FastPack® IP αGST Immunoassay

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES (U.S. ONLY).

For the Quantitative Measurement of Alpha Glutathione S-Transferase (αGST) in Human serum and plasma

INTENDED USE

The FastPack® IP αGST Immunoassay is a paramagnetic particle immunoassay for the *in-vitro* quantitative determination of αGST in human serum and plasma. The FastPack® IP αGST Immunoassay is designed for use with the FastPack® IP System.

SUMMARY

In the liver, alpha glutathione S-transferase is located in the hepatocytes whereas pi GST (πGST) is confined to the intrahepatic bile duct cells. This heterogenous GST subclass distribution suggests that the isoenzymes have unique *in vivo* functions in different hepatic regions and that the detection of GST subclass levels in biological fluids would be of significant use in studying the integrity of specific hepatic regions.

Currently, hepatic regions are studied by the measurement of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A disadvantage of these markers is that they are not distributed uniformly throughout the liver, the periportal concentration being greater than the centrilobular. In contrast, αGST has been found to be equally distributed in both the centrilobular and periportal regions. Therefore, αGST quantitation can be used to study the hepatocellar status of individuals.[1-7]

TEST PRINCIPLE

The FastPack® IP αGST Immunoassay is a chemiluminescence assay based on the "sandwich" principle.

- Primary incubation: Antibody solution (mixture of a biotinylated monoclonal αGST-specific antibody and a monoclonal αGST antibody labeled with alkaline phosphatase) [100 µL] reacts with αGST from the patient's sample, control or calibrator [100 µL].
- Secondary incubation: Streptavidin-coated paramagnetic particles [150 µL] are combined with the reaction mixture. During this incubation, the sandwich complex is bound to the solid-phase via the interaction of biotin and streptavidin.
- Removal of unbound materials: The paramagnetic particles are repeatedly washed with wash buffer [0.2 mL/wash] to remove unbound materials.
- Substrate addition and detection: Chemiluminogenic substrate [140 µL] is added to the solid-phase bound complex and results in "glow" chemiluminescence, which is measured using the FastPack® IP System.
- The amount of bound labeled-antibody is directly proportional to the concentration of αGST in the sample.

REAGENTS – Content and Concentration

Each FastPack® IP carton contains:

- 30 FastPack® IPs

Each FastPack® IP Contains:

- Paramagnetic Particles, 150 µL
  Streptavidin-coated paramagnetic particles in buffer containing 0.1% ProClin® 300 as a preservative.
- αGST Antibody Solution, 100 µL
  Antibody solution containing a mixture of a biotinylated mouse monoclonal anti-αGST antibody and a second mouse monoclonal anti-αGST antibody labeled with alkaline phosphatase in a protein matrix containing ProClin® 150 as preservative.
- Wash Buffer, 2.0 mL
  Tris buffer containing surfactants.
- Substrate, 140 µL
  ImmuGlow™ Plus: Indoxyl-3-phosphate and lucigenin in buffer containing preservatives.

Materials required but not provided

- FastPack® IP System
- FastPack® αGST RUO Calibrator Kit – Cat. No. 25000053
- FastPack® αGST RUO Sample Diluent Kit – Cat. No. 25000054

Optional materials

- FastPack® αGST RUO Control Kit – Cat. No. 25000052

WARNINGS AND PRECAUTIONS

- **For *in-vitro* use only.**
- Do not pipette by mouth.
- Do not eat, drink or smoke in designated work areas.
- Wash hands thoroughly after handling specimen.
- HAMA Interference: some individuals have antibodies to mouse protein (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice[8].
- FastPack® IP reagents are stable until the expiration date on the label when stored and handled as directed. Do not use FastPack® IP reagents beyond the expiration date.
- Discard used FastPacks into a Biohazard container.
- The components containing ProClin® are classified per applicable European Economic Community (EEC) Directives as: Irritant (Xi). The following are appropriate Risk (R) and Safety (S) phrases for ProClin®:

R36/38	Irritating to eyes and skin
R43	May cause sensitization by skin contact
S24/25	Avoid contact with skin and eyes
S36/37	Wear suitable protective clothing and gloves
S60	This material and/or its container must be disposed of as hazardous waste

STORAGE INSTRUCTIONS

Store at 2 – 8 °C.

