CHOLINESTERASE (BTC)

Kinetics determination of cholinesterase in serum and plasma

TEST SUMMARY

Cholinesterase is an enzyme that hydrolyses acetylcholine and other choline esters.
There are two types of cholinesterases: acetylcholinesterase (AChE) or cholinestersase I, and butyrolcholinesterase (BChE) or pseudocholinesterase or cholinesterase II.

Acetylcholinesterase is involved in the transmission of nerve impulses. It hydrolyse acetylcholine, a substance used as a mediator of nerve transmission, and allows preparation for a subsequent impulse.
The decrease in acetylcholinesterase activity can lead to hyperstimulation of the innervations present in the tissues and organs involved.

Pseudocholinesterase is present not only in the blood but also in the liver, pancreas and heart.
Pseudocholinesterase is able to hydrolyze a wider range of choline esters than acetylcholinesterase, including some drugs used to achieve muscle relaxation during anesthesia conducted with artificial ventilation.
In the presence of reduced pseudocholinesterase activity in plasma, the paralyzing action of these drugs could be prolonged beyond what is necessary.
There may be an increase in pseudocholinesterase in various diseases such as diabetes, nephrotic syndrome, hyperthyroidism and acute and chronic liver disease
A decrease in activity can be caused by acute infections, myocardial infarction, liver disease, anemia.

PRINCIPLE OF THE TEST

Sericeous cholinesterase (pseudocholinesterasi) catalyzse the hydrolysis of butyrylthiocholine (BTC) forming Butyrate and Thiocholine, which reduces ferricyanide ions (III) to ferrocyanide (II). The decreasing of absorbance at 405 nm is proportional to the enzymatic activity of the sample.

SAMPLES

Serum, plasma (EDTA or heparine). Do not utilize sodium fluoride as anticoagulant as it inhibits enzyme activity. Avoid the haemolysis. Immediately separate serum or plasma from erythrocytes as they contain cholinesterase. Cholinesterase activity increase of about 25-30% a day if serum or plasma are in contact with red blood cells.
Stability: 30 days at 2-8°C.

REAGENTS

Reagent A:	Sodium pyrophosphate 75 mM, potassium ferricyanide (III) 2 mM, stabilizers, pH 7.6.
Reagent B:	Goods Buffer 25 mM, Butyrylthiocolyne 75 mM, stabilizers, pH 4.0.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Distilled water.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Add 10 ml of Reagent B to a vial of Reagent A. Stability of Work Reagent: 3 weeks at 2-8°C away from light source.
Reagent A and Reagent B are stable until expiration date on label away from light source at 2-8°C.

Stability after first opening: ≥ 60 days at 2-8°C.

PROCEDURE (SAMPLE STARTER)

Kind of analysis:	Kinetics (decrease)
Reading time:	60, 120, 180, 240 sec.
Wavelength:	405 nm
Temperature:	37°C
Lightpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Work reagent	1000 µl
Preincubate at 37°C for 5 minutes	
Sample	15 µl
Mix, after 60 seconds measure the absorbance against water, incubate at 37°C. Execute other 3 readings at 60 seconds distance. Calculate the ΔA/min.	

CALCULATION

Serum/Plasma

Sericeous cholinesterase total (U/l) = ΔA/min. x 74200

EXPECTED VALUES

Total Cholinesterase

Men	5600 – 11200 U/l
Women	4200 – 10800 U/l

Dibucaine number:

Normal homozygous	> 75 %
heterozygous	35 – 75 %
Atypical homozygous	< 35 %

Every laboratory should establish own reference intervals in accordance with own population.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATIN/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

CC03100 10 x 5 ml
Control Sera normal values

CC03200 10 x 5 ml
Control Sera pathological values

TEST PERFORMANCE

Precision
Reproducibility studies of intra and inter assay precision gave CV% < 1.5.
A correlation study with similar commercial method gave the following results: y = 1,0107x– 79,75 with r = 0.9995.

Sensibility/limit of detenction
The method is able to discriminate up to 432.3 U/l.

Linearità
The method is linear up to 15000 U/l.
If ΔA/min is exceeded to 0.25 is suggested to dilute the sample 1+9 with physiological solution and perform again the test, multiplying the results by 10.

Interferences
No interference was observed by the presence of:

hemoglobin	≤ 180 mg/dl
Triglycerides	≤ 1000 mg/dl

Some medicines can interfere with the Cholinesterase dosage.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC01200	(200 TESTS)	
Reagent A	4 x 40 ml	(liquid)
Reagent B	1 x 40 ml	(liquid)

REFERENCES

Kaplan, LA., Pesce, A.J. : Clinical Chemistry, Mosby Ed. (1996).
Eur.J.Clin.Chem.Clin.Biochem. Vol. 30, 1992, 162-170.
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).
Tietz NW,: Clinical guide to laboratory tests, Second Edition, Saunders Co., (1991).

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SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

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