Transferases- transaminases (alanine and asparagine aminotransferase)

E.C.2.6.1.2.
Alanine Aminotransferase
(ALT)

The aim of exercise:

The evaluation of indicative enzyme – asparagine aminotransferase in plasma from healthy and ill cows

Localisation:

Intracellular enzyme localised in cytoplasm

- Blood plasma
- Liver
- Heart
- Muscles

Mechanism of action:

Reversible reaction of the transfer of amino group from alanine to ketoacid (alpha ketoglutarate) by use of phosphate pirydoxal (PLP)

The determination of activity:

- Is based on the difference in absorbance of 2,4-dinitrophenylhydrasones of alpha ketoglutaric acid in basic environment

Clinical meaning:

Indicative enzyme which is characteristic for liver diseases (inflammation, cirrhosis, jaundice) and damage to skeletal muscles (crushing, atrophia). Significant increase in ALT activity in plasma is detected in the course of these diseases.

E.C.2.6.1.1. Asparagine Aminotransferase (AST)

Localisation:

Intracellular enzyme localised in mitochondria and cytosol

- Heart
- Liver
- Muscles

Mechanism of action:

Reversible reaction of the transfer of amino group from asparagine to ketoacid (alpha ketoglutarate) by use of phosphate pirydoxal (PLP)

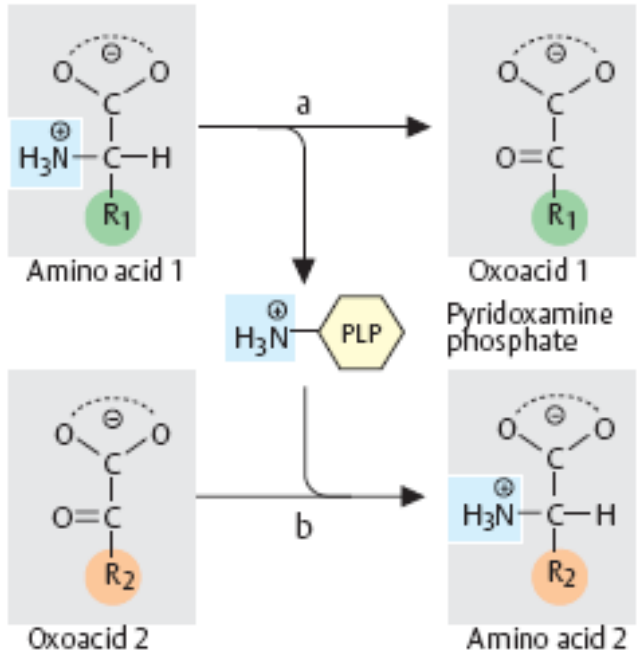
The determination of activity:

- Is based on the difference in absorbance of 2,4-dinitrophenylhydrasones of alpha ketoglutaric and pyruvatic acid in basic environment

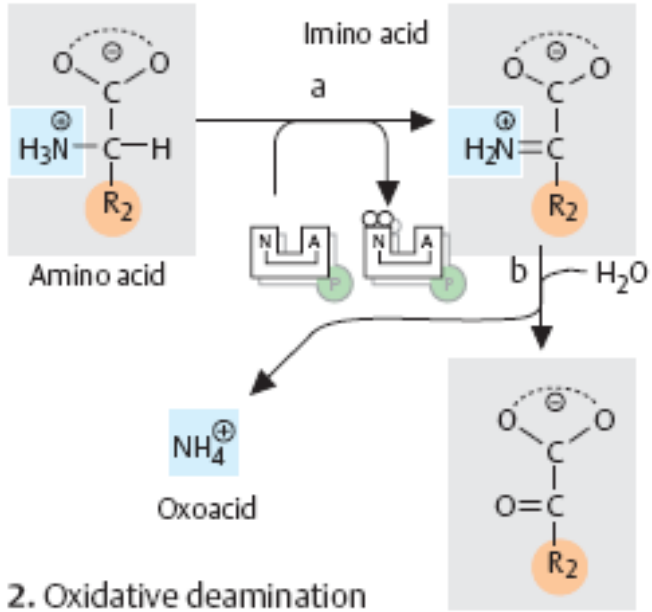
Clinical meaning:

Indicative enzyme characteristic for diseases of heart muscle (infarct, inflammation), liver (inflammation, cirrhosis, jaundice) as well as damage to skeletal muscles (crushing, atrophia). Significant increases in activity of AST in plasma are detected in the course of these diseases.

