

GlycoMark™

Closer Diabetes Monitoring

For the quantitative measurement of
1,5AG (1,5-anhydroglucitol) in serum or plasma

For in vitro diagnostic use

Intended Use

The GlycoMark™ test provides quantitative measurement of 1,5-anhydroglucitol (1,5AG) in serum or plasma. The test is for professional use, and is indicated for the intermediate term monitoring of glycemic control in people with diabetes.

Summary and Explanation of Test

As early as 1981, Akanuma, et al. observed diminished plasma concentrations of 1,5AG in patients with insulin-dependent diabetes mellitus (IDDM) in comparison to healthy controls¹. This observation was confirmed in a 1983 study by Yoshioka et al.². In the 1983 study, plasma 1,5AG was measured by GC-LC in 21 diabetic patients prior to initiation of insulin therapy and 13 patients receiving insulin. 1,5AG was generally undetectable in the patients not receiving insulin, but was measurable in the population on therapy. At the time, Yoshioka hypothesized that the absence of 1,5AG was a marker of diabetic metabolism. However, it is now known that the method used at the time was merely insufficiently sensitive to detect 1,5AG in diabetic patients not receiving insulin therapy.

These early results spawned more intensive investigations of the clinical utility of 1,5AG as a marker of glycemia. Those investigations employed more sensitive detection methods such as GC-MS. For instance, in a 1989 longitudinal study by Yamanouchi et al.³, 14 patients on various anti-diabetic therapies including insulin, glyburide, gliclazide, and diet modification were monitored for up to 12 months. Hemoglobin A1C (A1C) and fasting plasma glucose were also determined by standard methods, and the results showed excellent agreement between A1C and 1,5AG, i.e., all 14 patients showed reductions in A1C values with time, and these reductions were correlated with time-dependent increases in 1,5AG values. Further, the time course of normalization of glycemia was established. Specifically, both A1C and 1,5AG values stabilized at 6-10 weeks post-initiation of treatment.

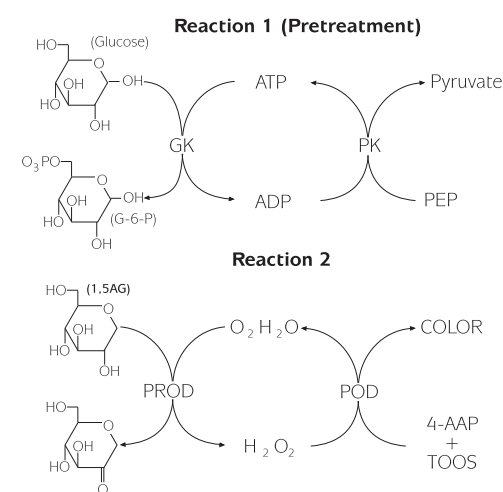
In a 1996 longitudinal study by Yamanouchi, et al.⁴ employing an enzymatic method, 56 patients newly diagnosed with type 2 diabetes were monitored for four weeks following initiation of oral anti-hyperglycemic medications. At the end of the four week period, half of the patients continued on treatment while the rest were discontinued. The results showed that 1,5AG increased in value vs. baseline for the first four week period for the population. Following discontinuation of treatment, 1,5AG values sharply decreased, and the values at the six week time point were significantly different from those in the subgroup that continued on therapy.

Results from this 1996 study were similar for mean daily and fasting plasma glucose, and fructosamine. A1C was responsive to therapy in the initial four weeks, but did not show a significant difference between subjects maintaining therapy versus subjects discontinuing therapy. This finding is indicative of the fact that A1C measurements are reflective of glycemic control occurring 1-2 months previously, whereas the other markers display faster response to changes in glycemia. In comparing A1C, fructosamine and 1,5AG, 1,5AG displayed the most rapid response to glycemic changes. These findings indicated the ability of 1,5AG to reflect changes in glycemia over a 1 to 2-week time course. Moreover, the changes in 1,5AG reflect changes in glucose in both directions, i.e. progressions both towards and away from euglycemia.

Principle of the Assay

GlycoMark™ is an enzymatic method consisting of a two-reagent test kit (Reagent 1 and Reagent 2). The test has been developed to be used with a fully automated chemistry analyzer. The test system also includes the 1,5AG calibration standard solution (50 µg/mL), and a two-level control set ("Low" and "High"). The calibration standard solution and control set are purchased separately.

