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Comparison of Two Assays for Serum 1,5-Anhydroglucitol

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Dear editor:

1,5-anhydroglucitol (1,5-AG) is a monosaccharide, originating mainly from foods and closely resembling glucose in structure. Blood concentrations of 1,5-AG are thought to reflect glucose excursions and short-term (1–2 week) glycemic control in the diabetes patient (1–5). The attractiveness of 1,5-AG for use in diabetes care is that it may capture additional information on glycemic excursions not reflected in values of hemoglobin A1c, the most common measure of glucose control. Only one commercial assay for 1,5-AG (GlycoMark™) is approved and marketed for clinical use in the U.S. This assay is available at major laboratories and is reimbursed by some insurers including Medicare. We sought to directly compare the performance of the GlycoMark assay (GlycoMark, Inc) to the Determiner-L 1,5-AG assay manufactured by Kyowa Medex (Tokyo, Japan). We also assessed the effect of a single freeze-thaw cycle on the GlycoMark assay value.

We utilized stored serum specimens obtained from a subsample of participants in the Atherosclerosis Risk in Communities (ARIC) Study in 2005–06 (6). All samples were stored at –70°C until time of measurement. To assess the impact of a freeze-thaw cycle, we conducted measurements using the GlycoMark assay on separate days (4 months apart) using identical methodology. At the time of the second GlycoMark measurement, we simultaneously conducted measurements using the Determiner-L Kyowa Medex assay in the same samples so as to have head-to-head data on the comparative performance of these 2 brands.

These enzymatic colorimetric assays were implemented on the Roche Modular P800 (Roche Diagnostics Corp., Indianapolis, IN) with materials from GlycoMark, Inc and Kyowa Medex. The GlycoMark assay is a 2-step, end point enzymatic method consisting of a 2-reagent system. In the first step, the specimen is incubated with a reagent mixture (glucokinase, pyruvate kinase, phosphoenol pyruvate) that converts endogenous glucose to glucose-6-phosphate. The second reagent (pyranose oxidase) oxidizes 1,5 AG, producing hydrogen peroxide. Peroxidase catalyzes the formation of a colored end product from 4-aminoantipyrine and TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium

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dehydrate), with the absorbance measured at 546 nm. In the Kyowa Medex assay 1,5-anhydro-D-glucitol (1,5AG) is first converted to 1,5-anhydro-glucitol-6-phosphate (AG-6-P) by ADP-dependent hexokinase (ADP-HK) and adenosine-5'-diphosphate (ADP). AG-6-phosphate dihydrogenase (AG6P-DH) reacts with AG-6-P and acid β -NADP⁺, producing β -NADPH. Diaphorase (DIP) then promotes the reaction of NADPH and the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium sodium to produce a water-soluble formazan pigment, measured at 450 nm to determine the 1,5-AG concentration.

We used Student's *t*-test to compare mean 1,5-AG values for the GlycoMark assay before and after the freeze-thaw and to compare the GlycoMark results to those obtained with the Determiner-L assay. We calculated the CVs for the differences between the assays (CV_D). We also calculated the Pearson's and intra-class correlation (ICC) coefficients to compare the different measurements and visually displayed the agreement using scatter and Bland-Altman plots (7). We also fit linear regression models to these data. Institutional review boards approved the study protocol and written informed consent was obtained from all participants.

Of the 70 samples selected for measurement, 66 had sufficient sample volume and were assayed using both methods (mean age 63 y, 52% female, 32% diabetic). The intra- and inter-assay CVs for GlycoMark were 0.7% and 2.7%, respectively, and 1.9% and 5.1% for the Determiner-L assay, respectively.

Effect of Freeze-Thaw on GlycoMark 1,5-AG Results: The mean (SD) of the first run of the GlycoMark assay was 15.9 ug/dl (6.7) as compared to 16.1 ug/dl (6.9) in the same samples after 4 months of storage. The mean difference was small, but statistically significant: -0.22 ug/dl (95%CI -0.30, -0.14), *p*-value <0.0001. The CV_D was 1.84%. The high correlation but systematic difference between the measurements at the two time points is shown in the scatter and Bland-Altman plots (Fig. 1A and B). The Pearson's correlation and ICC for the measurements at the two time points were 0.999 and 0.997, respectively. The difference (bias) was not entirely consistent over the range of values as can be seen in the Bland-Altman plot with fitted regression line (Fig. 1B) with a slope that was statistically significant from zero (*p* =0.0021).

Comparison of GlycoMark and Determiner-L Kyowa 1,5-AG Assays: The mean (SD) of the GlycoMark was 16.1 ug/dl (6.9) as compared to 16.5 ug/dl (7.1) for the Determiner-L Kyowa 1,5-AG assay (mean difference, -0.38 ug/dl (95%CI -0.54 to -0.12), *p*-value <0.0001). The high correlation but systematic difference between the two assays conducted at the same time is shown in the scatter and Bland-Altman plots (Fig. 1C and D). The Pearson's correlation and ICC for the two assays were 0.996 and 0.985, respectively. The difference between assays (bias) was not consistent across the range of values as can be seen in the Bland-Altman plot with fitted regression line (Fig. 1D). The slope of the regression line was statistically significant from zero (*p*<0.0005).