SPECIMEN COLLECTION/PREPARATION

1. Serum, EDTA or lithium-heparin plasma samples can be used for the FastPack® IP αGST Immunoassay.
2. The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for handling, processing and storing blood.[9,10]
   A. Collect all blood samples observing routine precautions for venipuncture.
   B. For plasma samples:
      - Collect samples in an EDTA (lavender top) or heparinized (green top) tube.
      - Mix the tube immediately after collection by gently inverting it several times.
      - Plasma should be separated from the cells by centrifugation within 3 hours from time of collection and stored at 2 – 8 °C. Transfer the plasma from the original tube for storage.
      - If not tested within 24 hours, the sample should be frozen at –20 °C or colder.
   C. Samples should be free of red blood cells, or other particulate material for optimal results.
   D. Samples showing turbidity and particulate matter should be centrifuged prior to use.
   E. Ensure the samples are free of bubbles.

ASSAY PROCEDURE

See the FastPack® IP System Procedure Manual for detailed instructions for running the FastPack® IP assays.

INSTRUMENTATION

FastPack® IP System

DETAILS OF CALIBRATION

During the FastPack® IP production process, Qualigen generates a master standard curve and places this information in the barcode of each FastPack® IP label, where it can be read by the FastPack® IP System analyzer during the testing sequence. The FastPack® IP System analyzer must be calibrated by the end user so that it is properly adjusted for the particular lot of FastPacks that are being used. For the FastPack® IP αGST Immunoassay, the FastPack® IP System analyzer must be calibrated once every 30 days or whenever a new lot of αGST FastPacks are to be used.

Whenever the user performs a calibration for a particular lot of FastPacks or uses a new lot of calibrator, 2 FastPacks must be run for calibration (duplicates). When the calibration expires (30 days after initial calibration) 2 FastPacks must be run for calibration. See the FastPack® IP System Procedure Manual for “Running a Calibration”.

Use FastPack® αGST RUO Calibrator Kit - Cat. No. 25000053

RESULTS

The FastPack® IP System analyzer uses the information from the barcode to construct a lookup table of x,y values that represent the standard curve and estimates the concentration of unknown samples by linear interpolation.

QUALITY CONTROL

Quality control materials are essential for monitoring the system performance of the assay. To ensure that all reagents and protocols are performing properly, at least two levels of quality control materials should be used. See the FastPack® IP System Procedure Manual for "Control Testing".

Controls available: FastPack® αGST RUO Control Kit – Cat. No. 25000052

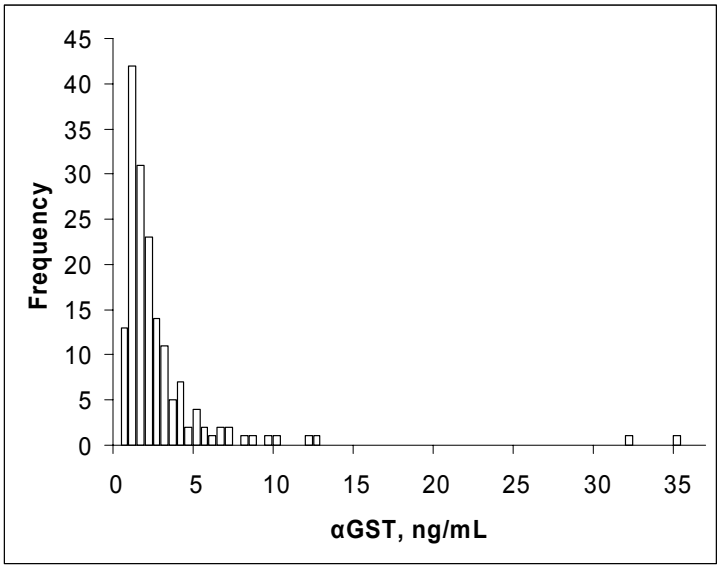
LIMITATION OF PROCEDURE

- Plasma samples to be collected using EDTA or lithium-heparin as the anticoagulant.
- Specimens can be measured within the reportable range of the limit of quantitation (0.5 ng/mL) and the upper end of the calibration range of 200 ng/mL.
- Samples >200 ng/mL should be reported as such or re-run after dilution. Samples may be diluted using the αGST Sample Diluent until values within range of the assay are obtained.
- The FastPack® IP αGST Immunoassay does not show a high-dose hook effect up to 5,000 ng/mL.
- Specimens from individuals who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits employing mouse monoclonal antibodies.
- Heterophilic antibodies in a sample have the potential to cause interference in immunoassay systems. Infrequently, αGST levels may appear depressed due to heterophilic antibodies present in the individual's sample or to nonspecific protein binding. If the αGST level is inconsistent with clinical evidence, additional αGST testing is suggested to confirm the result.

