum/Plasma ChE, or Cholinesterase II which is present in serum/plasma and is found mostly in the liver, heart, and to a lesser extent in the pancreas.¹

Both ChE isoenzymes are required in the process of sending nerve signals. The activity of these two ChE isoenzymes will be decreased by exposure to toxic chemical agents, insecticides such as organophosphates or carbamates, anesthetic agents, and drug therapy for Alzheimer's disease (Donepezil or Rivastigmine).

Therefore, AChE and BChE are potential biomarkers of suppression and increased activity of the central and peripheral nervous systems. In addition, measurement of serum ChE enzyme activity helps assess liver function, among other parameters. The normal value of ChE in men is 4,620-11,500 U/L, while in women it is 3,930-10,800 U/L. Serum ChE activity is decreased in impaired liver synthesis function, chronic liver disease, and hypoalbumin because albumin acts as a ChE transport protein. The decrease in ChE is more specific than albumin for assessing hepatic synthetic function because it is less influenced by factors outside the liver. In acute and chronic hepatitis, ChE decreases by about 30%-50%. A decrease in ChE of 50%-70% can be seen in cirrhosis, hepatocellular carcinoma (HCC), and carcinomas that metastasize to the liver. Serial ChE measurements can help to assess the prognosis of liver disease patients and monitor liver function after liver transplantation.¹⁻³

ChE enzyme activity can be measured by decreasing substrate concentration or increasing product concentration. These reactions are usually followed continuously over time intervals, which makes it possible to verify whether the increase or decrease in concentration is linear during the test. Some methods are limited to the endpoint method, two-point method, and rate method.⁴

Many methods have been developed to measure the activity of ChE as an enzyme. However, of these methods, the most popular and the gold standard is the photometric method based on increasing the concentration of Thiocoline, which is also known as Ellman's photometric method. This method is the most popular although it has some limitations. This thiocholine is reacted with Ellman's reagent, namely 5,5-dinitrobis-2-nitrobenzoic acid (DTNB) to produce 5-thio-2-nitrobenzoate (TNB) which is yellow. ChE enzyme activity was measured according to the increase in yellow color on the photometer using a wavelength (λ) of 412 nm. There is also a rapid test for measuring ChE activity using the ChE Check Mobile tool. In this paper, the ChE test using Ellman's photometric method will be discussed.⁴⁻¹²

AIM

The purpose of this test is to determine the principle and method of examining ChE using Ellman's photometric method using a BioChem SA semi-automatic spectrophotometer.

METHOD

A. Pre-Analytic 13-16

1) Patient preparation

No special preparation is needed.

2) Sample preparation

The sample is a sample of venous blood. The sample used is serum or plasma. Avoid samples that are lipemic, hemolyzed, and icteric. Serum or plasma samples can be stored at 2-4°C for 1 week. Samples must be tightly closed.

3) Reagent preparation

Reagents hold up to their expiration date when stored at 2-8°C

4) Instruments and materials

Instruments:

- BioChem SA semi-automatic spectrophotometer
- Volumetric pipettes P1000, P200, blue and yellow tip pipettes
- Rack and test tube
- Centrifuge
- Incubator
- Timer

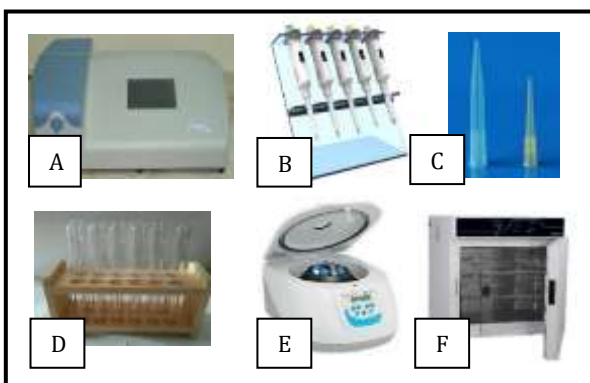


FIGURE 1: Instruments used in ChE test.

- Spectrophotometer, B. Volumetric Pipette, C. Tip Pipette, D. Rack & Test Tube, E. Centrifuge, F. Incubator

Materials:

- Reagent I (Substrate)
- 75 mM Acetylthiocholine Iodide (21.67 mg/ml) or 75 mM Butyrylthiocholine Iodide (21.67 mg/ml) in H₂O
- Reagent II (Buffer/Chromogen-Color Indicator)
- DTNB: 10 mM 5-5 Dithiobis (2-Nitrobenzoic Acid) (3.96 mg/ml) in 100 mM NaHPO₄, pH 7.0-8.0, contains 1.5 mg NaH₂CO₄.
- Calibrator or Control Material
- Aquabidest or 0.9% NaCl



FIGURE 2: Materials used in ChE test¹⁶

B. Analytic

1) Test principle

Measurement of ChE activity using Ellman's photometric method. Acetylthiocholine (ATCh) artificial substrate was hydrolyzed by ChE enzymes into Acetate and Thiocoline. Thiocoline is then reacted with Ellman's reagent, namely 5,5-dithiobis-2-nitrobenzoic acid (DTNB) to produce 5-thio-2-nitrobenzoate (TNB) which is yellow. ChE enzyme activity was measured according to the increase in yellow color production using a photometer using endpoint methods at a wavelength (λ) of 412 nm. It is based on the reaction (Figure. 3 and 4)⁴⁻¹¹

