DO NOT USE THE REAGENT IF:
1. The reagent is turbid.
2. The reagent has an optical density greater than 1.0 at 405 nm.

SPECIMEN COLLECTION AND STORAGE
1. Use non-hemolyzed serum or EDTA plasma.
2. Serum Gamma-GT is stable for seven days at 2-8°C and two months frozen (-20°C) and protected from evaporation.

INTERFERENCES
Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:
No significant interference (±10%) from hemoglobin up to 100 mg/dL.

Bilirubin:
No significant interference (±10%) from bilirubin up to 23.0 mg/dL.

Lipemia:
No significant interference (±10%) from lipemia up to 880 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
1. LIASYS 330 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control material such as those provided by AMS Diagnostics.

Gamma-GT Reagent is used in the LIASYS-330 Clinical Chemistry System. This method is based on the kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Principle
L-Gamma-Glutamyl-3-carboxy-4-nitroanilide + Glycylglycine → Gamma-GT

L-Gamma-Glutamyl-gluyl-4-nitroanilide + 5-amino-2-nitrobenzoic acid

Gamma-GT in the sample catalyzes the transfer of the glutamyl group from L-Gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine according to the above reaction. The amount of 5-amino-2-nitrobenzoic acid formed is proportional to Gamma-GT activity and may be measured kinetically at 405 nm by the increasing intensity of the yellow color formed.

REAGENT COMPOSITION

Active Ingredients
Concentrations
Reagent 1
Glycylglycine 150 mM

Reagent 2
L-Gamma-glutamyl-3-carboxy-4-nitroanilide 6.0 mM

Concentrations are those in the working reagent.

Precautions and Warnings:
1. For in vitro diagnostic use only.
2. DO NOT pipette by mouth. Avoid contact with skin and eyes. If spilt, thoroughly wash affected area with water. For further information, consult the AMS Diagnostics Gamma-GT Reagent Material Safety Data Sheet.
3. Reagent contains Sodium Azide as a preservative. In a dry state may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.
4. Do not re-use the reagent after the expiration date printed on the kit.

REAGENT PREPARATIONS
Reagents are supplied in a two vial, ready to use, liquid form.

REAGENT STORAGE
1. Store the reagents at 2-8°C (refrigerated).
2. The reagents are stable until the expiration date when stored at 2-8°C.
3. Working reagent is stable for 4 weeks when stored at (2-8°C).
4. Do not freeze the reagents.
5. Reagent 2 should be protected from light.

REAGENT DETERIORATION
1. The reagents are stable until the expiration date when stored at 2-8°C.
2. Working reagent is stable for 4 weeks when stored at (2-8°C).
3. Do not freeze the reagents.
4. Reagent 2 should be protected from light.

INTENDED USE
This reagent is intended for the in vitro quantitative kinetic determination of gamma glutamyltransferase (Gamma-GT) in human serum.

SUMMARY AND EXPLANATION
Gamma-glutamyltransferase (Gamma-GT) is an enzyme present in liver and bile duct which is the most sensitive indicator of hepatobiliary diseases. Due to a high negative predictive value for these diseases the measurement of Gamma-GT is widely used to rule out an hepatic or biliary origin. Together with other enzymes Gamma-GT is a valuable tool for the differential diagnosis in liver diseases.

METHODOLOGY
Methods for determining Gamma-GT are based on the use of glutamyl derivatives of aromatic amines as substrate material. Orito and Meiser introduced Gamma-Glutamyl-p-nitroanilide as a substrate in 1963 with Kulhanek and Dimov (1966) adding glycylglycine and significantly increasing the speed of the reaction.

PERFORMANCE
Linearity:
When run as recommended the assay is linear from 2 to 1000 U/L.

Studies performed between this procedure and a similar procedure yielded the following results:

Number of samples pairs: 43

METHOD COMPARISON
No significant interference (±10%) from bilirubin up to 23.0 mg/dL.

No significant interference (±10%) from hemoglobin up to 100 mg/dL.

Hemoglobin:
L-Gamma-Glutamyl-3-carboxy-4-nitroanilide could be substituted for the L-gamma-glutamyl-p-nitroanilide, producing a more water soluble and stable reagent. AMS Diagnostics Gamma-GT reagent uses this soluble 3-carboxy derivative. This method is based on the kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Gamma-GT (Liquid)

TEMPERATURE: 37°C
WAVELENGTH: 405 nm
ASSAY TYPE: Rate/Kinetic
DIRECTION: Increase

e.g. Sample Vol. 0.050 mL (50 mL)

Reagent 1 Vol. 1.0 mL (1000 mL)

Reagent 2 Vol. 0.250 mL (250 mL)

DELAY/LAG TIME: 1 Min
READ TIME: 3 Min

Procedure Notes:
1. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.
2. Samples with values exceeding linearity should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calculation
One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions. For example:

U/L = AAbs./min. x 1000 x 1.270 = AAbs./min. x 5333
9.5 x 1 x 0.06

Where:
AAbs./min. = Absorbance change
1000 = Conversion of U/ml to U/L
1.270 = Total reaction volume (mL)
9.5 = Millimolar absorptivity of 5-amino-2-nitrobenzoate.
1 = Light path in cm
0.020 = Sample volume (mL)

Example: If your ΔAbs / min. = 0.06
then 0.06 x 5333 = 320 U/L.

NOTE:
If test parameters are altered the factor has to be recalculated using the above formula. To convert to SI units (kat/L) multiply U/L by 0.1667.

CALIBRATION
The procedure is calibrated by means of the millimolar absorptivity of 5-amino-2-nitrobenzoate. As such the 9.5 at 405nm should be recalculated with the above formula.

To convert to SI units (kkat/L) multiply ΔAbs by 0.1667.

QUALITY CONTROL
The integrity of the reaction should be monitored by use of a two level control with known Gamma-GT values.

EXPECTED VALUES (37°C)

Adults: Female 9 - 36 U/L Male 12 - 64 U/L

Children / Adolescents: Female 1 - 10 U/L Male 1 - 19 U/L

1 day – 6 months Female 15 - 132 U/L Male 12 - 122 U/L
6 months – 1 year Female 1 - 39 U/L Male 1 - 39 U/L
1 – 12 year(s) Female 4 - 22 U/L Male 3 - 22 U/L
13 – 18 years Female 4 - 24 U/L Male 2 - 42 U/L

It is strongly recommended that each laboratory determine its own reference range.

REFERENCES
8. Orlowski and Meiser introduced Gamma-glutamyltransferase (Gamma-GT) in 1963 with Kulhanek and Dimov (1966) adding glycylglycine and significantly increasing the speed of the reaction.

Sensitivity / Limit of Detection:
A sensitivity of approximately 0.001 ΔAbs per U/L was obtained. The lower Limit of Detection was found to be 2 U/L.

For further information, consult the AMS Diagnostics Gamma-GT Reagent Material Safety Data Sheet.

MANUFACTURED FOR:
Ams Diagnostics
117 Old State Road
Brookfield, CT 06804 — Tel 1 866 419 7839
Fax 1 843 277 0903
www.amsdiagnostics.com