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Liquid ALT (SGPT) Reagent Set

Intended Use
For the quantitative determination of Alanine Aminotransferase (ALT) in serum using the Mindray BS-200 analyzer.

Clinical Significance
ALT is widely distributed in tissues with the highest concentrations found in the liver and kidneys. Even so, ALT is considered more liver-specific than AST. Elevated levels of ALT are often only observed in liver diseases such as cirrhosis, hepatitis, or metastatic carcinoma. However, there can be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis.[1]

Method History
UV methods for ALT determination were described by Henley[2] in 1955 and Wroblewski and La Due[3] in 1956. The procedure was improved and optimized by Henry et al[4] in 1960. In 1974, the Scandinavian Society for Clinical Chemistry[5] recommended optimized reaction conditions. The International Federation of Clinical Chemistry (IFCC)[6] published a proposed recommended method in 1980 utilizing the LDH-NADH coupled assay. The procedure described herein is based on that method.

Principle
$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+ + H_2O$$

ALT catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

Reagents
After combining R1 and R2, the reagent contains: L-alanine >450mM, α-ketoglutaric acid >14mM, LDH(microbial) >2000IU/L, NADH >0.18mM, buffer, sodium azide 0.28%, Stabilizers.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use reagent if:
1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions
1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.28%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Storage
1. Hemolyzed samples cannot be used as red cells contain ALT.[7]
2. ALT in serum is stable for three days at room temperature (15-30°C), seven days refrigerated (2-8°C), and thirty days frozen (-20°C).[7]

Interferences
1. A number of drugs and substances affect ALT activity. See Young, et al.[8]
2. Bilirubin to at least 30 mg/dl, and hemoglobin to at least 400 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided
ALT (SGPT) Reagents R1 and R2

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry control, catalog number C7592-100

BS-200 Test Parameters

Test :	ALT	R1:	180
No.:	003	R2:	45
Full Name:	ALT	Sample Volume:	9
Standard No.:		R1 Blank:	
Reac. Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	340nm	Linearity Range:	0 - 500
Sec. Wave:	405nm	Linearity Limit:	0.2
Direction:	Decrease	Substrate Limit:	
Reac. Time:	3 / 11	Factor:	4700
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	3	□ Prozone check	
Unit:	U/L	q1: q2: q3: q4:	
Precision:	Integer	PC:	Abs:

Calibration Parameters

Rule:		Calibrator 1:	
Sensitivity:		Calibrator 2:	
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:	0		

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Limitations

1. Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05ml (50ul) of sample and multiplying the final answer by two.
2. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calibration

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculation (Example)

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{ALT (IU/L)} = \frac{\Delta\text{Abs./Min. x 1.10 x 1000}}{\text{6.22 x 0.10 x 1.0}} = \Delta\text{Abs./min. x 1768}$$

Where ΔAbs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.10 = Total reaction volume (ml)
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then 0.12 x 1768 = 212 IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[9]

4 to 24 IU/L (30°C)
4 to 36 IU/L (37°C)
Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance

1. Linearity: 0-500 IU/L.
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.999 and a regression equation of y=0.94x + 5.8. (n=33).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Run (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
35.4	1.3	3.6	41.8	1.5	3.6
89.5	1.1	1.3	111.9	2.6	2.3

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and serums with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the ALT (SGPT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L ALT activity gives a ΔAbs/Min. of 0.0004.

References

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Use by (YYYY-MM) | LOT Lot and batch code | REF Catalog number | Manufacturer | IVD *In vitro* diagnostic medical device
Temperature limitation | Consult instructions for use | CE mark | EC REP Authorized representative in the European Community