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CORMAY CHOLINESTERASE

Kit name	(EN) Cat. No
CORMAY CHOLINESTERASE mini	2-056
CORMAY CHOLINESTERASE 30	2-057
CORMAY CHOLINESTERASE 60	2-058
CORMAY CHOLINESTERASE 120	2-059

INTENDED USE

Diagnostic kit for determination of cholinesterase activity intended to use for manual assay (Sample Start and Reagent Start method) and on several automatic analysers.
The reagents must be used only for *in vitro* diagnostic, by suitably qualified laboratory personnel, only for the intended purpose, under appropriate laboratory conditions.

INTRODUCTION

There are two cholinesterases (CHE and ACHE) differing in substrate specificity, tissue of origin and biological role. The term cholinesterase (ACHE), also known as acetylcholine acetylhydrolase, is found in erythrocytes, in the lungs, spleen and in grey matter of the brain. The pseudocholinesterase (CHE), also referred to as acylcholine acylhydrolase, is found in serum, the liver, pancreas, heart and in the white matter of brain. The assay of serum cholinesterase (CHE) is useful to diagnose: liver disorders, hepatitis, cirrhosis, carcinoma with metastasis, sensitivity to succinylcholine administration and pesticide poisoning. Levels decrease in all of the diseases above.

METHOD PRINCIPLE

Optimized kinetic method according to Deutsche Gesselschaft für Klinische Chemie (DGKC).
The method uses butyrylthiocholine as the specific substrate for cholinesterase (CHE). Cholinesterase catalyses the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine, in presence of potassium hexacyanoferrate (III). Thiocholine reduces potassium hexacyanoferrate (III) (yellow colour) to potassium hexacyanoferrate (II) (colourless). The decrease in absorbance is directly proportional to the CHE activity in the sample.

REAGENTS

Package

	CORMAY CHOLINESTERASE mini	CORMAY CHOLINESTERASE 30	CORMAY CHOLINESTERASE 60	CORMAY CHOLINESTERASE 120
1-REAGENT	2 x 24 ml	5 x 24 ml	5 x 48 ml	5 x 96 ml
2-REAGENT	1 x 12 ml	1 x 30 ml	1 x 60 ml	1 x 120 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. On board stability of the reagents depends on type of analyser used for analysis.Working reagent is stable 4 weeks at 2-8°C

Concentrations in the test

1-REAGENT

pyrophosphate buffer, pH 7.7	65 mmol/l
hexacyanoferrate (III)	2 mmol/l

2-REAGENT

Good's Buffer pH 4.0	20 mmol/l
butyrylthiocholine iodide	65 mmol/l

Warnings and notes

- Do not freeze reagents.
- Protect from direct sunlight, evaporation and avoid contamination!
- Immediately after use, recap the bottles and store at 2-8°C.
- Do not use after expiry date.
- Do not interchange caps.
- Reagents should be mixed before use by gentle inverting the bottle several times.
- The appearance of turbidity or control sera values outside the manufacturer's acceptable range may indicate of reagent instability.

SPECIMEN

Fresh serum free from haemolysis, plasma (EDTA, heparin) not hemolyzed.
Serum / plasma should be separated from red blood cells as soon as possible after blood collection. Do not use citrate, borate, oxalate and fluoride as an anticoagulant because it inhibits cholinesterase activity. It is recommended to follow CLSI procedures regarding specimen collecting and handling.
Sample may be stored for up to 15 days at 2-8°C or 12 months at -20°C.
Nevertheless it is recommended to perform the assay with freshly collected samples!

ADDITIONAL EQUIPMENT

- automated analyser or photometer able to read at 405 nm (400 – 440 nm);
- thermostat at 37ºC;
- general laboratory equipment.

PROCEDURE

Assay can be performed with use of separate 1-REAGENT and 2-REAGENT or with use of working reagent.
For working reagent preparation mix gently 4 parts of 1-REAGENT with 1 part of 2-REAGENT
Applications for automatic analysers are available on request.

Manual procedure

wavelength	405 nm (400 – 440 nm)
temperature	37°C
cuvette	1 cm
reaction type	Kinetic

Sample Start method

Pipette into the cuvettes:

	reagent blank (RB)	test (T)
working reagent	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

distilled water	15 µl	-
sample	-	15 µl

Mix well, after 1 minute incubation at 37°C read absorbance A of the reagent blank (RB) and test (T) against air. Repeat the reading after next 1, 2 and 3 minutes against reagent blank (RB).
Calculate the mean absorbance change per minute (ΔA/min.).

Reagent Start method

The determination can be also performed with use of separate
1-REAGENT and 2-REAGENT.
Pipette into the cuvettes:

	reagent blank (RB)	test (T)
1-REAGENT	800 µl	800 µl

Bring up to the temperature of determination. Then add:

distilled water	15 µl	-
sample	-	15 µl

Mix well and incubate 1 minute at 37°C, then add:

2-REAGENT	200 µl	200 µl

Mix well and after exactly 4 min. incubation at 37°C read absorbance A of the reagent blank (RB) and test (T) against air. Repeat the reading after next 1, 2 and 3 minutes against reagent blank (RB).
Calculate the mean absorbance change per minute (ΔA/min.).

Calculation

cholinesterase activity [U/l] = ΔA/min. x 66844

REFERENCE VALUES [4,5]

serum / plasma	37°C	
female	4000 – 12600 U/l	67 – 210 µkat/l
male	5100 – 11700 U/l	85 – 195 µkat/l

In infants up to 6 months of age, cholinesterase activity is 40% to 50% higher than in adults. In young adult (< 35 years) women, the enzyme activity is approximately 64% to 74% of that in adult males. The activity decreases during pregnancy.
It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) **or** CORMAY MULTICALIBRATOR LEVEL 2 (Cat. No 5-175; 5-177) **is recommended depending on the calibrator lot number.**
Calibration stability depends on type of analyser used for analysis. The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 223 U/l (3.717 µkat/l).
- **Linearity:** up to 21000 U/l (350 µkat/l).

For higher activity dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor

- **Specificity / Interferences**

Haemoglobin up to 5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	4832	18.70	0.39
level 2	6966	64.96	0.93

Reproducibility (day to day) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	7167	113.19	1.58
level 2	4905	77.94	1.59

- **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 77 samples gave following results:
y = 1.1039 x - 566.16 U/l;
R = 0.999 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders, 708-11, (1999).
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3. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO. Publication WHO/DIL/LAB/99.1 (Rev.2. Jan. 2002).
4. Kaplan LA. Pesce AJ: "Clinical Chemistry", Mosby Ed. 967, (1996).
5. Internal reference range studies.

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