The method uses the enzyme pyranose oxidase (PROD) as an oxidase of 1,5AG to oxidize the 2nd position hydroxyl group of 1,5AG and to detect the generated hydrogen peroxide by colorimetry using peroxidase (POD). As PROD also reacts with glucose, the sample is pretreated by enzyme reaction using glucokinase (GK). Glucose is converted into glucose-6-phosphate (G-6-P), a species non-reactive with PROD. To drive the reaction to completion, an adenosine triphosphate (ATP)-regenerating system consisting of pyruvate kinase (PK) and phosphoenol pyruvate (PEP) is utilized. As ATP is converted to adenosine diphosphate (ADP), PK, in the presence of PEP, catalyzes the phosphorylation of ADP back to ATP. Following the conversion of glucose to G-6-P, the assay is rendered specific for 1,5AG. The following figure presents a schematic of the assay.



Package Components (Materials Supplied)

Reagent 1 (Pretreatment reagent) – Small Size (1 bottle, 20 mL), Large Size (1 bottle, 68 mL)

- 4-aminoantipyrine (4-AAP)
- Glucokinase (GK, from Bacillus stearothermophilus)
- Pyruvate kinase (PK, from pig heart)
- Adenosine triphosphate (ATP)
- Phosphoenol pyruvate (PEP)
- Sodium azide (1.0 mg/ml)
- various buffers, water, and BSA

Reagent 2 (Coloring reagent) – Small Size (1 bottle, 10 mL), Large Size (1 bottle, 34 mL)

- Pyranose oxidase (PROD from Polyporus obtusum)
- Peroxidase (POD, from horseradish)
- N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium dihydrate (TOOS)
- Sodium chloride
- Sodium azide: (0.2 mg/ml)
- various buffers and water

Warnings and Precautions

- Directions must be followed for optimal results.
- The pretreatment reagent (Reagent 1) and the coloring reagent (Reagent 2) contain sodium azide as a preservative. Avoid contact by mouth, with the skin, or any mucous membranes. In the case of contact with the reagents, immediately wash the affected areas with large amounts of water.

- Sodium azide reacts with lead and copper pipes to generate an explosive metal azide compound. When disposing leftover reagents down a sink, large amounts of water should be used to flush the pipes.
- As microbial contamination and residues from decomposed reagents are possible, reagents should not be replenished while a procedure is in process.
- The reagents should not be used if flocculation or discoloration occurs.
- Do not use reagents past their expiration dating.
- Reagents are to be stored at refrigerated temperatures (2-8°C) until expiration. Opened vials are usable for one month past the date they are opened if stored at 2-8°C.
- Do not combine different lots of Reagent 1 and Reagent 2.
- Do not dilute the reagents.
- As with all biological specimens, care should be taken to avoid exposure to infectious diseases.
- After GlycoMark™ analysis, the containers should be discarded in accordance with rules of the facility, and in accordance with local, State, and Federal regulations.

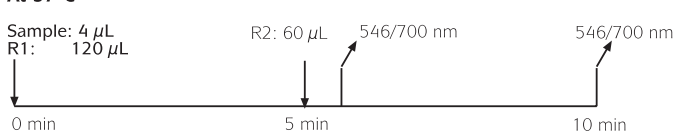
Materials Required But Not Supplied

- Calibration standard- Catalog No. NK-8320
- 2-level GlycoMark™ control set- Catalog No. NK-8330
- Saline reagent blank (0.9% NaCl in deionized water)
- Automated chemistry analyzer, Roche/Hitachi 917 (Roche Diagnostics Corporation, Indianapolis, IN), or other appropriate open systems

Procedure

The assay is based on a kinetic determination principle. All reagents are used without dilution or other preparation. The reaction is performed at 37°C on the Hitachi 917 automated analyzer. At time zero, 4 µL of standard, control, reagent blank, or sample is added to 120 µL of Reagent 1. After five minutes, 60 µL of Reagent 2 is added. The absorbances at 546 nm (primary wavelength) and 700 nm (secondary) are determined immediately after the addition of Reagent 2. The absorbances at both wavelengths are determined again at time = 10 minutes. The absorbance value at 700 nm is subtracted from the absorbance at 546 nm to correct for noise. This is performed for readings both at the 5 minute and 10 minute time points. Once this calculation is completed, the kinetic difference in absorbance (value at 10 minutes minus the value at 5 minutes) is calculated. Concentrations of 1,5AG in the reagent blank, control, or sample are determined by comparison to a two point calibration based on the kinetic change in absorbance of the reagent blank and standard (50 µg/ml 1,5AG). Conduct the measurement according to the following diagram.