EXPECTED VALUES

Samples were obtained from 167 individuals. Samples were obtained from apparently healthy blood donors without any clinically abnormal indications. αGST levels were determined using the FastPack® IP αGST Immunoassay in conjunction with the FastPack® IP System in order to establish the αGST concentration in the normal population. The reference interval (5^{th} to 95^{th} percentiles) for the FastPack® IP αGST Immunoassay is 0.24 – 11.4 ng/mL. The reference intervals reflect the donor population of this study group. The distribution of the values is presented in the histogram below.

Sample Type	Reference Group	n	Median Age (years)	Age Range	Median Concentration (ng/mL)	95% Reference Interval (ng/mL)
Serum	Males and Females	167	35	18 - 55	1.4	0.24-11.4



SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Three control serum pools spiked with varying amounts of αGST were assayed using three lots of FastPack® IP αGST reagents and four FastPack® IP System analyzers. Each control was tested in duplicate determinations per day over a period of 10 days for a total of 80 replicate determinations per control. The data was analyzed using the analysis of variance (ANOVA) technique and within-assay and total imprecision were determined. Mean, standard deviation (SD), and percent coefficient of variation (%CV) were calculated for each control solution.

Within-Assay Imprecision

	Control 1	Control 2	Control 3
Mean ng/mL αGST	7.3	37.6	180.5
SD	0.34	1.5	10.4
%CV	4.7	4.0	5.8

Total Imprecision

	Control 1 (n = 80)	Control 2 (n = 80)	Control 3 (n = 80)
Mean mIU/mL αGST	7.6	37.6	180.5
SD	0.80	3.0	25.4
%CV	11.1	7.9	14.1

Range of Linearity.

For αGST tested by FastPack® IP αGST, based on the CLSI EP-6A evaluation, the method has been demonstrated to be linear from 1.3 to 26.7 ng/mL, with 5.0 ng/mL up to 66.8 ng/mL, and 25.0 ng/mL in the interval 66.8 and 267.4 ng/mL αGST. Linearity determined by diluting of human serum clinical sample spiked with known high endogenous αGST concentration. The FastPack® IP αGST is linear over the range of 0.5 ng/mL (LOQ) to 200 ng/mL. (% Recovery = [Measured value/Expected Value] X 100).

Linearity Upon Dilution

A serum sample spiked with high endogenous αGST concentration was diluted 90% to 0.5% with the serum sample and quantified in triplicate determinations with one FastPack® αGST reagent lot on a single FastPack® IP System analyzer. Measured values of the dilutions compared to their expected values (determined in the same assay) based on the measured value of the Neat sample and respective dilution. (% Recovery = [Measured value/Expected Value] X 100).

%dilution	Expected αGST, ng/mL	Measured αGST, ng/mL	% Recovery
100.0%	N/A	267.4	N/A
90.0%	240.6	229.2	95.2
75.0%	200.5	173.8	86.7
50.0%	133.7	122.1	91.3
25.0%	66.8	54.8	82.0
12.5%	33.4	29.0	86.8
6.3%	16.7	14.3	85.6
3.0%	8.0	6.8	85.0
1.0%	2.7	2.5	92.6
0.5%	1.3	1.2	92.3

In addition, three serum samples with high endogenous αGST levels were diluted with FastPack® αGST Sample Diluent containing phosphate buffered saline solution and Tween 20. The measured values of the dilutions were compared to their expected values.

Serum 1	Expected (ng/mL)	Measured (ng/mL)	% Recovery
Neat	N/A	>200	N/A
1:160	N/A	68.5	N/A
1:320	34.3	32.9	96.6
1:640	17.1	16.4	95.9
1:1280	8.6	7.9	91.9
1:2560	4.3	4.2	97.7

Serum 2	Expected (ng/mL)	Measured (ng/mL)	% Recovery
Neat	N/A	>200	N/A
1:320	N/A	99.9	N/A
1:640	50.0	52.0	104.0
1:1280	25.0	23.9	95.6
1:2560	12.5	12.2	97.6
1:5120	6.25	5.4	86.4

Serum 3	Expected (ng/mL)	Measured (ng/mL)	% Recovery
Neat	N/A	>200	N/A
1:20	N/A	148.9	N/A
1:40	74.4	70.7	95.0
1:80	37.2	35.8	96.2
1:160	18.6	18.2	87.8
1:320	9.3	9.1	97.8

INTERFERING SUBSTANCES

The effect of icteric, lipemic, and hemolyzed serum samples on quantification of αGST was investigated by spiking one serum pool with an αGST concentration of ~60.0 ng/mL with known concentrations of bilirubin, triglycerides, and hemoglobin, and another serum pool with endogenous αGST concentration of ~3.0 ng/mL with the same concentrations of the interfering substances. The values obtained for the serum pools with each interfering substance were compared to the values obtained for the serum pools without the interfering substance and the percentage bias or absolute bias in ng/mL determined. These compounds did not show interference at the levels indicated.

