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Evaluation of an assay for serum 1,5-anhydroglucitol (GlycoMark™) and determination of reference intervals on the Hitachi 917 analyzer

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Abstract

Background: 1,5-Anhydroglucitol (1,5-AG) is a glucose analogue, which is decreased in hyperglycemic individuals. We report the technical performance of an assay (GlycoMark™) on a chemistry analyzer, evaluation of analyte stability and determination of reference intervals for 1,5-AG in a non-diabetic US population.
Methods: NCCLS protocols were followed to evaluate the reagent on a Hitachi 917 chemistry analyzer.
Results: Intra- and interassay imprecision ranged from 1.3% to 3.8% and 0.79% to 3.7%, respectively. The assay was linear to 110 μg/ml. Interference from triglyceride, hemoglobin and bilirubin was <10% to concentrations of 12.6 mmol/l, 12.1 and 911.4 μmol/l, respectively. Correlation coefficients between lot numbers on the Hitachi 917 and between analyses on the Hitachi 917 and the Hitachi 7170 analyzers were >0.99. The lowest limit of detection was 0.49 μg/ml (mean±2 S.D.). 1,5-AG was stable at 4 °C for 7 days, at 22 °C for 5 days, at −80 °C for 14 days and for three freeze–thaw cycles at −80 °C. The US reference intervals (nonparametric 2.5th–97.5th percentiles) were 10.2–33.8 μg/ml (males) and 5.9–31.8 μg/ml (females).
Conclusions: The performance of the GlycoMark™ assay for the measurement of 1,5-AG was acceptable on the Hitachi 917 analyzer.

Keywords: 1,5-Anhydroglucitol; 1-Deoxyglucose; Diabetes; Hyperglycemic; Biomarker

Abbreviations: 1,5-AG, 1,5-anhydroglucitol; CLCS, Core Laboratory for Clinical Studies; PK, Pyruvate kinase; PEP, phosphoenolpyruvate.

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1. Introduction

1,5-Anhydroglucitol (1,5-AG), or 1-deoxyglucose, is an unmetabolizable glucose analogue present in human blood due predominantly to dietary ingestion. In general, cereal foods, beef and pork contain relatively high 1,5-AG levels with soybeans containing an unusually high 1,5-AG concentration [1]. A small but significant fraction of 1,5-AG (~10%) appears to be derived from endogenous synthesis [1]. Medical interest in the measurement of 1,5-AG derives from its potential use as a marker for glycemic control in the monitoring of diabetes mellitus [2–11]. In the normal non-diabetic state, 1,5-AG concentration is attributed to a steady state of ingestion, distribution and excretion of unmetabolized 1,5-AG. However, in periods of glucosuric hyperglycemia, the 1,5-AG excretion rate is increased, apparently due to competition for reabsorption by increased glucose [3,12,13]. Poor glycemic control can therefore be accompanied by a significant overall decrease in 1,5-AG. In principle, serial 1,5-AG monitoring on appropriate time scales can be used to monitor the success of intervention in diabetes, as evidenced by reestablishment of a euglycemic state, or to indicate episodic hyperglycemia, as evidenced by decreases in 1,5-AG or an alteration in the rate of repletion of 1,5-AG to normal values [14,15]. The information derived from 1,5-AG monitoring is distinct from monitoring of glycated proteins: whereas glycated hemoglobin (gHb) provides an averaged measurement of plasma glucose over a period of 6–8 weeks [16,17], and fructosamine 1–3 weeks, 1,5-AG changes reflect changes in glycemic control on a shorter time scale (days to weeks) and can, in principle, provide information about excursions of glucose above the glucosuric threshold that might not be apparent from gHb or fructosamine [3,15].

