Serum or Plasma Glucose Using the Infinity Glucose Hexokinase Liquid Stable Reagent (Thermo Scientific TR15421)

10-31-2017 wk/jb; rev 11-13-2017 jb; rev 7-8-2020 MR; rev 1-5-2021 wk

*Read package insert (**Addendum Figure**) before running this analysis. If the 2012 package insert has been updated, this procedure must be re-evaluated. wk*

Specimen: Serum or plasma (heparin, i.e.., green top blood collection tube) separated from cells as soon as possible; non-hemolyzed. Store frozen (-20°C) until analysis. ***For samples with lower glucose concentrations, such as allantoic and amniotic fluids, see **Table 2** for preparation of working standard set.

Reagents:

Glucose Hexokinase Liquid Stable Reagent: Supplied ready to use. Store refrigerated at 4°C. Stable until expiration date.

1000 mg/dL Glucose Standard (VWR470108-606, Ward's Science, Rochester NY): Supplied ready to use. Store refrigerated at 4°C. Stable until expiration date. Not available as of 12-21-2020.

18 MOhm water (18MΩ H_2O): from water purification system in Lab 109/110.

Control. In-house pooled plasma or serum sample. Store frozen at -20°C. Thaw only the amount needed for the day; use a new aliquot each day. Control ranges (average ± 2SD) to be determined.

0.85% (w/v) Sodium chloride (for diluting high glucose samples) [saline]: In a 500-mL volumetric flask, dissolve 4.25 g NaCl (MW = 58.44 g/mol) in ~ 400 mL 18MΩ H_2O. Mix. Bring up to 500 mL with 18MΩ H_2O. Mix. Store in a labeled container refrigerated at 4°C; stable 6 months.

Procedure:

1. Check that the Synergy H1 Microplate Reader (Biotek, Winooski, VT) in Hultz 135 is available for use. Sign up to use it on the Departmental Microplate Calendar. Turn on the computer that controls the plate reader. The password is "Nutrition135."

2. Thaw serum or plasma SAMPLEs and CONTROL. Vortex gently to mix.

3. Remove **Glucose Hexokinase Liquid Stable Reagent** from the refrigerator and allow it to come to room temperature. Before use, gently invert the reagent bottle a few times to thoroughly mix. *Do not shake*.

4. Prepare the **Working Glucose STANDARDs** fresh, daily, in 1.5-mL microtubes following the **Table 1** below.

Table 1. Working Glucose standard set preparation using a 1000-mg/dL Glucose Standard for a total volume of 1000 µL of each Working Glucose STANDARD.			
Standard number	**Amount of 1000 mg/dL Glucose Standard (µL)**	**18MΩ H_2O (µL)**	**Final Glucose Working STANDARD concentration (mg/dL)**
BLANK	0	1000	0
Std 1	25	975	25
Std 2	50	950	50
Std 3	75	925	75
Std 4	100	900	100
Std 5	150	850	150
Std 6	200	800	200
Std 7	300	700	300

5. Plan the layout of a 96-well microtiter plate (96-well flat bottom, medium binding, polystyrene without lid; Greiner Bio-one 655101) according to the existing protocol. See **Figure 1** plate layout example.