	Interfering Substance		
	Bilirubin (30 mg/dL)	**Lipemia (1000 mg/dL)**	**Hemoglobin (500 mg/dL)**
Non-spiked aliquot	3.8 ng/mL	3.0	3.9
Spiked aliquot	3.9 ng/mL	3.2	3.8
Absolute bias, ng/mL	+0.1	+0.2	-0.1

	Interfering Substance		
	Bilirubin (30 mg/dL)	**Lipemia (1000 mg/dL)**	**Hemoglobin (500 mg/dL)**
Non-spiked aliquot	66.5 ng/mL	57.7 ng/mL	58.7
Spiked aliquot	66.8 ng/mL	56.2 ng/mL	56.2
% Bias	+0.5%	- 2.6%	- 4.3%

Cross-reactivity

No significant cross reactivity (<0.5 ng/mL) was obtained when a FastPack® αGST sample was spiked with the following cross-reactants: mμ GST, at 250 ng/mL, and pi GST at 2000 ng/mL.

Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantitation (LOQ)

The limit of blank (LOB, the highest measurement likely to be observed for a blank sample), limit of detection (LOD, the lowest amount of analyte in a sample that can be detected with type I and II error rates set to 5%), and limit of quantitation (LOQ, the lowest amount of analyte in a sample that can be reliably detected and at which the total error meets the pre-specified requirement for accuracy) were determined according to CLSI EP17-A for the FastPack® IP αGST assay. In this study, the limit of blank was determined from 20 replicate determinations of the FastPack® αGST sample on each of three different FastPack® IP System analyzers. Raw RLUs from the assays were converted to apparent ng/mL based on the calibration curve for each assay. The LOB was determined as the maximum observed value. This value was 0.12 ng/mL αGST.

The LOD was estimated from 60 replicate determinations of a low control sample per the CLSI EP17-A guideline. In this study, the LOD was found to be 0.21 ng/mL αGST.

For the LOQ analyses, the prospectively defined goals for accuracy of ≤20% CV. In this study, a control sample precision shown to meet this criterion for CV, thus the LOQ was found to be 0.5 ng /mL αGST.

REFERENCES

1. Trull, A.K. *et al.* (1994). Serum α-Glutathione S-Transferase- A sensitive marker of hepatocellular damage associated with acute liver allograft rejection. Transplantation, 58 (12), 1345-1351.
2. Kobayashi, H. *et al.* (2000). α-Glutathione-S-Transferase as a new sensitive marker of hepatocellular damage in biliary atresia. Pediatr. Surg. Int., 16: 302-305.
3. Mulder, T. P.J. *et al.* (1999). Variability of Glutathione S-Transferase a in human liver and plasma. Clinical Chemistry 45 (3), 355–359.
4. Rees, G.W. *et al*. (1995). Evaluation of an enzyme-immunometric assay for serum α-Gultathione S-Transferase. Ann. Clin. Biochem. 32, 575-583.
5. Hughes, V.F. *et al.* (1997). Randomized Trial to evaluate the clinical benefits of serum α-Glutathione S-Transferase concentration monitoring after liver transplantation. Transplantation 64 (10), 1446-1452.
6. Beckett, G.J. and Hayes, J.D. (1993). Glutathione S-Transferases: biomedical applications. Adv. Clin. Chem., 30, 281–389.
7. Platz K.P. *et al.* (1997). Determination of alpha- and Pi-Glutathione-S-Transferase will improve monitoring after liver transplantation. Transplant Proc. 1997 Nov; 29(7):2827-9.
8. Schroff, R.J. *et al*. Human anti-mouse immunoglobin responses in patients receiving monoclonal antibody therapy. Cancer Res, 45:879 – 885, 1985.
9. Procedures for the collection of diagnostic blood specimens by venipuncture. Approved Standard – Sixth Edition: H3-A6: 27(26), 2007, Clinical and Laboratory Standards Institute (CLSI).
10. Procedures for the handling and processing of blood specimens; Approved guideline – Third Edition, H18-A3; 23 (38), 2004. Clinical and Laboratory Standards Institute (CLSI).

Qualigen, Inc.
Carlsbad, CA 92011 USA
Technical Support
(760) 918-9165
(877) 709-2169