The performance of both the Determiner-L (Kyowa Medex, Tokyo, Japan) and GlycoMark (GlycoMark, Inc, Winston-Salem NC.) assays was excellent. Our results demonstrate that the Determiner-L and GlycoMark assays are comparable but give slightly different results in the same samples, with the Determiner-L results being slightly higher by 0.4 ug/dl in our study. This small but consistent difference is likely due to differences in calibration between the Kyowa Medex and GlycoMark 1,5-AG assays suggesting that this assay would benefit from closer consideration of calibration issues and organized efforts to ensure international standardization.

A single freeze-thaw cycle after 4 months of storage had a small effect on the values from the GlycoMark assay, with the results from the second run being, on average, 0.2 ug/dl higher compared to the first. Nonetheless, the high correlations indicate high reliability and that the relative ranking of individuals would be essentially unchanged by either freeze-thaw and regardless of assay manufacturer. Consequently, relative measures of associations such as odds ratios and relative risks in and in clinical and epidemiologic studies would be unaffected by the differences observed here.

In summary, our data suggest that these two 1,5-AG assays are comparable and the GlycoMark assay is largely robust to freeze-thaw effects. Investigators interested in measuring 1,5-AG can use the GlycoMark and Kyowa Medex assays interchangeably but we recommend that 1,5-AG measurements be conducted in a single run to ensure consistency across the range of values.

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References

1. True MW. Circulating biomarkers of glycemia in diabetes management and implications for personalized medicine. *J Diabetes Sci Technol* 2009;3(4):743–747. PMID: PMC2769973. [PubMed: 20144323]
2. Pal A, Farmer AJ, Dudley C, Selwood MP, Barrow BA, Klyne R, et al. Evaluation of serum 1,5 anhydroglucitol levels as a clinical test to differentiate subtypes of diabetes. *Diabetes Care* 33(2): 252–257. PMID: PMC2809258. [PubMed: 19933992]
3. Suwa T, Ohta A, Matsui T, Koganei R, Kato H, Kawata T, et al. Relationship between clinical markers of glycemia and glucose excursion evaluated by continuous glucose monitoring (CGM). *Endocr J* 57(2):135–140. [PubMed: 19926921]
4. Won JC, Park CY, Park HS, Kim JH, Choi ES, Rhee EJ, et al. 1,5-Anhydroglucitol reflects postprandial hyperglycemia and a decreased insulinogenic index, even in subjects with prediabetes and well-controlled type 2 diabetes. *Diabetes Res Clin Pract* 2009;84(1):51–57. [PubMed: 19187997]
5. Stettler C, Stahl M, Allemann S, Diem P, Schmidlin K, Zwahlen M, et al. Association of 1,5-anhydroglucitol and 2-h postprandial blood glucose in type 2 diabetic patients. *Diabetes Care* 2008;31(8):1534–1535. PMID: PMC2494656. [PubMed: 18426859]
6. Wagenknecht L, Wasserman B, Chambless L, Coresh J, Folsom A, Mosley T, et al. Correlates of Carotid Plaque Presence and Composition as Measured by MRI: The Atherosclerosis Risk in Communities Study. *Circ Cardiovasc Imaging* 2009;2(4):314–322. [PubMed: 19808612]
7. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1(8476):307–310. [PubMed: 2868172]

Figure 1.

Panel A. Scatterplot Comparing the GlycoMark 1,5-anhydroglucitol Assay Before and After Freeze-Thaw

Panel B. Bland-Altman Plot Comparing the GlycoMark 1,5-anhydroglucitol Assay Before and After Freeze-Thaw

Panel C. Scatterplot Comparing GlycoMark and Kyowa Medex 1,5-anhydroglucitol Assays

Panel D. Bland-Altman Plot Comparing GlycoMark and Kyowa Medex 1,5-anhydroglucitol Assays

Table 1

Summary Statistics GlycoMark (ug/dl) Before and After Freeze-Thaw, N=66 pairs

GlycoMark Run #1, Mean (SD)	15.88 (6.73)
GlycoMark Run #2, Mean (SD)	16.10 (6.85)
Mean difference, (95% CI)	-0.22 (-0.30, -0.14)
<i>P</i> -value for difference in means*	<0.0001
CV _{Difference}	1.84%
ICC	0.997
Pearson's correlation	0.999
Summary Statistics Comparing GlycoMark (ug/dl) and Kyowa Medex Determiner-L (ug/dl) 1,5-anhydroglucitol assays, N=66 pairs	
GlycoMark, mean (SD)	16.10 (6.85)
Kyowa Medex, mean (SD)	16.48 (7.13)
Mean difference, (95% CI)	-0.38 (-0.54, -0.21)
<i>P</i> -value for difference in means*	<0.0001
CV _{Difference}	3.72%
ICC	0.985
Pearson's correlation	0.996

* Paired *t*-test of the hypothesis that the means are equal.