At 37°C



The following describes the instrument parameters for the Hitachi 917 automatic analyzer. (The assay may also be performed on open systems following the assay procedure described above.)

[Analysis]

Analytical method/Measurement point (2 point end)	(10)(17)(34)(0)(0)
Wavelength (sub/main)	(700)(546)
Sample volume (standard)	(4.0)
Sample volume (decrease)	(2.0)
Sample volume (increase)	(8.0)
Diluent	(water)(0)
Reagent volume (R1)	(120)(0)(*)(0)
Reagent volume (R2)	(0)(0)(*)(0)
Reagent volume (R3)	(60)(0)(*)(0)
Reagent volume (R4)	(0)(0)(*)(0)
Absorbance limit	() ()
2 item analysis	()
Prozone limit	(-32000)(minimum)
Alkaline cleaner	(Designate)
Acid cleaner	(Not designate)

[Calibration]

Calibration method	(linear)()
Point	(2)
Span point	(2)
Weight factor	(0)
Auto-calibration	

blank	(0)	time out	()	change over	()
span	(0)			inter lot	()
2 point	(0)			inter bottle	()
whole points	(0)				

Focusing tolerance abs.	(0.1)
Dispersion tolerance abs.	(1000)
Sens. tolerance abs.	(0)
1st standard soln. abs. range	(-32000)(32000)

[Range]

Test item code	(*)
Unit	(µg/ml)
Report name	(*)
Data mode	(measurable)
Control sample measurement interval ()	
Instrument constant (Y=aX+b) a=(1.0) b=(0.0)	
Panic value	(-999999)(999999)
Normal value range	
Male	
() (year) () ()	Qualitative judgment
() (year) () ()	(not appoint)
() ()	(1) () ()
() ()	(2) () ()
Female	(3) () ()
() (year) () ()	(4) () ()
() (year) () ()	(5) () ()
() ()	(6) () ()

[Standard soln. conc.]

<Standard soln.>	(1) (2) (3) (4) (5) (6)
Conc.	(0.0) (50.0) (0) (0) (0) (0)
Position	(99) (*) () () () ()
Sample volume	(4.0) (4.0) (0) (0) (0) (0)

<Pre-dilution>

Sample volume	(1) (2) (3) (4) (5) (6)
Diluent volume	(0) (0) (0) (0) (0) (0)
Dilutor code	(0) (0) (0) (0) (0) (0)

* : User select

Note: Use separately sold 1,5AG standard solution (50 µg/ml) as the standard solution

Results

Results are expressed in $\mu\text{g/mL}$ 1,5AG.

Specimen Collection and Storage

GlycoMark™ provides equivalent results with serum or plasma samples.

Studies have shown that serum samples are stable at room temperature or 2-8°C for up to one week. For longer storage, freezing of the serum sample is recommended. Aliquots of frozen samples may be thawed and re-frozen for up to three cycles.

Kit Storage and Stability

GlycoMark™ is stable at refrigerated temperature (2-8°C) until the expiration date noted on the outer box. Once the reagents have been opened, they are stable for 30 days (at 2-8°C).

Limitations

- GlycoMark™ is to be used with serum or EDTA plasma; performance in other matrices has not been evaluated.
- Persistently positive urinary glucose levels, or oxyhyperglycemia after gastrectomy, may result in a low 1,5AG value. Low values have also been observed in pregnancy, terminal stage renal failure, dialysis patients, advanced cirrhosis, and prolonged incapability of oral ingestion of food. Abnormal values have also been noted in individuals with abnormal glomerular filtration rates.^{5,6}
- For some patients with severe hyperglycemia, the internal pool of 1,5AG may tend to remain depleted as a result of persistent glucosuria. In these cases, measurements of 1,5AG may be less indicative of initial recovery following initiation of anti-diabetic treatment.
- 1,5AG values may be increased when some Chinese medicines, such as Polygala Tenuifolia and Senega syrup, are administered. Values may also be increased during intravenous hyperalimentation. 1,5AG values may be lower in patients undergoing therapy with steroids.⁷
- As with all diagnostic tests, GlycoMark™ results should be interpreted along with clinical findings and results from other diagnostic methods.

