

CHOLINESTERASE FL (DGKC)

CH F245 CH

12 x 24 ml

SUMMARY OF TEST

Two related enzymes have the ability to hydrolyze acetylcholine. One is acetylcholinesterase, which is called true cholinesterase, or choline esterase I. True cholinesterase is found in erythrocytes, in the lungs and spleen, in nerve endings, and in the gray matter of the brain. It is responsible for the prompt hydrolysis of acetylcholine released at the nerve endings to mediate transmission of the neural impulse across the synapse. The degradation of acetylcholine is necessary to the depolarization of the nerve so that it can be repolarized in the next conduction event.

The other cholinesterase is acylcholine acylhydrolase; it is usually called pseudocholinesterase, benzoyl cholinesterase, or choline esterase II. Although it is found in the liver, pancreas, heart, white matter of the brain, and serum, its biological role is unknown. The serum enzyme is the one whose assay is clinically useful.

The cholinesterase present in normal sera can be separated by electrophoresis into 7 to 12 bands, the number obtained depending on the experimental technique used. The isoenzymes of SChE differ in molecular size and appear to be aggregates of different numbers of the same basic unit.

The normal, most common phenotype is designated as E^a₁E^a₁ or UU. The gene E^a₁ is referred to as the atypical gene, the sera of persons homozygous for this gene (E^a₁E^a₁ = AA) are only weakly active toward most substrates for cholinesterase and possess increased resistance to inhibition of enzyme activity by dibucaine.

The mutations that give rise to the atypical and dibucaine-resistant SChE variants involve a change in the structure of the active center. The variant isoenzymes are less effective catalysts than the usual form; the affinity of the enzymes for the substrates is reduced, and affinity for competitive inhibitors such as dibucaine or fluoride is similarly decreased. This gives rise to the characteristic dibucaine- or fluoride-resistant properties of the genetic variants that are exploited in their characterization. The homozygous forms, AA or FF, are found in only 0.3 to 0.5% of the white population.

Cholinesterase levels in serum are useful as a test of liver function, as an indicator of possible insecticide poisoning, or for the detection of patients with atypical forms of the enzyme. The spread of values encountered in apparently healthy people is rather wide, but the level in any given person is fairly constant. Levels at birth are only one fourth of those of adults but reach adult levels by the second month of life. No enzyme is found in urine.

Measurements of SChE activity can serve as sensitive measures of the synthetic capacity of the liver if a patient's normal (baseline) level is known, which unfortunately is rarely the case. In the absence of known inhibitors, any decrease in activity in serum reflects impaired synthesis of the enzyme by the liver, as in acute hepatitis and in chronic hepatitis of long duration. Decreases of 50 to 70% occur in advanced cirrhosis and carcinoma with metastases to the liver. Essentially normal levels are noted in chronic hepatitis, mild cirrhosis, and obstructive jaundice. Decreased levels of serum enzyme are also found in patients with acute infections, pulmonary embolism, muscular dystrophy, chronic renal disease and in pregnancy, as well as after surgical procedures. After a myocardial infarction, the enzyme level decreases until the fifth day and then begins a slow rise to normal.

Among the organic phosphorus compounds that inhibit cholinesterase activity are many organic insecticides, such as Parathion, Sarin, and tetraethyl pyrophosphate.

Succinylcholine (suxamethonium) and mivacurium are drugs used in surgery as muscle relaxants. Because succinylcholine is very similar to acetylcholine, it is also hydrolyzed by cholinesterase, and its physiological effect persists only long enough (30-50 min) to meet the needs of the surgical procedure. In patients with low levels of enzyme activity or in those with the atypical, weakly active enzyme variants, destruction of this drug does not occur rapidly enough, and patients may enter a period of prolonged apnea requiring mechanical ventilation until the drug is eliminated by other routes. Preoperative screening has been advocated to identify patients in whom suxamethonium administration may lead to complications.

Measurements of total SChE activity as well as determination of the "dibucaine number" are needed to characterize cholinesterase variants fully. The latter parameter indicates the percentage inhibition of enzyme activity toward specified substrates in the presence of a standard concentration of inhibitor. The average values of the dibucaine numbers for normals, heterozygotes, and homozygotes (E^a₁ gene) are 78, 60, and 16%, respectively, when benzoylcholine is used as substrate. The phenotypes most susceptible to apnea after succinylcholine administration are AA, AS, FF, FS, SS, AF, and to some extent UA.

PRINCIPLE OF THE METHOD

This reagent is formulated according to DGKC recommendations. Cholinesterase catalyzes the hydrolysis of butyrylthiocholine, forming butyrate and thiocholine, which reduces the ferricyanide ions to ferrocyanide.

The decrease in absorbance is followed at 405 nm and it is proportional to cholinesterase activity in examined sample.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: 12 x 20 ml (liquid) blue cap

Reagent B: 3 x 16 ml (liquid) red cap

Composition in the test: sodium pyrophosphate 75 mM, pH 7.6, potassium ferricyanide 2 mM, butyrylthiocholine 15 mM, stabilizers.

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Serum as starter procedure:

Add 4 ml of reagent B to a vial of reagent A.

Stability of working reagent: 15 days at 2-8°C, away from light sources.

Reagent as starter procedure:

use separate reagents ready to use.

Stability: up to expiration date on labels at 2-8°C;

Stability since first opening of vials: ≥ 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma (EDTA, heparinate only). Avoid hemolysis.

ChE is stable in sample for at least 14 days whether the sample is stored at room temperature or under refrigeration.

TEST PROCEDURE (sample as starter)

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1200 µl
preincubate at 37°C for 5 minutes.	
add sample:	20 µl
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 30 seconds intervals. Calculate the ΔA/min.	

TEST PROCEDURE (reagent as starter)

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
add sample:	20 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	200 µl
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 30 seconds intervals. Calculate the ΔA/min.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

Calculation in U/l: ΔA/min x 65800

Calculation in µkat/l: U/l x 0.0167 = µkat/l

EXPECTED VALUES

Total SChE:
Men: 5600 - 11200 U/l
Women: 4200 - 10800 U/l

Dibucaine number:
Normal homozygotes: > 75%
Heterozygotes: 35 - 75%
Atypical homozygotes: < 35%

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

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

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml

with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml

with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 25000 U/l.

If a ΔA/min of 0.30 is exceeded, it is suggested to dilute sample 1+9 with saline solution and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 432.3 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 500 mg/dl

bilirubin ≤ 40 mg/dl

Lipids ≤ 800 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	5972.9	122.8	2.1
sample 2	5743.8	57.5	1.0

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	5808.4	113.4	2.0
sample 2	5753.5	99.6	1.7

Methods comparison

a comparison between Chema Cholinesterase FL and a commercially available product gave the following results:

SChE Chema = x
SChE competitor = y
n = 107

y = 0.985x + 51.7 U/l r=0.996

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.








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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions