Liquid GGT (γ-glutamyl transferase) Reagent Set

Intended Use
For the quantitative kinetic determination of gamma glutamyl transferase (GGT) activity in serum using the Mindray BS-480 analyzer.

Clinical Significance
GGT measurements are used in the diagnosis and treatment of liver diseases such as alcoholic cirrhosis, and primary and secondary tumors. Elevated GGT levels appear earlier and are more pronounced than those of other liver enzymes, in cases of obstructive jaundice and metastatic neoplasms.[1]

Test Summary
Methods for determining GGT are based on the use of glutamyl derivatives of aromatic amines as substrate material.[2] Orlowski and Meiser introduced γ-Glutamyl-p-nitroanilide as a substrate in 1963[3] with Kulhanek and Dimov (1966) adding glycylglycine and significantly increasing the speed of the reaction.[4] In 1969, Szasz published a kinetic procedure for GGT[5] on whose principle the present procedure is based. Szasz and Persijn[6] later reported that the 3-carboxyl derivitive, L-γ-glutamyl-3-carboxy-4-nitroanalide (GLUPA-C) could be substituted for the L-γ-glutamyl-p-nitroanilide, producing a more stable reagent. The MedTest DX Liquid GGT reagent uses this soluble 3-carboxyl derivative.

Principle
L-γ-Glutamyl-3-carboxy-4-nitroanilide + Glycylglycine $\xrightarrow{\text{GGT}}$ L-γ-glutamylglycylglycine + 5-amino-2-nitrobenzoate

GGT in the sample catalyzes the transfer of the glutamyl group from GLUPA-C to glycylglycine according to the above reaction. The amount of 5-amino-2-nitrobenzoate formed is proportional to GGT activity and may be measured kinetically at 405nm.

Reagent Composition
In addition to a stabilizer, the combined R1 and R2 reagent contains: Tris buffer: <89 mmol/L, Glycylglycine: <126 mmol/L, GLUPA-C: 4.0 mmol/L, Sodium Azide: 0.095%

Reagent Preparation
Reagents are supplied as ready to use liquids.

Reagent Storage and Stability
Store reagents at 2-8°C. The reagents are stable until the expiration date if stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.
NOTE: The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature. Return reagent to 2-8°C as soon as possible after use.

Precautions and Hazards
1. This reagent is for *in vitro* diagnostic use only.
2. Do not use the reagent if the initial absorbance of the working reagent is greater than 0.800 when measured at 405 nm against water or if the reagent fails to meet stated parameters of performance.
3. Do not pipette by mouth. Avoid ingestion and contact with skin.
4. Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drainlines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CSC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

Hazards:
R1 and R2: Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.
Refer to the Safety Data Sheet for this product (SDS-GGT600) available at www.medtestdx.com.

Specimen Collection and Storage
1. Use serum only. GGT activity is inhibited by most anticoagulants.
2. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.
3. Serum GGT is reported stable in serum for up to seven days when stored at 2-25°C, up to one month when stored at 4°C, and up to one year at (-20°C) and protected from evaporation.[7]
4. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Ed., 1988, HHS Publication No. (CDC) 88-8395.

Interferences
1. Most anticoagulants used in blood collection tubes inhibit GGT activity.[8]
2. Anti-epileptic drugs (phenytoin and barbituates) may falsely elevate GGT levels.[9,10]
3. Bilirubin to the level of 20 mg/dl has been found to exhibit negligible interference (< 5%) in this assay.
4. Hemoglobin from 100-500 mg/dl has been found to show minimal depression (approximately 5-7%) of recovered GGT activities.
   NOTE: GGT level was 45 U/L for the bilirubin study and 48 U/L for the hemoglobin study.
5. For a comprehensive list of drug interferences, see Young et al.[11]

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Materials Provided
GGT reagents (R1 and R2)

Materials Required but not Provided
1. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry control, catalog number CHEQ480

Limitations
Samples that exceed the linearity limit (800 U/L) should be diluted with an equal volume of saline and re-assayed and the final results multiplied by two.

Calibration
The procedure is calibrated by means of the millimolar absorptivity of 5-amino-2-nitrobenzoate which is 9.5 at 405nm under the specified conditions. Results are based on the change in absorbance per minute. All parameters must be known and controlled.

Quality Control
The validity of the reaction should be monitored by the use of control serums with known normal and abnormal GGT values. These controls should be run at least with every working shift in which GGT 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 [12]
Male: 8-37 U/L at 30°C, 9-54 U/L at 37°C
Female: 6-24 U/L at 30°C, 8-35 U/L at 37°C
Due to a wide range of conditions (dietary, geographical, age, etc.) believed to affect normal ranges, it is strongly recommended that each laboratory determine its own reference range.

Performance
1. Assay Range: 1-800 U/L. Samples that exceed 800 U/L should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: A study was performed between the Mindray BS-480 and a similar analyzer and method, resulting in the following:

Method	GGT
N	85
Mean GGT (U/L)	130.6
Range (U/L)	6-728
Standard Deviation	193.2
Regression Analysis	y = 1.049x – 2.3
Correlation Coefficient	0.9998

3. Precision: Precision studies were performed following the modification of the guidelines contained in NCCLS document EP5-T2.[13]

Within Run

Sample	LOW	MID	HIGH
N	20	20	20
Mean	42.8	146.2	687.0
Standard Deviation	0.6	0.7	1.5
Coefficient of Variation (%)	1.3%	0.5%	0.2%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	42.4	147.3	691.4
Standard Deviation	0.6	15.8	4.6
Coefficient of Variation (%)	1.5%	1.1%	0.7%

4. Sensitivity: 2SD Limit of Detection (95% Conf) = 1 U/L

References

1. Tietz, N.W., editor, Fundamentals of Clinical Chemistry, 3rd Ed., W.B. Saunders Co., 391 (1987).
2. Demetriou, J.A., Drewes, P.A., Gin, J.B., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper Row, pp 872-873 (1974).
3. Orlowski, M., Meister, A., Biochem, Biophys. Acta 73:679 (1963).
4. Kulhanek, V., Dimov, D.M., Clin. Chem. Acta 14:619 (1966).
5. Szasz, G., Clin. Chem. 15:124 (1969).
6. Szasz, G., Persijn, J.P., et al, A Klin. Chem. Klin. Biochem. 12:228 (1974).
7. Zern, M., and Discombe, G., Lancet 2:748 (1971).
8. Wolf, P.L., et al, Practical Clinical Enzymology and Biochemical Profiling, New York, Wiley-Interscience p.37 (1973).
9. Rosalki, S.B., et al, Lancet 2:376 (1971).
10. Whitfield, J.B., et al, Gut 13:702(1972).
11. Young, D.S., et al, Clin. Chem. 21:1D (1975).
12. Kaplan, L.A., Pesce, A.J. Clinical Chemistry, 2nd Ed., St. Louis, C.V. Mosby Company, (1992).
13. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	GGT	No.:	217	Sample Type:	Serum
Chemistry:	Gamma Glutamyl Transferase			Print Name:	GGT
Reaction Type:	Kinetic			Reaction Direction:	Positive
Pri Wave:	412			Sec Wave:	660
Unit:	U/L			Decimal	0
Blank Time:	0 0			Reaction Time:	56 71

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	7.0 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 30 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	1	800	Linearity Limit:	0.2
Linearity Range (Decreased)	____	____	Substrate Depletion:	25000
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	GGT	0	U/L

Calibration Setup

Chem: GGT

Calibration Settings

Math Model:	K Factor		
Factor:	2933	Replicates:	1

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF GGT480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	