Quality Controls

Good laboratory practice includes the assaying of controls at regular intervals. It is recommended to use the Tomen two-level control set ("Low" and "High"), and this control set is available from Tomen America, Inc. Users should also follow applicable Federal, state, and local requirements for quality control testing.

Performance Characteristics

The following performance parameters were established on the Roche Hitachi 917 analyzer.

Analytical Sensitivity

To determine the analytical sensitivity, twenty-one replicates of a saline reagent blank were analyzed as unknowns in one assay run. The analytical sensitivity is estimated to be 0.2 $\mu\text{g/ml}$, and this is defined as the mean 1,5AG concentration plus one standard deviation.

Expected Values

A study was done with a presumptively normal population in order to determine GlycoMark™ reference ranges for 1,5AG. The study included serum samples from 82 males between the ages of 18 and 39, 82 females between the ages of 18 and 39, and 30 males and 30 females of age 40 or greater, for a total of 224 individuals. Ethnic backgrounds included African Americans, Caucasians, Asians, and Hispanics. The data did not demonstrate differences in ages, but there were gender differences. The following table provides the male and female ranges, based on non-parametric 5th-95th percentiles.

GlycoMark™ Reference Ranges

	Mean (SD) $\mu\text{g/mL}$ 1,5AG	Reference Interval $\mu\text{g/mL}$ 1,5AG
Males	22.5 (5.8)	10.7-32.0
Females	17.7 (6.2)	6.8-29.3

Each laboratory should establish their own reference ranges.

Linearity

Linearity was evaluated in a series of experiments using spiked samples. The concentrations of 1,5AG in the samples ranged from 0 $\mu\text{g/mL}$ to 113 $\mu\text{g/mL}$. The samples were tested in quadruplicate with GlycoMark™, and the averaged obtained result (y-axis) was compared to the expected result (x-axis) by linear regression. The data indicated that GlycoMark™ is linear to at least 110 $\mu\text{g/mL}$ 1,5AG.

Interference Testing/Specificity

Studies were performed to evaluate the effects of high levels of hemolysis (hemoglobin), lipemia (triglycerides), and icterus (bilirubin) on the GlycoMark™ test. The data showed that GlycoMark™ is unaffected by hemoglobin up to 125 mg/dL, triglycerides up to 1153 mg/dL, and bilirubin up to 53 mg/dL. GlycoMark™ results were also unaffected by the following substances at their noted concentrations: glucose- 1000 mg/dL; maltose- 500 mg/dL; ascorbic acid- 25 mg/dL; uric acid,- 20 mg/dL; creatinine- 10 mg/dL, urea- 20 mg/dL.

The following anticoagulants did not interfere with the GlycoMark™ test, at their noted concentrations: EDTA- 1.0%; heparin- 100 units/mL; sodium fluoride- 0.25%; sodium citrate- 2.0%.

Precision

Precision studies were performed to evaluate both within-assay and between-assay precision. The summarized results are described below.

Within-Assay Twenty (20) replicates of the GlycoMark™ controls were assayed according to standard procedure. Mean, standard deviation, and percent coefficient of variation (%CV) were calculated for each control solution. The within-assay precision ranged from 1.3 to 3.8%.

Within-Assay Precision

	Control Low n = 20	Control High n = 20
Mean $\mu\text{g/ml}$ 1,5AG	4.63	14.67
Standard Deviation	0.18	0.19
% CV	3.83	1.28

Between-Assay (Day-to-Day) Two (2) replicates of each of the GlycoMark™ controls and two serum pools were assayed twice daily with one (1) lot of reagents according to standard procedure for a total of 10 days. Mean, standard deviation, and percent coefficient of variation (%CV) for each sample over the entire 10 day set were calculated from their respective daily standard calibrations. The between-assay %CVs ranged from approximately 1% to approximately 4%.