A. Transamination and deamination

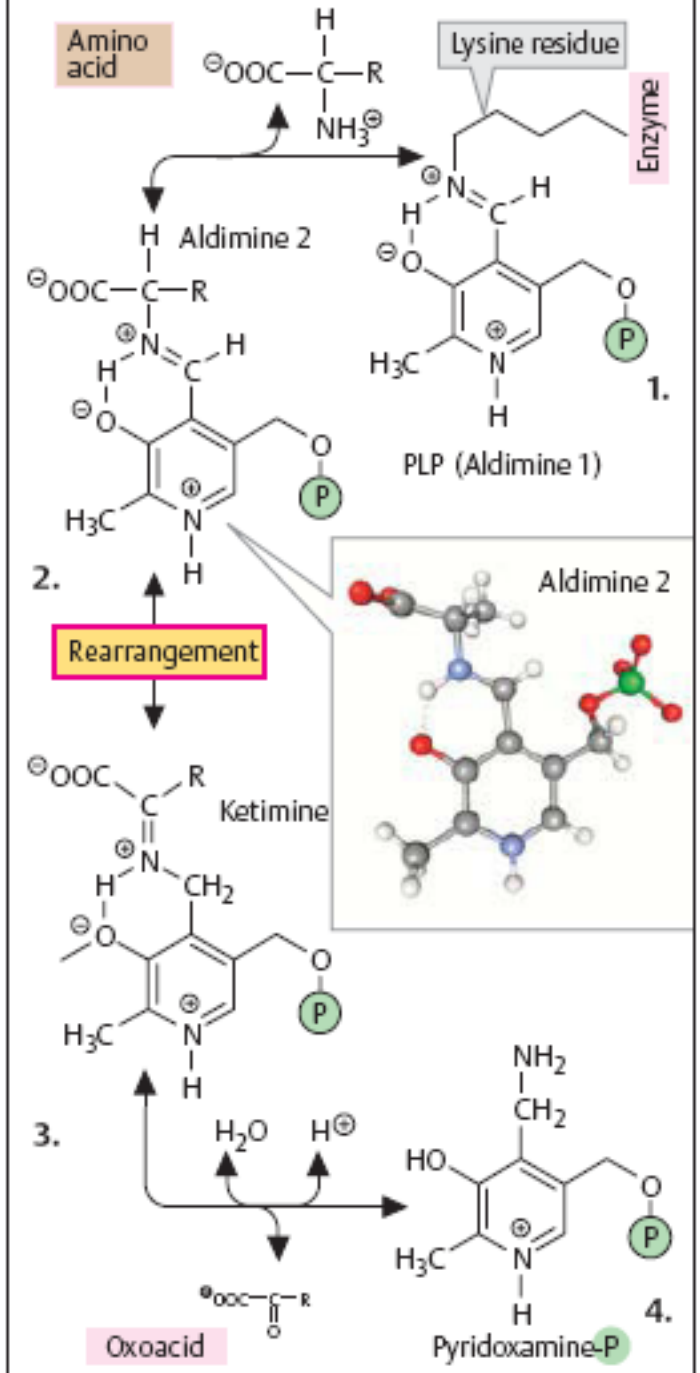


1. Transamination



2. Oxidative deamination

B. Mechanism of transamination



Alanine Aminotransferase (ALT, ALAT, GPT)

Activity of ALT in plasma (U/l)

Horses	Cattle	Sheep	Goats	Pigs
3-25	25-74	5-17	23-44	9-43
Dogs	**Cats**	**Rabbits**	**Guinea pigs**	**Chomiki syryjskie**
3-50	20-107	31-53	25-60	22-128

The increase in activity from reference value to 200 U/l

- Cancers of liver.
- Inflammation of pancreas.
- Hemolysis in vitro and in vivo.

