ALAT/ALT/GPT
(COLORIMETRIC)

INTENDED USE

Vitro ALT reagent is intended for the in vitro quantitative determination of alanine aminotransferase (EC 2.6.1.2) activity in serum on manual systems.

METHOD

Colorimetric method according to Reitman and Frankel[1].

CLINICAL SIGNIFICANCE

Alanine aminotransferase (glutamate pyruvate transaminase) belongs to the group of transaminases, which catalyze the conversion of amino acids to the corresponding α-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy, and damage to internal organs. Increased levels of ALT however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis, and obstructive jaundice. Levels of ALT are only slightly elevated in patients following a myocardial infarction[2].

ASSAY PRINCIPLE

Colorimetric methods based on formation of the chromogenic dinitrophenylhydrazone of pyruvate have been in wide use. However, the accuracy of these methods is limited, and since dinitrophenylhydrazine reacts with α-ketoglutarate as well as pyruvate, high reagent blanks are obtained[1]. The series of reactions involved in the assay system is as follows:

1. The amino group is enzymatically transferred by ALT present in the specimen from alanine to the carbon atom of α–oxoglutarate yielding pyruvate and L-glutamate.
2. Pyruvate formed is measured in its derivative form, 2.4-dinitrophenylhydrazone.

$$\text{DL-Alanine} + \alpha\text{-Oxoglutarate} \xleftrightarrow{\text{ALT/GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

The intensity of the color produced is directly proportional to the enzyme activity. It is determined by measuring the increase in absorbance at 530 – 550 nm.

EXPECTED VALUES

Serum Up to 12 U/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALT results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Pyruvate standard	2.0 mmol/l
R_2	Phosphate buffer pH 7.4 DL-Alanine α-oxoglutarate	100 mmol/l 100 mmol/l 4.0 mmol/l
R_3	2,4-dinitrophenyl-hydrazine HCl	4.0 mmol/l 1 N
R_4	NaOH	4.0 N

- **Reagent Preparation & Stability**

The pyruvate standard (R_1), the buffer (R_2) and the color reagent (R_3) are ready for use and stable up to the expiry date given on label when stored at 2–8°C. The sodium hydroxide reagent (R_4) should be completed to 1 liter before use.

SPECIMEN

Serum is the only accepted specimen. Avoid hemolysis

Specimen Preparation & Stability

- **For serum specimen**

Separate serum from clot/cells within 8 hours at room temperature or 48 hours at 2–8°C.

ALT activity is stable at 2-8 °C for 7 days. Freezing of the samples is not recommended.

PROCEDURE

- **Manual Procedure**

Wavelength	546 nm (530 – 550)
Cuvette	1 cm light path
Temperature	37 °C
Zero adjustment	against reagent blank
Specimen	Serum

"Transaminase activities in some sera are stimulated by high concentrations of aldehydes, ketones, or oxoacids. Measurement against a sample blank instead of reagent blak is highly recommended to avoid the risk of finding such artifacts".

- **Method (1) using table**

	Blank	Specimen
R_2	0.5 ml	0.5 ml
Incubate at 37°C for 5 minutes		
Specimen Dist. H_2O	…… 100 µl	100 µl ……
Mix, incubate for ***exactly 30 minutes at 37°C.***		
R_3	0.5 ml	0.5 ml
Mix, incubate for ***exactly 20 minutes at 20-25°C***		
R_4 (NaOH 0.4 N)	5.0 ml	5.0 ml

Mix, read the absorbance of specimen ($A_{specimen}$) against reagent blank after 5 minute.

The color development is stable for 60 minutes.

