UNIVERSITY of LIFE SCIENCES in Lublin
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF BIOCHEMISTRY
20-033 Lublin, Akademicka 12
Phone +48 81 445 66 08
www.biochwet.up.lublin.pl

Transferases -transaminases

Alanine and aspartate aminotransferase

Exercise 1

The aim is to determine the activity of AST and ALT and to be able to interpret obtained results.

The principle of method

The principle of method is based on the differences in absorbancy of 2,4-dinitrophenylohydrazones of alfa oxoglutaric acid and pyruvic acid in basic environment. As the result of the reaction catalysed by AST oxalacetic acid is synthesised which in turn by the proces of spontaneous decarboxylation creates pyruvic acid. Pyruvic acid reacts with 2,4-dinitrophenylohydrazine (DNFH) and forms hydrazone of pyruvic acid.

The measure of activity of aminotransferases is the increase of the content of pyruvic acid in tubes incubated with plasma samples in comparison with controls.

The reactions catalysed by aminotransferases:

$$\text{alanine : 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate : glutamate}$$

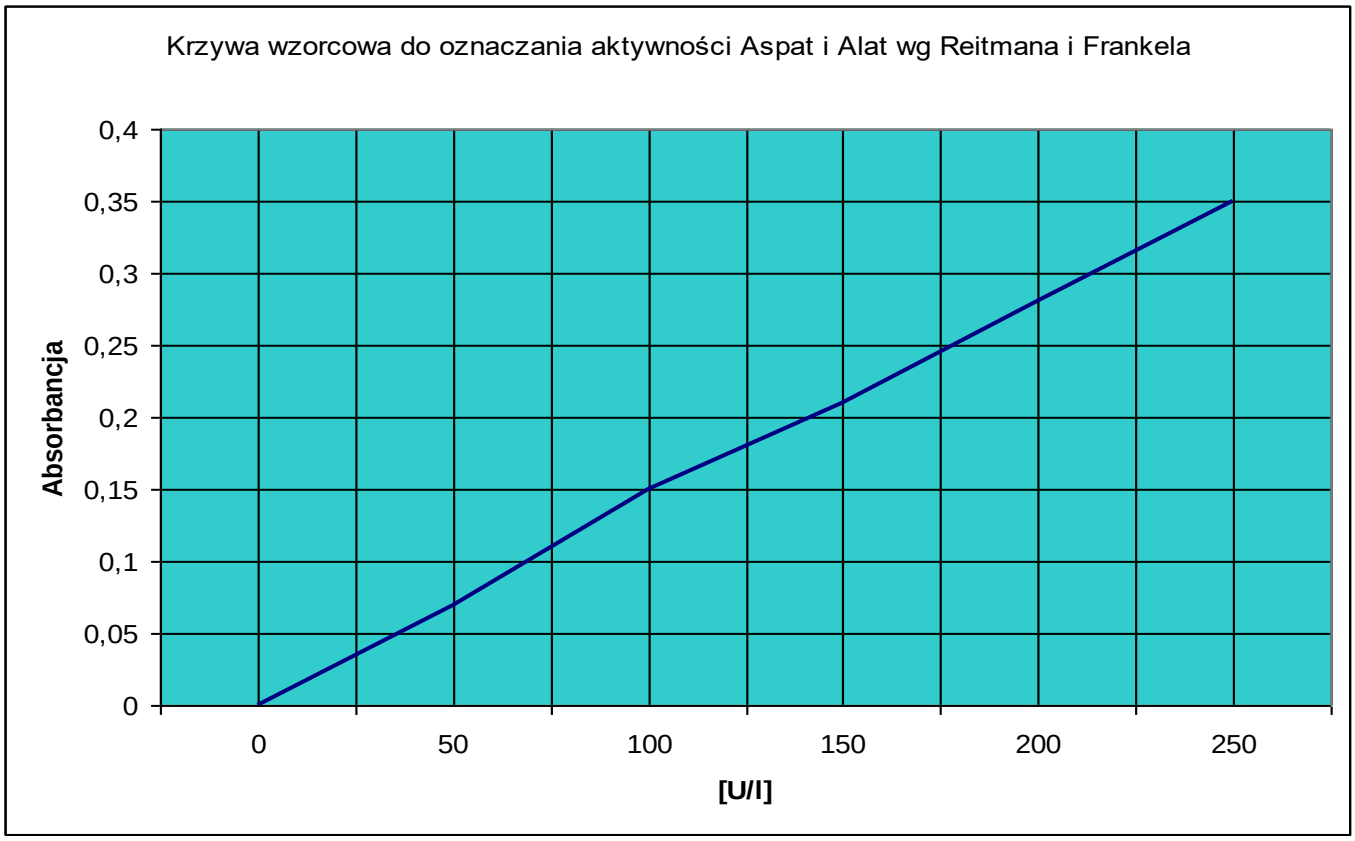
$$\text{aspartate : 2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxalacetate : glutamate}$$

Protocol

Mentioned below chemicals should be pipetted into 2 tubes marked as 1-control and 2-examined and followed the procedure given in the table.

Chemicals	(AST)		(ALT)	
	1-Control	2-Examined	1-Control	2-Examined
Substrate Solution of NaCl 0,9%	0,5 0,1	0,5 -	0,5 0,1	0,5 -
Incubation in water bath in temp. of 37° C	5 min.		5 min.	
Plasma	-	0,1	-	0,1
Incubation	30 min. in water bath in temp. of 37° C		60 min. in water bath in temp. of 37° C	
DNFH	0,5	0,5	0,5	0,5
Incubation	20 min. in room temp.		20 min. in room temp.	
0,4 mol/dm^3 NaOH	5	5	5	5

After 10 min the absorbancy of examined tubes should be measured against appropriate control tubes at 505 nm wave length. Obtained values should be recalculated into units of enzyme activity based on standard curve.



Activity of AST in blood plasma [U/l]

Horses	Cattle	Sheep	Goats	Pigs	Rats
205-555	58-100	40-96	122-321	16-65	190-260
Dogs	Cats	Rabbits	Guinea pigs	Hamsters	
1-37	6-44	42-98	27-68	28-122	