The increase in activity between 200 U/l and 400 U/l

- Liver Cholestasis.
- Cirrhosis (the increase of AST in parallel)
- Treatment with high doses of salicylates.

The increase of activity between 200 U/l do 4000 U/l

- Viral liver inflammation.
- Toxic damage to liver.
- Circulation insufficiency.

Given values are related to optimal conditions of determination in 37°C. Activity of ALT in different conditions might be lower of 30%.

Hemolysis may result in artificial increase of results. The decrease in activity of 10 % in plasma after 3 days of storage in 4°C is observed. Plasma can be stored in -20°C up to 7 days without any change.

The increase in ALT activity in plasma may indicate rather on diffuse damage to cells than the disturbance in the function of organ.

In dogs like: doberman, Bedington terier, West highland white terier the increased activity of ALT may indicate on copper storage disease.

Asparagine Aminotransferase (AST, AspAT, GOT)

Activity of AST in plasma (U/l)

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

The increase from reference value to 200 U/l

(the exception horses and goats)

- Liver cirrhosis.
- Pancreatic inflammation.
- Hemolysis in vitro and in vivo.

The increase between 200 U/l and 400 U/l

- Diseases of skeletal muscles.
- Chronic liver inflammation.
- Surgery.
- Parasites.
- Insufficiency of selenium and vitamin E.

The increase between 200 U/l and 4000 U/l

- Infarct.
- Viral liver inflammation.
- Toxic liver damage.
- Cancers.
- Intense effort in sport horses.

Given values are related to the determination in optimal conditions at 37°C. Activities of AST in plasma and serum are similar. Hemolysis may resultin artificial increase of results.

The increase in activity of AST in plasma is rather related to diffuse damage to cells than the disturbance of function of organ.

De Ritis coefficient is useful in some diseases of dogs. It represents the ratio of AST and ALT. Physiological values are between 1,2 do 1,8.

Task 1

Metoda oznaczania aktywności AST i ALT oparta jest na różnicy absorbancji 2,4-dwunitrofenylohydrazonów kwasu alfa oksoglutarowego i pirogronowego w środowisku zasadowym. W wyniku reakcji katalizowanej przez AST powstaje kwas szczawiooctowy, który ulega samoistnej dekarboksylacji i tworzy się z 2,4-dwunitrofenylohydrazyną (DNFH) także hydrazon kwasu pirogronowego.

Miarą aktywności aminotransferaz jest zatem przyrost stężenia kwasu pirogronowego w probówkach inkubowanych z surowicą w porównaniu z probówkami kontrolnymi.

Reakcje katalizowane przez aminotransferazy:

$$\text{alanina : 2-oksoglutaran} \xRightarrow{\text{ALT}} \text{pirogronian : glutaminian}$$