Given the established principle of close monitoring of glucose and gHb as standards of care in diabetes [16], further study of the information provided by 1,5-AG monitoring in diabetes care is certainly warranted. The overwhelming majority of data on clinical use of 1,5-AG comes from Japan where reduced concentrations of 1,5-AG in serum of hyperglycemic patients in comparison to euglycemic subjects [2] have been demonstrated. Additionally, a gradual normalization of 1,5-AG values for patients responding to anti-diabetic therapies [7] has been demonstrated, and studies have shown that 1,5-AG measurements reflect glycemic status over the previous 48 h to 2 weeks. The early Japanese research studies of 1,5-AG employed measurement via chromatographic methods. For instance, gas–liquid chromatography (GC-LC) and coupled gas chromatography–mass spectrometry (GC-MS) were utilized [18,19]. With either method, it was necessary to remove plasma proteins and glucose prior to measurement of 1,5-AG. These methods were proven to be sensitive and precise for clinical research studies, but the multistep chromatographic procedures were time consuming and cumbersome. An automated 1,5-AG assay (Lana® 1,5-AG Auto Liquid, Nippon Kayaku, Japan), using a two-step enzymatic method for measurement [20,21], has been commercially available in Japan since 1991 and is used extensively there. The test has also recently received clearance from the Food and Drug Administration (FDA) in the United States as a tool for intermediate term monitoring of glycemic control under the trade name GlycoMark™. Although the test has been well characterized in Japan, the performance characteristics and analyte stability have not been extensively evaluated in the US. Furthermore, because of its derivation from diet, it is important to establish population-dependent reference intervals for 1,5-AG, as there are no firm data on the differences in 1,5-AG consumption in Japanese and American diets. Our objectives in this study were thus threefold: first, to establish performance characteristics of the assay using the automated Hitachi 917 chemistry analyzer; second, to evaluate the stability of 1,5-AG during collection and storage of human blood; and third, to determine reference intervals for individuals residing in the US for comparison to reference intervals established in Japanese studies using the same assay.

2. Materials and methods

2.1. Reagents and analyzer

The GlycoMark™ kits were provided by Tomen America (New York, NY). This product contains a two-reagent system. One hundred twenty microliters of Reagent 1 [4.0 kU of glucokinase, 3.0 kU of pyruvate kinase (PK), 5.0 kU of ascorbate oxidase, 1 mmol of

ATP, 8 mmol of phosphoenolpyruvate (PEP), 1.5 mmol of 4-aminoantipyrine, 7.5 mmol of $MgCl_2$ and 30 mmol of KCl per liter of 2-morpholinoethane sulfonic acid buffer (17.8 mmol/l, pH 6.4)] is combined with 4 μl of serum or Na-EDTA plasma and allowed to react for 5 min. In the second step, 60 μl of Reagent 2 [40 kU of pyranose oxidase, 67.5 kU peroxidase, 6 mmol *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium dihydrate per liter of HEPES buffer (56 mmol/l, pH 8.0)] is added, and the reaction is continued for an additional 5 min. The primary reaction with glucokinase renders the system specific for 1,5-AG as all glucose present in the sample is converted into glucose-6-phosphate, a species unreactive with pyranose oxidase. To drive the reaction to completion, an ATP-regenerating system consisting of PK and PEP is utilized. As ATP is converted to ADP, PK in the presence of PEP catalyzes the phosphorylation of ADP to ATP. Oxidation of the C-2 hydroxyl group of 1,5-AG generates hydrogen peroxide leading to color development, which is bichromatically monitored at 546/700 nm. All reactions were performed on a Hitachi 917 Analyzer (Roche Diagnostics, Indianapolis, IN) at 37 °C. Instrument parameter settings, commercial calibrators (0, 50 μg/ml) and quality control materials were provided by Tomen America.

2.2. Samples

Unless otherwise indicated, all human serum samples were collected from adult volunteers under informed consent according to policies approved by the Institutional Review Board at Washington University School of Medicine, Saint Louis, MO. Serum samples were collected using BD Vacutainer™ SST™ Tubes (Becton Dickinson, Franklin Lakes, NJ) and processed according to the manufacturer's instructions. Samples not immediately analyzed were stored at −80 °C in 2-ml screw-capped polypropylene vials (SARSTEDT, Newton, NC).

3. Analytical methods

3.1. Precision

Intra-assay imprecision was evaluated with two levels of quality control material. Twenty aliquots were analyzed in one analytical run. Interassay imprecision was evaluated according to NCCLS protocol EP5-A [22]. Briefly, two levels of quality control material and two levels of human serum were prepared as individual pools and aliquoted into 20 0.4-ml samples in 2-ml screw-capped cryo vials. All samples were immediately stored at −80 °C. Duplicate 1,5-AG analyses were performed on each pool in two separate runs per day for 10 days. Precision was evaluated as the coefficient of variation calculated from the data series mean and standard deviation.

