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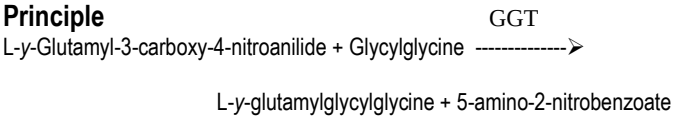
Liquid GGT (γ-glutamyl transferase) Reagent Set

Intended Use
For the quantitative kinetic determination of gamma glutamyl transferase (GGT) activity in serum using the Mindray BS-200 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 Pointe Scientific Liquid GGT reagent uses this soluble 3-carboxyl derivative.

Principle


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.
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
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 as toxicity has not been established.
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.

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]

Materials Provided
GGT 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:	GGT	R1:	180
No.:	017	R2 Volume:	45
Full Name:	GGT	Sample Volume:	9
Standard No.:		R1 Blank:	
Reac. Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	405nm	Linearity Range:	0 - 800
Sec. Wave:	670nm	Linearity Limit:	0.2
Direction:	Increase	Substrate Limit:	
Reac. Time:	3 / 11	Factor:	2642
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	3	□ Prozone check	
Unit:	U/L	q1: q2: q3:	q4:
Precision:	Integer	PC:	Abs:

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(γ-glutamyl transferase)
Reagent Set

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		

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.

Calculation (Example)
GGT activity is expressed as units/liter. At 37°C, one Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined conditions.

$$\frac{\Delta \text{ Abs/min} \times TV \times 1000}{MMA \times SV \times LP} = \text{U/L GGT in sample}$$

Δ Abs/min……Change in absorbance per minute.
TV……Total assay volume (1.100ml).
1000……..Conversion of ml to L.
MMA……millimolar absorptivity of 5-amino-2-nitrobenzoate(9.5).
SV……..Sample volume (0.100ml).
LP……..Light path (1cm).

$$\frac{\Delta \text{ Abs/min} \times 1.100 \times 1000}{9.5 \times 0.100 \times 1.0} = \Delta \text{ Abs/min} \times 1158$$

Then: Δ Abs/min x 1158 = U/L of unknown
Example: If Δ Abs/min = .06, then .06 x 1158 = 69 U/L

Note: If any of the above parameters are changed, a new factor must be recalculated.

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. Linearity: 0-800 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-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.998 and the regression equation was y=1.02x+4.8.
3. Precision: Precision studies were performed following the modification of the guidelines contained in NCCLS document EP5-T2.[13]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
25.4	0.70	2.6	28.9	1.1	3.8
71.7	0.90	1.2	76.8	2.4	3.1

4. Sensitivity: The sensitivity for the Liquid GGT reagent was investigated by reading the change in absorbance for a saline sample, and serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Liquid GGT reagent showed little or no drift on a zero sample. Under the reaction conditions described, 1 U/L gives an absorbance movement of 0.0003.

References

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12. Kaplan, L.A., Pesce, A.J. Clinical Chemistry, 2nd Ed., St. Louis, C.V. Mosby Company, (1992).
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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