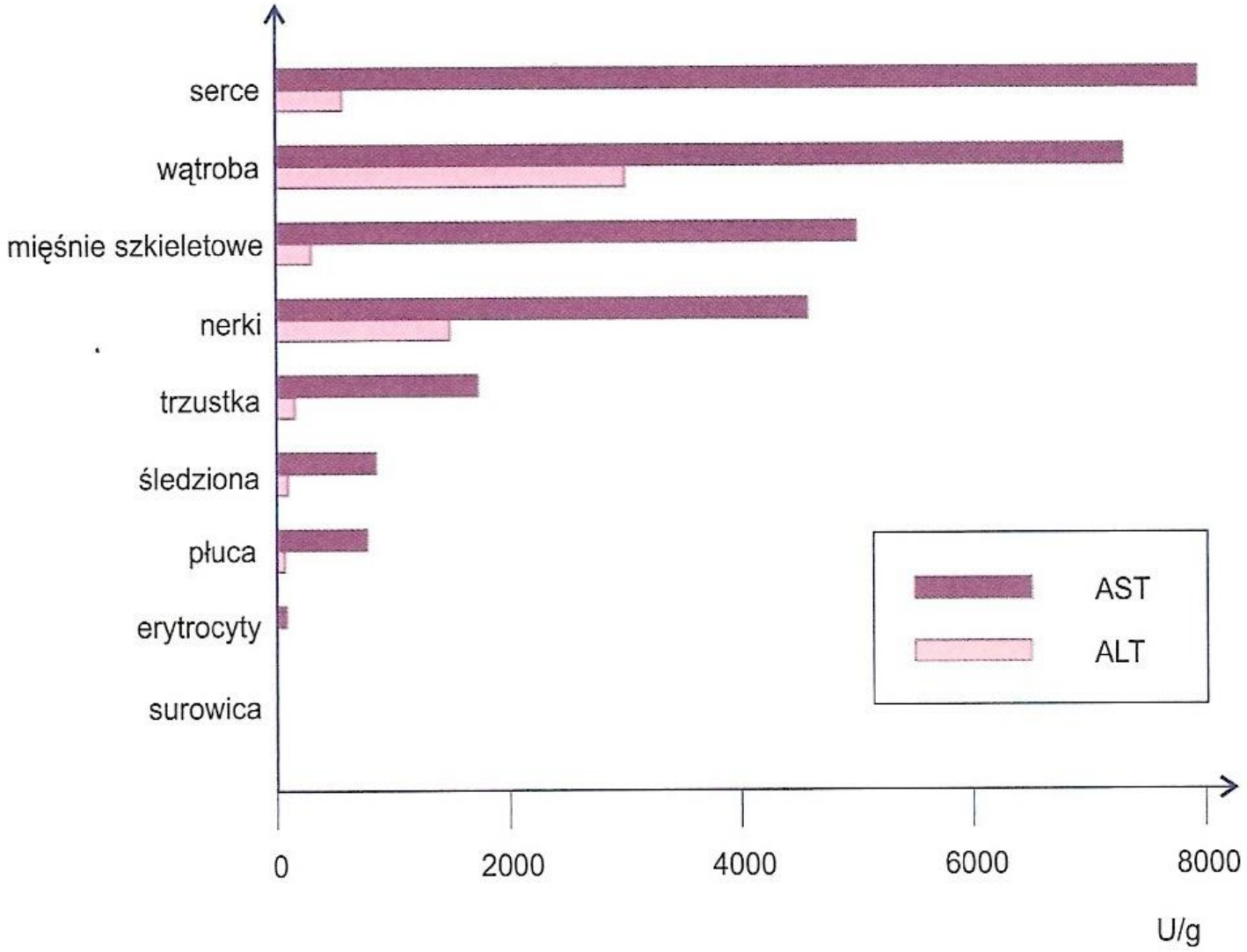
$$\text{aparaginian : 2-oksoglutaran} \xRightarrow{\text{AST}} \text{szczawiooctan : glutaminian}$$

Task 1

Wykonanie.

Do 2 probówek: 1 (kontr.), 2 (bad.) dodawać odczynniki zgodnie z poniżej zamieszczona tabelą.

Odczynniki	AST		ALT	
	Kontrola	Badana	Kontrola	Badana
Substrat Roztwór NaCl 0,9%	0,5 0,1	0,5 -	0,5 0,1	0,5 -
Inkubacja w łaźni wodnej w temp. 37°C	5 min.		5 min.	
Osocze	-	0,1	-	0,1
Inkubacja	30 min. w łaźni wodnej w temp. 37° C		60 min. w łaźni wodnej w temp. 37° C	
DNFH	0,5	0,5	0,5	0,5
	pozostawić w temp. pokojowej na 20 min.		pozostawić w temp. pokojowej na 20 min.	
0,4 mol/dm^3 NaOH	5	5	5	5
Po upływie 10 min. zmierzyć wartość absorbancji prób badanych wobec odpowiednich prób kontrolnych przy długości fali 505 nm, następnie odczytać liczbę jednostek aktywności z krzywych wzorcowych				



Ryc. 9.1. Rozkład aktywności transaminaz w narządach

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:

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

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

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/dm³ 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	