3.2. Linearity

1,5-AG deficient serum was prepared by dialysis. Briefly, 30 ml of fresh serum was collected from an adult volunteer. The 1,5-AG concentration was 19.8 μg/ml. To deplete the serum of 1,5-AG, 20 ml of this serum was dialyzed against phosphate-buffered saline (4 °C, 16 h), in Spectra/Por CE dialysis tubing with a molecular weight cutoff of 1000 Da (Spectrum Laboratories, Rancho Dominguez, CA). The 1,5-AG concentration after dialysis was 0.1 μg/ml. To 3.0 ml of 1,5-AG deficient serum, 65 μl of 0.9% saline was added to form the low pool. To another 3.0 ml of 1,5-AG deficient serum, 65 μl of concentrated 1,5-AG stock solution was added (4600 μg/ml prepared in 0.9% saline; Sigma-Aldrich Chemicals, St. Louis, MO). This preparation was used as the high pool. The high pool was combined with the low pool to generate a series of dilutions. Each sample was analyzed in four replicates according to NCCLS EP6-P [23]. The series dilution was analyzed neat (0–110 μg/ml) and also as a 1:1 dilution (0–55.2 μg/ml) with 0.9% saline. Linearity was evaluated from regression statistics using the statistical program EP Evaluator™ Release 3.11e (David G. Rhoads Associates, Kennett Square, PA).

3.3. Interference studies

1,5-AG was evaluated for common interferences including those due to lipemia, hemolysis and bilirubinemia. Each potential interferent was evaluated by supplementation of human serum with the indicated agent to create a high or low interferent pool followed by serial dilution with the high to the low pool to create a dilution series. Lipemia was inves-

tigated using triglyceride-rich lipoprotein subfractions as an interferent. Sera (10 ml) containing elevated triglyceride concentrations were pooled and centrifuged (18 h, 18 °C, 105,000×*g*). Fractions concentrated in triglyceride were collected. This material (0.7 ml, 27.1 mmol/l triglyceride) was pooled, and the centrifugation was repeated. The triglyceride-rich fraction was physically isolated from the remaining sample with a Beckman CentriTube slicer (Beckman Coulter, Fullerton, CA) and collected (0.12 ml, 129.6 mmol/l triglyceride). A high triglyceride pool was prepared by adding 60 μl of this triglyceride-rich material to 550 μl of fresh human serum (net triglyceride=12.6 mmol/l) (1,5-AG=14.3 μg/ml). A low triglyceride pool was prepared by adding 218 μl of saline to 2000 μl of the same human serum (triglyceride=1.0 mmol/l). A series of samples varying in triglyceride concentration was created by serial dilution of the high to the low triglyceride pool.

Hemoglobin interference was evaluated by preparing a dilution series with hemoglobin obtained from fractured red blood cells. After centrifugation (15 min, 4 °C, 3500×*g*) of 16 ml of lithium heparin blood, the plasma was discarded and the packed cells were washed with two volumes of 0.9% saline. After centrifugation, the wash was twice repeated for a total of three washes. The packed cells were resuspended in an equal volume of deionized water and stored at −80 °C for 16 h. The mixture was thawed and centrifuged. The hemolysate supernatant was collected, and the hemoglobin concentration was determined (2279 μmol/l). To 1864 μl of fresh human serum (1,5-AG=18.4 μg/ml), 136 μl of the hemolysate supernatant was added [high hemolysate pool (Hb)=166 μmol/l]. The low hemolysate pool was prepared by adding 102 μl of deionized water to 1398 μl of non-hemolyzed fresh human serum. A series of samples varying in added hemoglobin concentration was created by serial dilution of the high pool to the low hemolysate pool.

Bilirubin interference was evaluated by preparing a dilution series of serum with varying concentrations of commercial bilirubin. Bilirubin (National Bureau of Standards 916a Bilirubin Clinical Standard) was prepared in 0.1 mol/l NaOH to a concentration of 342,000 μmol/l. Fresh human serum (1,5-AG=19.4 μg/ml) was prepared with this material having a bilirubin concentration of 911.4 μmol/l (high pool). The low bilirubin pool was unadulterated fresh human serum (5.1 μmol/l bilirubin). A series of samples varying in added bilirubin concentration was created by serial dilution of the high to the low bilirubin pool.