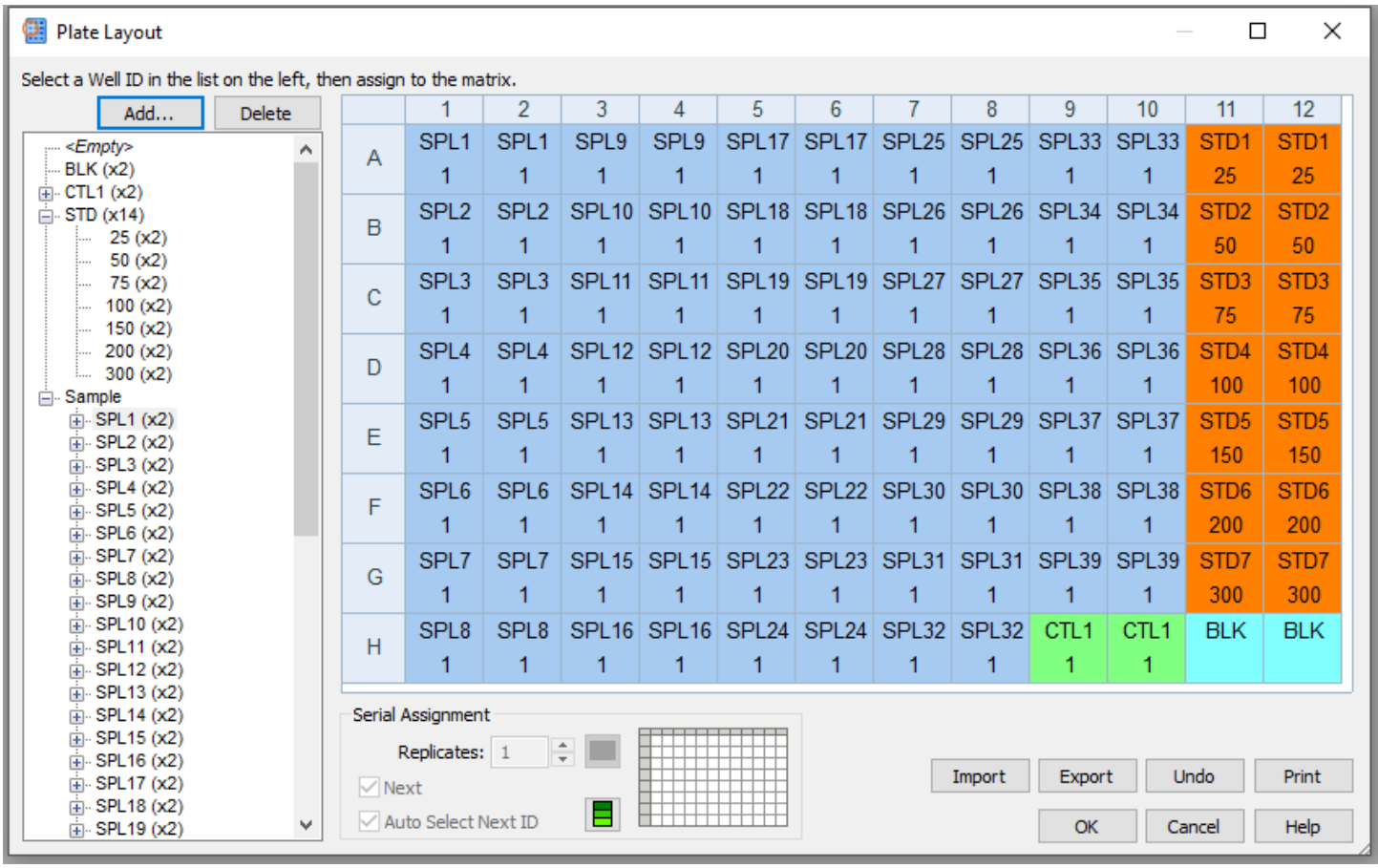

Figure 1. Glucose plate layout example. SAMPLES, STANDARDS, BLANKS, and CONTROL are run in duplicate. In this example, SAMPLES all have dilution factors of 1.

6. Turn on the Synergy microplate reader (right, bottom, front side). Open Gen5 2.04 program located on the desktop. Allow the instrument to do start up functions. Door opens when complete. Manually close door. Preheat the microplate reader to 37°C.

[Task Manager] menu

[Experiments] menu

[Create using existing protocol]. To find protocol go to [Documents] folder; [Lab 135] folder; [Protocols] folder; [Glucose] folder; filename Infinity Glucose Hexokinase Protocol Lab 135.prt.

Check the [Protocol] for correct [Procedure]: [Temperature: setpoint 37°C; Shake: Linear for 15 s; Delay for 15 min.; Shake: Linear for 15 s; Read(A)340].

Check [Plate Layout]; change accordingly. Check dilution factors. To change dilution factors, double click on samples (e.g., SPL 1) [Well ID] menu; [Type: Dilutions]. Enter dilution factor in SPL1:1. [OK].

