CHYMOTRIPSIN

Colorimetric determination of chymotripsin in faeces

TEST SUMMARY
Chymotripsin present in the sample hydrolyses the substratum forming a yellow compound.

SAMPLES
Faeces. Stability: 10 days at 15-25°C.

REAGENTS
Reagent
Chromogen: Succ-Ala-Ala-Pro-Phe-pNA 0.5 mM, Tris 100 mM pH 9,0 $CaCl_2$ 20 mM; NaCl 250 mM.

Diluent
for samples: Chloride of lauril-trimetil ammonium NaCl 500 mM $CaCl_2$ 100 mM.

MATERIAL REQUIRED BUT NOT SUPPLIED
Normal laboratory equipment. Spectrophotometer UV/VIS. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

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
Dissolve a vial of Chromogen with 2 ml of distilled water.
Reagents are stable until expiration date on label if stored at 2-8 °C
Reagent A reconstituted is stable 5 days at 2-8 °C.

SAMPLES PREPARATION
Dilute the sample 1 at 100 with the diluent (ex. 100 mg with 10 ml), homogenize, centrifuge and use the surnatant for the analysis.
Chymotripsin into surnatant is stable 3 hours at 15-25 °C and 24 hours at 2-8 °C.

PROCEDURE
Method:	Kinetic
Reading time:	1,2,3 minutes
Wavelength:	405 nm (400-420)
Temperature:	25, 30, 37°C
Pathlength:	1 cm
Zero:	air

REAGENTS	CONTROL	SAMPLE
Sample Chromogen	-- 2 ml	100 µl 2 ml
Mix carefully, wait for 1 minute and read absorbances after 1, 2, 3 minutes.		

CALCULATION
Calculate average value of extinction differences for min. (ΔE/min).

U/g (25/30/37°C) faeces = 212 x ΔE/min

EXPECTED VALUES
	25°C U/g	30°C U/g	37°C U/g
Normal Interval	>6	>8,4	>13,2
Interval that needs a control	3-6	4.2-8.4	6.6-13.2
Pathologic Interval	< 3	< 4.2	< 6.6

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

NOTE
- For faeces particularly solid, it is advisable to let rest sample with diluent before homogenisation for 10-15 minutes.
- Suspend supply of medicine containing pancreatic enzymes at least 5 days before test execution.
- Don't give laxative.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL
It is suggested to perform an internal quality control. For this purpose on request are available the following control sera.

CC03730 **2 x 1 ml**
Chymotripsin-Control Set
(2 levels)

TEST PERFORMANCE
Precision

In series (n = 10)	Mean (U/g)	SD (U/g)	CV%
Sample 1	7.40	0.51	7.0
Sample 2	18.23	0.87	4.8
Sample 3	56.62	1.64	2.9

Among series (n = 20)	Mean (U/g)	SD (U/g)	CV%
Sample 1	2.32	0.10	4.3
Sample 2	17.15	1.50	8.7
Sample 3	55.15	2.60	4.7

Sensitivity
The method is sensitive until 0.2 U/g.

Linearity
If Δ absorbance/min is > 0.030 dilute 100 µl of surnatant with 400 µl of diluent and repeat the determination. Multiply the result by 5.

Methods comparison
A comparison with an available commercial method gave following results on 20 samples compared:

LTA = x
Competitor = y
n = 20

y = 0,409 + 0,941x r = 0,98

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

PACKAGING
CODE CC03700	**(20 TESTS)**	
Chromogen	20 x 2 ml	(liophile)
Diluent for samples	2 x 100 ml	(liquid)

CODE CC03720		
Diluent for samples	6 x 100 ml	(liquid)

REFERENCES

Kasper, P., G. Moeller, A. W. Wahlefeld e. F. Staehler, Freserius Z. Anal. Chem. 311 (1982) 391-392.
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Ammann, R., Portschritte in dar Pankreasfunktionsdiagnostik, Springer Verlag, Berlin, Haidelberg, New York, 1967.

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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

Mod. 01.06 (ver. 1.1 - 05/12/2005)

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