Interference was calculated as the percent change in 1,5-AG concentration in the presence of the interferent compared to the 1,5-AG concentration in the absence of supplemented interferent. Each value represents the mean of three measurements. A change of >10% was considered significant.

3.4. Method and between lot comparisons

A comparison of the performance of the GlycoMark™ assay on two different analyzers was completed according to NCCLS EP9-A [24]. A split sample comparison was performed on 50 fresh patient samples, which were provided by the reagent manufacturer in Tokyo, Japan. Samples were stored at −80 °C until received in the United States. Each laboratory analyzed 10 samples in duplicate per day on either a Hitachi 917 (St. Louis, MO) or a Hitachi 7170 (Tokyo, Japan). Three different reagent lot numbers were compared on the Hitachi 917. The results were evaluated by linear regression analysis.

3.5. Detection limit

The minimum detectable 1,5-AG concentration was assessed as the measured analyte concentration mean plus 2 standard deviations of replicate samples of the zero calibrator (saline). The minimum detection limit was calculated as the mean from four separate analytical runs of 21 replicates.

3.6. Analyte stability studies

1,5-AG stability was evaluated for several storage conditions. Fresh serum was collected from six volunteers representing a range of 1,5-AG concentrations. Immediately after collection and processing, baseline serum 1,5-AG value assignments were made for each sample as the mean of triplicate measurements. The remaining serum was aliquoted into 2-ml screw-capped cryo vials and stored at the indicated conditions protected from light. Analyte stability was evaluated for (1) three freeze–thaw cycles over 7 days (−80 °C); (2) 4 °C for 7 days; or (3) 22 °C for 7 days.

Table 1
Population descriptive statistics for reference interval study participants

N	All	Males		Females	
		18–39 years	All males	18–39 years	All females
Total	224	82	112	82	112
African-American	102	31	46	41	56
Caucasian	88	36	44	36	44
Asian-South Pacific	29	13	19	5	10
Hispanic	5	2	3	0	2

Additionally, 1,5-AG stability at −80 °C with one freeze–thaw cycle was evaluated during the interassay imprecision protocol for 14 days. Changes in 1,5-AG concentration >10% from baseline were considered significant.

3.7. Reference interval studies

The reference interval study was designed according to the guideline in NCCLS C28-A [25]. Serum samples, which had been collected from healthy adult volunteers and stored at −80 °C for <1 year, were measured to determine the 1,5-AG distribution in non-diabetic American subjects. Inclusion criteria for study participants included no previous history of diabetes, ambulatory status, absence of any chronic conditions, glycated hemoglobin ≤6% and serum creatinine concentrations which were within the normal reference interval for the indicated age and gender. Participants were selected to obtain a cross-sectional representation of non-diabetic subjects from the recruitment areas (Table 1). Study sample size was based on the C28-A guideline in which partitions are hypothesized to exist. A minimum of 60 reference individuals in each partition was recruited. Because Japanese data have exhibited gender biases and suggested a possible difference in values as an effect of age, both gender and age were hypothesized to be partition criteria. With regard to the age partition, reference individuals were categorized as either 18–39 years of age or >40 years. To determine if gender or age-specific reference intervals were required, the parametric two-sample *t*-test was utilized, with $p<0.05$ considered significant. Reference intervals were calculated as the nonparametric 2.5th–97.5th percentile ranges independently for males and females. Statistical analyses were performed using the JMP Software package (SAS Institute, Cary, NC) and the NCSS software (NCSS, Kaysville, UT).

4. Results

4.1. Imprecision

Imprecision was evaluated as the coefficient of variation (CV%). Intra-assay imprecision was determined with two quality control materials. The largest intra-assay CV% observed was 3.83% (1,5-AG=4.6 μg/ml). Interassay imprecision was determined using quality control materials and human serum. The largest interassay CV% observed was 3.71% (1,5-AG=4.7 μg/ml). 1,5-AG imprecision for all samples is summarized in Table 2.