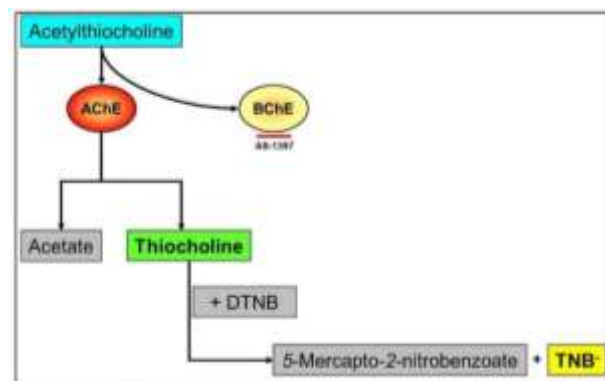


FIGURE 3: AChE Enzymatic Reaction Schematic with the Ellman's Method⁹

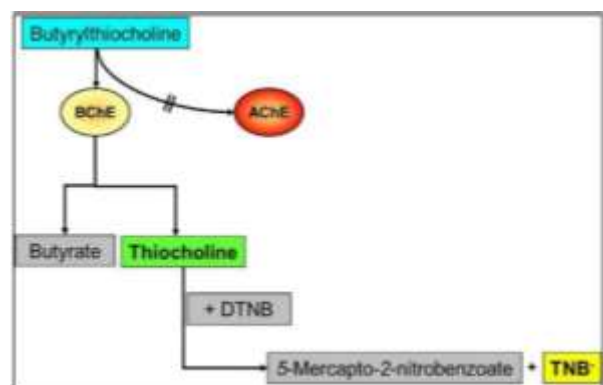


FIGURE 4: Schematic of BChE Enzymatic Reaction with the Ellman's Method⁹

2) The procedure with BioChem SA Semi-Automatic Spectrophotometer¹⁴⁻¹⁶

- a. The venous blood sample is centrifuged to obtain serum or plasma.
- b. Prepare reagents and samples at room temperature.
- c. Prepare 3 test tubes, namely blank tubes, calibrator tubes, and sample tubes.
- d. On the main menu of the spectrophotometer, select Edit Item → select the Liver Function examination panel → select the Cholinesterase test parameter by pressing CHE
- e. Set the configuration of the selected parameters → Method Endpoint, Wavelength: 412 nm, Unit: U/L, Measure Time: 2 sec, then press Save.
- f. Return to the main menu → select Test Items → select the Liver Function examination panel → select the Cholinesterase test parameter by pressing CHE, a screen will appear to measure the absorbance of the blank, the absorbance of the standard/calibrator, and the absorbance of the sample.
- g. Select Blank Test to measure the absorbance of the blank. Prepare a blank tube, pipette 200 µl of Reagent I ChE, and 20 µl of Aquabidest/ 0.9% NaCl. Mix well, then incubate the blank for 3 minutes at 37°C. After that add 200 µl of Reagent II ChE into the blank tube. Mix well, then incubate the blank for 5 minutes at 37°C. Then pull the blank by pressing the sipper to measure the absorbance.
- h. After measuring the absorbance blank, press Standard Test to continue checking the absorbance standard/calibrator. Prepare a standard tube/calibrator, pipette 200 µl of Reagent I ChE, and 20 µl of the calibrator. Mix well, then incubate the standard/calibrator for 3 minutes at 37°C. After that add 200 µl of Reagent II ChE into a standard tube/calibrator. Mix well, then incubate the standard/calibrator for 5 minutes at 37°C. Then pull the standard/calibrator by pressing the sipper to measure the absorbance.
- i. After measuring the absorbance of the standard/calibrator, press Sample Test to continue checking the absorbance of the sample. Prepare a sample tube, pipette 200 µl of Reagent I ChE, and 20 µl of the sample. Mix well, then incubate the sample for 3 minutes at 37°C. After that add 200 µl of Reagent II ChE into the sample tube. Mix well, then incubate the sample for 5 minutes at 37°C. Then pull the sample by pressing the sipper, and read the results of the ChE levels in the result (U/L).

TABLE 1: ChE Test procedure with BioChem SA Semi Semi-Automatic Spectrophotometer

Blank	Standard/Calibrator	Sample
I ChE 200 µl reagent	I ChE 200 µl reagent	I ChE 200 µl reagent
20 µl aquabidest	20 µl standard materials/ calibrator	20 µl sample material
Homogenize, incubate for 3 minutes at 37°C, then add		
II ChE 200 µl reagent	II ChE 200 µl reagent	II ChE 200 µl reagent
Homogenize, incubate for 5 minutes at 37°C		
Pull the blank	Pull the standard/calibrator	Pull the sample

C. Post-analytic^{11,16-19}

1) ChE reference value

Normal range:

- Male = 4.620-11.500 U/L (4.62-11.5 kU/L)
- Female = 3.930-10,800 U/L (3.93-10.8 kU/L)

2) Linearity

- a. Lowest linearity = 50 U/L
- b. Highest linearity = 20,000 U/L

3) Interpretation

Cholinesterase is slightly increased in diseases: thyrotoxicosis, schizophrenia, hypertension, acute emotional disturbances, and increases 2-3 times the normal value in nephrotic syndrome. Serum cholinesterase is decreased in impaired hepatic synthesis function, chronic liver disease, and hypoalbumin, and after the use of anticholinesterase drugs.

In acute and chronic hepatitis, ChE decreases by about 30%-50%. A decrease in ChE of 50%-70% can be seen in cirrhosis and carcinoma that metastasizes to the liver.

4) Limitations

ChE test using Ellman’s photometric method has several limitations, which are influenced by oxime reactivity and hemoglobin absorbance. The use of a semi-automatic spectrophotometer where pipetting, mixing reagents, and incubation are carried out manually, therefore there is the possibility of human error.

Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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