Obtain the activity of ALT activity in serum specimen from the table (p.2)

- **Method (2) using standard curve**
  - **Standard curve.**

1. Label tubes for standard curve from 1 to 10.
2. Pipette 200 □l of distilled water in all 10 standard tubes.
3. Add **R_2** (buffer reagent) and **R_1** (pyruvate standard) to the respective tubes as following:

Tube #	Pyruvate Standard (ml)	DI H2O (ml)	Buffer R2 (ml)
1	0.00	0.2	1.00
2	0.05	0.2	0.95
3	0.1	0.2	0.9
4	0.15	0.2	0.85
5	0.2	0.2	0.8
6	0.25	0.2	0.75
7	0.3	0.2	0.7
8	0.35	0.2	0.65
9	0.4	0.2	0.6
10	0.45	0.2	0.55

4. Pipette 1000 µl of **R_3** (color reagent) in all tubes, and incubate at 20 - 25°C for *exactly 20 minutes.*

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5. Pipette 10 ml of 0.4 N NaOH in all tubes. Mix, read the absorbance of all tubes against reagent blank after 5 minutes.
6. The color development is stable for one hour.

- **Assay.**

As same as Method (1) exactly but obtain the activity of ALT activity in serum specimen from standard curve.

CALCULATION

- **For Method (1)**

Obtain the activity of ALT in the serum specimen from the following table:

Absorbance	U/l	Absorbance	U/l
0.025	**4**	0.275	**48**
0.050	**8**	0.300	**52**
0.075	**12**	0.325	**57**
0.100	**17**	0.350	**62**
0.125	**21**	0.375	**67**
0.150	**25**	0.400	**72**
0.175	**29**	0.425	**77**
0.200	**34**	0.450	**83**
0.225	**39**	0.475	**88**
0.250	**43**	0.500	**94**

- **For Method (2)**

The absorbances of the increasing amounts of pyruvate standard correspond to the following transaminase activities in U/l.

Tube No.2	**09 U/l**	Tube No.7	**56 U/l**
Tube No.3	**18 U/l**	Tube No.8	**67 U/l**
Tube No.4	**27 U/l**	Tube No.9	**77 U/l**
Tube No.5	**37 U/l**	Tube No.10	**87 U/l**
Tube No.6	**46 U/l**		

The standard curve is obtained by plotting the measured absorbances against the transaminase activities in U/l.

Ordinate: Absorbance
Abscissa: Activity in U/l

Obtain the activity of ALT in the serum specimen from the standard curve.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established ALT/GPT values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Bilirubin:**

  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- **Drugs:**

  Youngs[3] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemoglobin:**

  No interference from haemoglobin up to a level of 500 mg/dl.
- **Haemolysis:**

  Erythrocyte contamination may elevate results, since ALT/GPT activities in erythrocytes are three to five times higher than those in normal sera.
- **Lipemia:**

  No relevant interference.
- **Pyruvate:**

  High levels of serum pyruvate may interfere with assay performance.

WARNING & PRECAUTION

- A Vitro reagent is for in vitro diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed.
- Incubation time and temperature are vital factor, instructions must be followed exactly.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Don't use the reagent if it is turbid.
- **Imprecision**

Reproducibility was determined using in an internal protocol. The following results were obtained.

PERFORMANCE CHARACTERISTICS

Method Comparison

Comparison studies were carried out using a similar commercially available GPT reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The results obtained did not show systematic differences when compared with other commercial reagents.

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable ALT/GPT concentration that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 4 U/l.

LINEARITY

When run as recommended, the assay is linear up to absorbance 0.5 or 94 U/l.

If result exceeds 94 U/l, specimen should be diluted 1+9 with 0.9% NaCl solution and reassayed. Multiply the result by 10.

BIBLIOGRAPHY

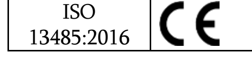
1. **Reitman, S, and Frankel, S.(1957):** Amer J Clin Path. 28: 56.
2. **Henry, JB, (1974):** Clinical Diagnosis and Management by Laboratory Methods. W.B. Saunders and Co., Philadelphia, PA. p 361.
3. **Young, Ds (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

SYMBOL DECLARATION

Symbol	Meaning
	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
12601	2 X 50 ml
12602	2 X 100 ml



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