Table 2
Imprecision of 1,5-AG measurements on the Hitachi 917 analyzer

	Intra-assay imprecision		Interassay imprecision			
	Quality control 1	Quality control 2	Quality control 1	Quality control 2	Human serum 1	Human serum 2
N	20	20	40	40	40	40
1,5-AG (μg/ml)	4.6	14.6	4.7	14.7	19.6	27.0
S.D.	0.18	0.19	0.18	0.20	0.23	0.21
CV%	3.83	1.28	3.71	1.35	1.17	0.79

4.2. Linearity

Because it was difficult to identify study participants with 1,5-AG concentrations at the extreme ends of the analytical measurement range, the concentration of 1,5-AG in fresh human serum from a single donor was either depleted or elevated in accordance with NCCLS guidelines [20]. Linearity was evaluated to a high-end range of 110 μg/ml (Fig. 1). The 1,5-AG assay was found to be linear to this range without sample dilution. Linear regression values of the observed 1,5-AG values plotted against theoretical values for the 0–110 μg/ml curve were: slope, 1.007 (±0.005); intercept, −0.49 (±0.27); and standard error of estimate, 1.05.

4.3. Interferences

Interference was defined as a >10% change in 1,5-AG concentration in the presence of the interferent compared to the 1,5-AG concentration in the absence of supplemented interferent. The acceptable interferent concentrations were observed at 12.6 mmol/l (triglyceride), 12.1 μmol/l (hemoglobin) and 911.4 μmol/l (bilirubin).

4.4. Method comparison (and lot–lot)

Ten different frozen patient samples were measured per day for 5 days for a total of 50 samples. Each sample was measured at the CLCS with three different lot numbers of 1,5-AG reagent. These values were compared to 1,5-AG values assigned by the reagent manufacturer in Japan. The greatest slope deviation in any of the compared lot numbers from 1.0 was 0.01. The greatest observed intercept deviation from 0.0 was 0.5279. Correlation coefficients were above 0.99 for comparisons (Fig. 2).

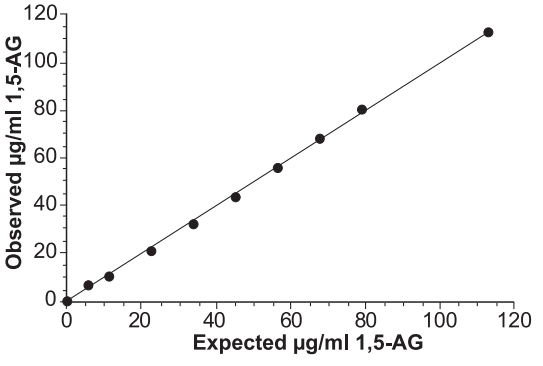

Fig. 1. 1,5-Anhydroglucitol linearity: observed values plotted vs. theoretical values. Each point represents the mean of four measurements. y=−0.49+1.07, R=0.999, standard error of estimate=1.05.

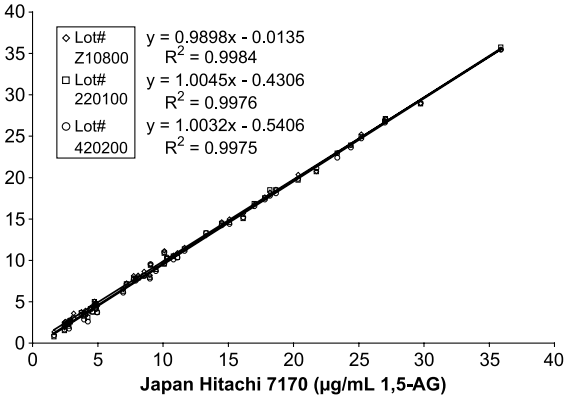

Fig. 2. Comparison of 1,5-anhydroglucitol analyzed by three different reagent lot numbers on the Hitachi 917 analyzer at the Core Laboratory for Clinical Studies (Y-axis). Target values were assigned by the manufacturer measured on a Hitachi 7170 analyzer.

4.5. Lowest limit of detection

The lowest limit of detection (LLD) of 1,5-AG measured on the Hitachi 917 analyzer was calculated as the mean plus 2 S.D. of replicate samples of the zero calibrator (saline). The LLD was determined to be 0.49 μg/ml.

