Cholinesterase

Butyrylthiocholine. Kinetic

Quantitative determination of cholinesterase (CHE)

IVD

PACKAGING

Ref.: 101-0286	Cont.: 20 x 2.5 mL

Store at 2-8ºC

CLINICAL SIGNIFICANCE

Cholinesterase is an enzyme present in plasma and synthesized by the liver. Its true physiological function is unknown, so its function may be to hydrolyze choline in plasma. Cholinesterase activity is usually measured for liver function, is a sensitive test of exposure to pesticides organophosphorus and identification of patients with the atypical form of enzyme whose presents high sensitivity to succinyl-choline[1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Cholinesterase hydrolysed butyrylthiocholine to butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form 5-mercapto-2-nitrobenzoic acid (5-MNBA) according the following reactions:

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Butyrate} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{DTNB} \longrightarrow \text{5-MNBA}$$

The rate of 5-MNBA formation, measured photometrically, is proportional to the enzymatic activity of cholinesterase in the sample[1,2].

REAGENTS

R 1 Buffer	Phosphate pH 7.7	50 mmol/L
R 2 Substrate	5,5-dithiobis-2-nitrobenzoic ac. (5,5 DTNB)	0.25 mmol/L
	Butyrylthiocholine	7 mmol/L

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR):

Dissolve (→) one tablet of R 2 Substrate in one vial of R 1.

Cap vial and mix gently to dissolve contents.

Stability: 2 hours at 2-8º C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use the tablets if appears broken.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm ≥ 1.20.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25º C, 30º C or 37º C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or heparinized plasma[1]: Stability 7 days at 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 405 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . 25º C / 30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	25 - 30º C	37º C
WR (mL)	1.5	1.5
Sample (µL)	10	--
Sample diluted 1/2 with NaCl (9 g/L) (µL)	--	10

4. Mix and wait 30 seconds.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 30 seconds intervals thereafter for 1.5 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per 30 seconds (ΔA/30 s).

CALCULATIONS

25º- 30º C ΔA / 30 s x 22710 = U/L

37º C ΔA / 30 s x 45420 = U/L

Calculating factor in automatic analyzers (ΔA/min.) is 22710 at 37º C.

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

TEMPERATURE CONVERSION FACTORS

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.24	1.55
30º C	0.81	1.00	1.26
37º C	0.64	0.80	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

25º C	30º C	37º C
3000 - 9300 U/L	3714 - 11513 U/L	4659 - 14443 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

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PERFORMANCE CHARACTERISTICS

Measuring range (at 37º C): From detection limit of 7 U/L to linearity limit of 9084 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/5 with NaCl (9 g/L) and multiply the result by 5.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	5992	3087	6277	3254
SD	70.0	56.1	50.5	66.0
CV (%)	1.17	1.82	0.80	2.03

Sensitivity: 1 U/L = 0.0002 ΔA/30 s.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Moderate haemolysis will not interfere in the results.[1,2]. A list of drugs and other interfering substances with cholinesterase determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

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