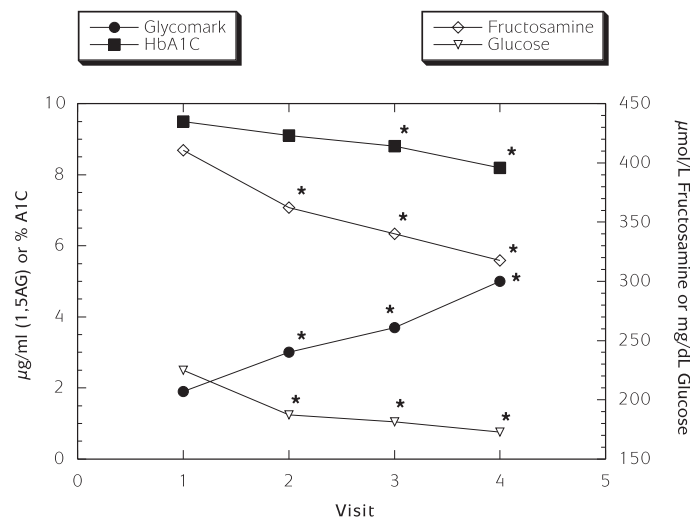
Between-Assay Precision

	Control Low	Control High	Pool 1	Pool 2
Number of replicates	40	40	40	40
Mean $\mu\text{g/ml}$ 1,5AG	4.70	14.70	19.60	27.00
Standard Deviation	0.18	0.20	0.23	0.21
%CV	3.71	1.35	1.17	0.79

Clinical Use

A prospective, longitudinal study was performed with 77 patients with diabetes (both type 1 and type 2). The patients exhibited suboptimal glycemic control (A1C level greater than or equal to 7%) at study entry, and these patients were monitored for eight weeks following initiation or modification of anti-hyperglycemic treatments. Measurements for GlycoMark™, A1C, fructosamine, and glucose were performed every two weeks for the first four weeks (Visits 1-3) and then at Week 8 (Visit 4).

The figure presents the mean values for each marker by visit. An asterisk (*) denotes significant changes vs. baseline values ($p < 0.05$, Wilcoxon signed-rank test). Significant changes vs. baseline appeared at Visit 2 for GlycoMark™, fructosamine, and glucose; whereas a significant change did not appear until Visit 3 for A1C.



Correlations of markers were also determined and are presented in the table. GlycoMark™ 1,5AG shows high association with A1C and fructosamine, two established markers used for the monitoring of glycemic control. There was understandably less of an association between glucose and the other three markers, as glucose can only provide a "snapshot" of glucose monitoring at the time of sampling.

Association between variables
Spearman's non-parametric analysis

	1,5AG	A1C	Fructosamine	Glucose
1,5AG				
A1C	-0.6459*			
Fructosamine	-0.6751*	0.6955*		
Glucose	-0.3358*	0.3334*	0.3529*	

* $p < 0.0001$

Lastly, concordance of time-dependent changes of GlycoMark™ 1,5AG values with the acknowledged gold standard method, A1C was determined. "Concordance" was defined as either increases in 1,5AG values with corresponding decreases in A1C values, or, conversely, decreases in 1,5AG values with corresponding increases in A1C values. 89.6% of the patients (69 of 77) displayed concordance in changes of GlycoMark™ and A1C values with time.

References

- Akanuma Y, Ogawa K, Yamanouchi T et al. Decreased plasma 1,5-anhydroglucitol in diabetic patients. Diabetes 1981;30:suppl 1:124A.
- Yoshioka S, Saitoh S, Negishi C et al. Variations of 1-deoxyglucose (1,5-anhydroglucitol) content in plasma from patients with insulin-dependent diabetes mellitus. Clin Chem 1983;29:1396-8.
- Yamanouchi T, Minoda S, Yabuuchi M, Akanuma Y, Akanuma H, Miyashita H, Akaoka I. Plasma 1,5-anhydroglucitol as new clinical marker of glycemic control in NIDDM patients. Diabetes 1989;38:6:723-9.
- Yamanouchi T, Ogata N, Tagaya T, Kawasaki T, Sekino N, Funato H, Akaoka I, Miyashita H. Clinical usefulness of serum 1,5-anhydroglucitol in monitoring glycemic control. The Lancet 1996;347:1514-8.
- Yamanouchi, S. Guide for Laboratory Test 1992: 597-599, 1992.
- Minoda S: Teikyo Medical Journal, 16 (4): 321-333,1993.
- Kato C: Clinical Pathology 44 (4): 369-399, 1995.

P/N (Small) NK-8300DI

P/N (Large) NK-8310DI

Rev. A

October 2003

Tomen America, Inc.
1285 Avenue of the Americas
New York, NY 10019
Phone/FAX: 800-658-3932
Email: support@glycomark.com