4.6. Sample stability

Stability studies were performed to determine whether 1,5-AG concentration was affected by delays in analysis after storage at various temperatures or by the freeze–thaw process. Such delays in analysis may occur due to transport time or the need to reanalyze stored samples at future times. Changes in 1,5-AG concentration >10% were considered significant. Previous in-house studies by the reagent manufacturer confirmed that 1,5-AG was stable for at least 36 months when stored at −20 °C. In this study, the largest change observed after three freeze–thaw cycles was −2.9% (13.7–13.4 μg/ml). At 4 °C storage, the largest percent change observed was +3.6% (13.7–14.2 μg/ml) after 7 days of storage. Samples were also

Table 3
1,5-Anhydroglucitol reference intervals partitioned by gender and age (gHb and glucose values presented for comparison)

	All males and females	Males		Females	
		18–39 years	All males	18–39 years	All females
1,5-AG (μg/ml)	20.1 (6.4)	23.9 (4.9)	22.5 (5.8)	18.4 (6.4)[†]	17.7 (6.2)[§]
1,5-AG 2.5th–97.5th RI (μg/ml)			10.2–33.8		5.9–31.8
gHb (%)	5.3 (0.3)	5.3 (0.3)	5.3 (0.3)	5.2 (0.3)	5.3 (0.3)
Glucose (mmol/l)	4.8 (0.8)	4.8 (0.8)	4.9 (0.8)	4.6 (0.9)	4.7 (0.8)

Conversion factor for gHb% to Hb fraction: 0.01.
[†] $p<0.005$ vs. males 18–39 years.
[§] $p<0.0001$ vs. all males.

analyzed at storage day 5 and the largest change was +2.9% (13.7–14.1 μg/ml). For samples stored at 22 °C, all samples at day 7 had changed by >5%, with the largest change being +10.2% (13.7–15.1 μg/ml). On day 5 (22 °C), all samples had changed by <5%, with the greatest change being +4.4% (13.7–14.3 μg/ml). When evaluating interassay imprecision, samples were aliquoted into 2-ml screw-capped cryo vials and stored at −80 °C. Two samples were removed daily for 1,5-AG analysis. During the 14-day protocol, the largest observed change in stored serum samples was −3.0% (19.7–19.1 μg/ml). In summary, 1,5-AG was acceptably stable for three freeze–thaw cycles at −80 °C, at 4 °C for up to 7 days, at 22 °C for up to 5 days or −80 °C for up to 14 days.

4.7. Reference interval

The overall distribution of 1,5-AG values in the US study population of 224 subjects (see Table 3) was found to be consistent with a Gaussian curve (Fig. 3). Therefore, comparative statistical analyses using the parametric two-sample *t*-test were used to determine the need for age- or gender-specific 1,5-AG reference intervals (Table 3). The results of the analyses show that there is a significant gender bias, but not a statistically significant bias as an effect of age. Therefore, two reference intervals are required, one each for males and females. The nonparametric 2.5th–97.5th reference intervals were determined to be 10.2–33.8 μg/ml (males) and 5.9–31.8 μg/ml (females).

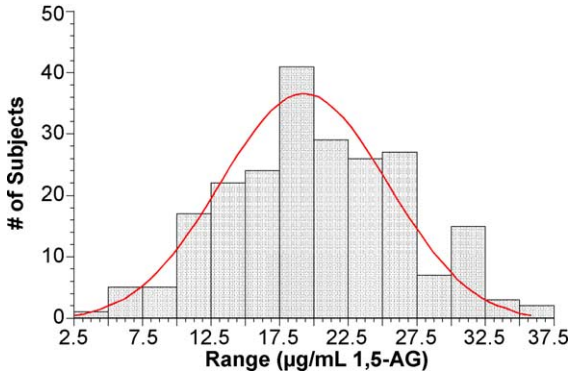

Fig. 3. Frequency distribution of 1,5-anhydroglucitol in males (n=112) and females (n=112) in a US population.

