INTRODUCTION

1. Infinite Liquid GOT (AST) is a reagent set for determination of GOT (AST) activity in serum and plasma based on **UV - Kinetic method.**
2. Infinite Liquid GOT (AST) is a **ready-to-use,** two liquid reagent system.
3. Infinite Liquid GOT (AST) estimates GOT (AST) activity in just **2 1/2 minutes**.
4. Infinite Liquid GOT (AST) is **linear** upto 800 IU/l.
5. Infinite Liquid GOT (AST) can be used on any **spectrophotometer, discrete semiautomated and automated analyzers.** Programme can be designed for any specific analyzer upon request.
6. Infinite Liquid GOT (AST) is **stable till expiry** at 2 - 8° C.

PRINCIPLE

α-ketoglutarate reacts with L-aspartate in presence of GOT (AST) to form oxaloacetate and L-glutamate. The increase in oxaloacetate is determined in an indicator reaction catalyzed by malate dehydrogenase. The conversion of NADH to NAD^+ at 340 nm. is proportional to the activity of GOT (AST) in serum/plasma and is determined kinetically as rate of decrease in absorbance.

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GOT (AST)}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightleftharpoons{\text{malate dehydrogenase}} \text{L-malate} + NAD^+$$

Abbreviations

AST = Aspartate transaminase
GOT = Glutamate oxaloacetate transaminase

PREPARATION OF WORKING SOLUTION

Prepare working solution by mixing **Reagent R_1 and Reagent R_2** in the ratio **4 : 1** as per requirement.

REAGENT STORAGE & STABILITY

The reagent kit should be stored at 2 - 8° C and is stable till the expiry date indicated on the label.

R_1 and R_2 reagents are stable till expiry at 2 - 8° C.

The working solution (4 R_1 + 1 R_2) is stable for 30 days at 2 - 8° C.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

The following components are present:

• Tris buffer, pH 7.8	80 mmol/l
• L-aspartate	240 mmol/l
• Lactate dehydrogenase	≥ 3000 IU/l
• Malate dehydrogenase	≥ 400 IU/l
• NADH	0.23 mmol/l
• α-ketoglutarate	10 mmol/l

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can be used. Samples with any visible haemolysis are not acceptable since erythrocytes contain approximately ten times the normal activity of GOT (AST) found in serum. GOT (AST) activity in serum/plasma is stable for 1 week at 2 - 8° C and 1 month at -20°C. The samples should be brought to room temperature prior to use.

PROCEDURE

☐ **Reaction type**	**UV - Kinetic**
☐ **Reaction direction**	**Decreasing**
☐ **Wavelength**	**340 nm.**
☐ **Flowcell temp.**	**37°C.**
☐ **Zero setting with**	**Distilled water**
☐ **Delay time**	**60 seconds**
☐ **No. of readings**	**4**
☐ **Interval**	**30 seconds**
☐ **Blank absorbance limit**	**≥ 0.900 Abs.**
☐ **Sample volume**	**0.05 ml (50 µl)**
☐ **Working solution volume (4 R_1 : 1 R_2)**	**1.0 ml (1000 µl)**
☐ **Factor**	**3339**
☐ **Linearity**	**800 IU/l**

MANUAL ASSAY PROCEDURE

Prewarm at 37°C the required amount of working solution before use. Perform the assay as given below:

1 ml procedure

Serum/plasma	**0.05 ml.**
Working solution	**1.0 ml (800 µl R_1 + 200 µl R_2)**

Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at 60th second and subsequently three more readings with 30 seconds interval at 340 nm.

Calculation:

Calculate the change in absorbance per minute.
(Δ Abs./30 seconds x 2)
Activity of GOT (AST) in IU/l = Δ Abs./min. x 3339

Conversion factors :

Following factors can be used for conversion of IU/l from one temperature to another :

Temperature Conversion

From 37 °C to 30 °C : 0.67
From 37 °C to 25 °C : 0.49

Note: Since temperature conversion factors are given only as an approximate conversion, it is suggested that values be reported at the temperature of measurement.

EXPECTED VALUES

Serum/Plasma

Temperature	at 25°C	at 30°C	at 37°C
MEN	≤ 18 IU/l	≤ 25 IU/l	≤ 37 IU/l
WOMEN	≤ 15 IU/l	≤ 21 IU/l	≤ 31 IU/l

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range.

PROCEDURE LIMITATIONS

1. If the GOT (AST) activity exceeds 800 IU/l, dilute the specimen with normal saline and repeat the assay. The result obtained should then be multiplied with the dilution factor to obtain correct GOT (AST) activity.
2. The working solution is considered unsatisfactory and should not be used if the absorbance is less than 0.900 at 340 nm. against distilled water.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include normal and abnormal commercial reference control serum. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

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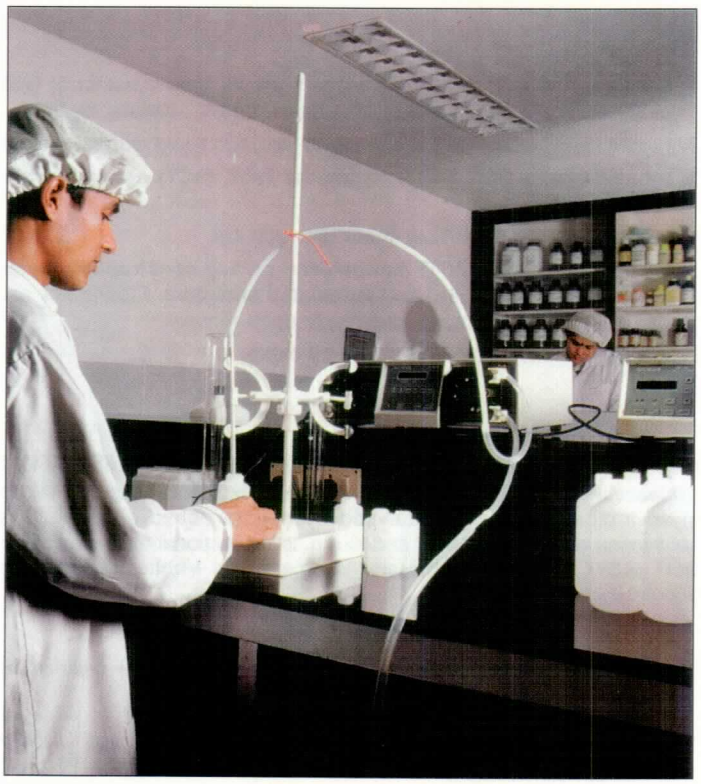

Liquid dispensing facility

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Clinical Chemistry

Infinite

GOT (AST)

UV-Kinetic

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