CHOLINESTERASE CE

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REF	1119005	CHOLINESTERASE	30 tests
For *in vitro* diagnostic use only			

CHOLINESTERASE

TOTAL AND INHIBITED

Enzymatic colorimetric method

KINETIC

PRINCIPLE

Cholinesterase (CHE) catalyzes the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine. The latter reduces 5,5´-mercaptobis-2-nitrobenzoic acid (DMNB) to 5-mercapto-2-nitrobenzoate (5-MNBA), a colored compound.
The reaction is monitored kinetically at 405 nm by the rate of formation of the yellow color produced, proportional to the activity of CHE in the sample[1].

$$\text{Butyrylthiocholine} + H_2O \xrightarrow[\text{pH 7.7}]{\text{CHE}} \text{Butyrate} + \text{Thiocholine}$$

$$\text{Tiocholine} + \text{DMNB} \longrightarrow \text{Oxidized thiocholine} + \text{5-MNBA} + H^+$$

Dibucain inhibition can be estimated by performing concurrent assays in which dibucaine is present in the substrate mixture. Percent inhibition is evaluated by comparison of activity in the inhibited system with that in the uninhibited system. The resulting dibucaine number allows the classification and identity of the homozygous and heterozygous variants.

REAGENT COMPOSITION

R1 **Buffer/Chromogen.** Phosphate buffer 50 mmol/L pH 7.7, DMNB 0.25 mmol/L. Powder.

R2 **Substrate.** Butyrylthiocholine iodide 7 mmol/L. Freeze-dried.

R3 **Dibucaine.** Dibucaine Clorhidrate 2.6 mmol/L.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagents are stable until the expiry date stated on the label.
Discard reconstitud reagents **R1** and **R2** if present an absorbance over 0.700 at 405 nm against distilled water.

REAGENT PREPARATION

Working reagents.

1. ***Buffer/Chromogen***. Add 25 mL of distilled water into a vial of **R1**. Cap. Shake. Stand for 15 min. before use. Stable for 6 weeks at 2-8ºC.
2. ***Substrate***. Add 2.0 mL of distilled water into a vial of **R2**. Mix. Stable for 6 weeks at 2-8ºC. Excess substrate may be frozen once.
3. ***Inhibitor reagent***. Mix 9 volumes of Buffer/Chromogen with 1 volumen of **R3**.

SAMPLES

Serum or heparinized plasma. Moderate hemolysis does not interfere. Cholinesterase in serum or plasma is stable for several weeks whether the specimen is stored at room temperature or under refrigeration, and for 3 months at –20ºC.

INTERFERENCES

- Specimens from patients who have displayed apnea after succinylcholine treatment should not be obtained until after paralysis has passed; metabolites of the drug appear to interfere with the assay.
- A list of drugs and substances wich cause changes in cholinesterase levels or interfere with its determination has been published.[2]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37ºC, capable to read at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagents and samples to reaction temperature (see NOTES).
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into labelled cuvettes:

Temperature	25/30ºC		37ºC	
Treatment	Without inhibitor	**With inhibitor**	Without inhibitor	**With inhibitor**
Buffer/Chromogen	1.5 mL	–	1.5 mL	–
Inhibitor reagent	–	**1.5 mL**	–	**1.5 mL**
Sample	10 µL	**10 µL**	–	–
Sample dil 1:2 with saline	–	–	10 µL	**10 µL**
Substrate	50 µL	**50 µL**	50 µL	**50 µL**

4. Mix gently by inversion. Insert cuvette into the cell holder, start stopwatch and record the initial absorbance.
5. Repeat the absorbance readings exactly after 30, 60 and 90 seconds.
6. Calculate the difference between absorbances.
7. Calculate the mean of the results to obtain the average change in absorbance per second (ΔA/30 sec).

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CALCULATIONS

Total cholinesterase

U/L = ΔA/30sec x 23111 (25/30ºC)
U/L = ΔA/30sec x 46222 (37ºC)

Samples with ΔA/30sec exceeding 0.250 at 405 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 16.67 = nkat/L

Inhibited cholinesterase

To express the dibucaine number apply:

$$\text{Percent inhibition} = \left[1 - \frac{\text{U/mL with inhibitor}}{\text{U/mL without inhibitor}}\right] \times 100$$

REFERENCE VALUES

Total cholinesterase[3]

Serum, plasma

Children Males and females > (40 years)	3.5-8.5 KU/L (58.3-141.7 µktal/L)
Females, (16-39 years) Nonpregnant, not taking contraceptives	2.8-7.4 KU/L (46.7-123.3 µktal/L)
Females, (18-41 years) Pregnant or taking contraceptives	2.4- 6.0 KU/L (40.0-100.0 µktal/L)

Inhibited cholinesterase

Dibucaine number	Percent inhibition
Normal homozygous	70-90
Heterozygous subjects	35-75
Atypical homozygous	0-20

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of cholinesterase. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of alkaline cholinesterase. Assayed.

CLINICAL SIGNIFICANCE

The cholinesterase of the serum (CHE) has been referred as pseudocholinesterase to distinguish it from the true acetylcholinesterase (AcCHE) of the red cells and nerve tissue.

Cholinesterase levels in serum has been used as a test for the liver function, as an indicator of possible insecticide poisoning and for the detection of patients with atypical forms of the enzyme. As a measure of hepatic disease function it a appears to add little to more commonly used laboratory measurements. However, CHE is a sensitive parameter to poisoning by inhalation or contact with organic phosphorus compounds that inhibit cholinesterase activity. Among them are many organic insecticides, such as Parathion, Sarin and tetraethylpyrophosphate[4].

The genetic control of serum CHE activity has some practical importance. Two forms of serum CHE have been recognized. One has been called “normal” and the other “atypical”. Individuals homozygous for the “atypical” gene can be distinguished ready from the homozygous “normal”. The homozygous abnormal has very low CHE levels and the abnormal CHE is not inhibited by dibucaine. The homozygous normal has much higher levels of serum cholinesterase inhibitable by dibucaine, while the heterozygous has intermediate levels and response to the inhibitors. This fact has clinical importance in regard to the administration of muscle relaxants (succinylcholine). Homozygous abnormals may develop prolonged apnea after they receive succinylcholine.

NOTES

Buffer/Chromogen and **Substrate** can be mixed proportionally in tests or analysers using the serum as starter. The mixture is stable for 2 hours at 15-25ºC.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 10 KU/L (37ºC)
- ***Precision***

KU/L	Within-run			Between-run		
Mean	4.1	6.8	140	4.1	6.8	140
SD	40.1	100.8	130.1	43.5	107.1	129.6
CV%	0.98	1.48	0.93	1.06	1.56	0.86
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: COBAS MIRA for 8 days.

- ***Sensitivity.*** Using this reagent and method an ΔA/30sec of 0.010 read at 405 nm is equivalent to 1,36 U/L of cholinesterase activity.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 25 r = 0.993 y = 0.95x+ 1.02

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