5. Discussion

The objectives of this study were threefold. First, the analytical characteristics of the GlycoMark™ enzymatic assay for 1,5-AG on the Hitachi automated analyzer were evaluated; second, the stability of 1,5-AG under various storage conditions was assessed; and third, reference intervals for 1,5-AG in a US population were determined. The analytical evaluation showed that the GlycoMark 1,5-AG assay is clinically acceptable for routine laboratory use, with performance characteristics comparable to those of most enzymatic assays used on automated analyzers. The linear range of the assay completely encompasses the range of expected values for 1,5-AG in both healthy and diabetic individuals. Significant analytical interferences were not observed for conditions of moderate hemoglobin, bilirubin or lipid concentrations. Although not evaluated in this study, the directional insert for the test reports a lack of interference of glucose to 55.5 mmol/l, maltose to 500 mg/dl,

ascorbic acid to 25 mg/dl, uric acid to 1190 μmol/l and creatinine to 884 μmol/l.

Investigation of the stability of 1,5-AG under a variety of storage conditions indicated that degradation of 1,5-AG is unlikely to be an issue for specimen collection and processing in routine clinical use. Determination of the reference interval for non-diabetic subjects with normal creatinine concentrations demonstrated the need for partitioning into gender-specific ranges. The reference intervals for the US populations were slightly lower than those observed in Japan. The Nippon Kayaku product insert for the Lana® 1,5-AG Auto Liquid assay indicates that the central 95% reference intervals for males and females are 12.2–41.0 and 9.5–33.5 μg/ml, respectively. The average absolute difference observed in the split sample comparison between the US and Japanese laboratories was 0.5 μg/ml, suggesting that the reference interval differences are, in fact, true regional differences rather than analytical. Variation in overall dietary intake of 1,5-AG is likely to be the major contributor to the difference in 1,5-AG reference intervals since evaluation of 1,5-AG values in the subpopulation of Asian subjects in this study gave similar or lower results than the overall mean of values; thus, an effect purely due to race is not indicated. Other lifestyle differences may also contribute to the observed reference interval deviations but were not investigated in this study.

The absolute range of the reference interval in all populations is very similar. To be clinically useful, it must be demonstrated that the 1,5-AG concentration in diabetics is distinctly different from that of healthy individuals. In addition, the marker will be most useful if the information provided is distinct from that obtainable from other monitored markers. The most striking difference between 1,5-AG and other markers (e.g., glycated hemoglobin) is the time frame in which physiological concentrations change in response to changes in blood glucose. In this respect, 1,5-AG most resembles fructosamine. To date, few prospective diabetic trials have compared the utility of the various potential diabetic blood markers. One study [15] demonstrated a greater sensitivity of serial 1,5-AG measurements compared to glycated hemoglobin and fructosamine to indicate deterioration in glycemic control in the short term (2 weeks) during a study involving pharmacologic intervention in newly diagnosed type 2 diabetics who also were overtly depleted of plasma 1,5-AG at the time of diagnosis. Availability of an automated 1,5-AG assay in the US will allow more extensive studies of the potential utility of 1,5-AG in diabetes management to be conducted.

Prospective studies with diabetic patients are ongoing to further evaluate the utility of 1,5-AG in the administration and monitoring of health care in diabetes. The addition of data from diabetic individuals will permit the determination of sensitivity, specificity and, ultimately, the judgment regarding the potential usefulness of 1,5-AG as a tool for screening and monitoring of glycemic control. The concept of diabetic screening using 1,5-AG measurements is of special interest as some early research studies concluded that the marker is sufficiently sensitive and specific, whereas others have not. For instance, Yamanouchi et al. [8] performed a multicenter study evaluating 342 subjects with normal glucose tolerance comparing the 1,5-AG results to those in 460 patients with diabetes. In this analysis, the 1,5-AG assay demonstrated 84% sensitivity and 93% specificity. Robertson et al. [26] demonstrated similar results in a hospital population, but were unable to confirm the results in a larger community-based cohort of Mauritian Chinese subjects [27]. Furthermore, 1,5-AG measurements appear to be of limited use in screening for gestational diabetes likely due to disturbances in renal function during pregnancy [8,28].

Currently, the GlycoMark test for 1,5-AG is indicated in the US only for monitoring of intermediate-term glycemic control, an intended use wherein there is clearly strong and supportive clinical data [29]. This limited regulatory clearance is prudent until such time as more data are available in the screening setting.

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