7. Following **Figure 1** plate layout, pipet, in duplicate, 5 µL of each SAMPLE into respective wells of the 96-well plate. Pipet 5 µL of each CONTROL, BLANK (18MΩ H_2O), or STANDARD into respective microwells. *Change pipet tips between every sample, do not rinse the pipet tip in the sample, and pipet directly to the bottom of the well for best results.*

8. Using a multichannel pipet with 300-µL tips attached, pipet 250 µL of **Glucose Hexokinase Liquid Stable Reagent** into all test wells. Check for bubbles in the wells. Pop any bubbles with a small hypodermic needle (e.g., 26 g x ½ inch). *To prevent bubbles, use only room temperature reagent, and pipet at a slight angle.*

9. To load the plate into the microplate reader, press the green triangular [Read] button on the top menu bar of the open Synergy Gen5 2.04 protocol file. The door to the microplate chamber should open. Place the microplate into the plate reader with well A1 on the upper left-hand side. Follow computer screen prompts. The instrument will perform the protocol: shake for 15s, delay at 37°C for 15 min shake for 15 s, read at (A) 340 nm. [*Read in progress*] will be displayed on the computer screen.

10. Results appear on the screen. The screen will prompt [SAVE]. Follow the guidelines below for editing/deleting points. Only edit results on [Plate 1] [Matrix] tab, [Data: Blank 340] dropdown menu.

a) On [Plate 1] [Matrix] tab, [Data: 340] dropdown menu, plate BLANK should be less than 0.500 OD at 340 nm. If it is not, then the reagent is expired. *This is assuming you have corrected for a 1-cm pathlenth! Do you know the pathlength of your volume of reagent in your plate? Do you want the program to do a pathlength correction?*

On [Plate 1] [Matrix] tab, [Data: Blank 340] dropdown menu, check that the BLANK values are at or near zero and are consistent. [Mask] any BLANK that does not meet these criteria.

b) Check the standard curve (*Raw OD340 data has been transformed as follows: Blank Transformation and then linear regression calculated from Working Glucose Standard concentration vs OD340*). [Plate 1] [Graphs] tab, [Results: StdCurve Fitting Results]. Is the R^2 between 0.995 and 1.00? Are the variables (slope and intercept) similar to previous Infinity Glucose Hexokinase assays? If not, delete the bad points to get the best and most consistent standard curve. To do this, go to [Plate 1] [Matrix] tab, [Data: Blank 340] dropdown menu. Click on [Mask] button and then click on values to be masked. [Apply changes] [Close] [Yes].

c) To check standard differences (assigned values vs values calculated and assigned based on the linear regression curve) go to [Plate 1] [Statistic] tab, [Data: Concentration] dropdown menu. Compare standard values in Conc/Dil column with those in the Mean column. Are these values acceptable? If not, go back and [Mask] the appropriate standard values.

d) Is the CONTROL within control limits? Go to [Plate 1] [Statistics] tab, [Concentration] x Dil dropdown menu.

e) Do the SAMPLE values fall within the standard curve values? Go to [Plate 1] [Statistics] tab, [Concentration] x Dil dropdown menu. If not, repeat with different dilutions of serum/plasma sample in saline.

f) Are the SAMPLE values in normal range and/or what you expected?

g) To check CV, go to [Plate 1] [Statistics] tab, [Concentration] x Dil dropdown menu. Are the CV (%) of the SAMPLE ≤ 7%? If not, repeat the analysis on that sample.

11. [SAVE] the .exp file in the [Project] folder.

12. Export the results to Excel (Paper with blue arrow Icon on top menu bar). Add identifying info to the report (date, tech initials, experiment name, type of sample, and catalog and lot numbers of all reagents used), [SAVE] the .xlsx file in the [Project] folder, and PRINT. Fill out an *Infinity Glucose Hexokinase Assay QC* sheet using the example below **(Figure 2)** for the project. Save QC files to the specific [Project] folder.

13. When finished using the microplate reader, close out of the program, and turn off the microplate master switch. Shut computer down at the end of the day, or when completed if you are the only user

Repeat analysis on sample duplicates with CV greater than 7%.

Linearity: at least 300 mg/dL Glucose.

Conversion: mmole/L * 18 = mg/dL

***For samples that are lower in glucose, such as amniotic and allantoic fluid, prepare the **LOW CONC Working Glucose STANDARDs** fresh, daily, in 1.5-mL microtubes following the **Table 2** below. Adjust the microplate protocol plate standard values accordingly.

Table 2. LOW CONC Working Glucose standard set preparation using a 1000-mg/dL Glucose Standard for a total volume of 1000 µL of each LOW CONC Working Glucose STANDARD

Standard number	**Amount of 1000 mg/dL Glucose Standard (µL)**	**18MΩ H_2O (µL)**	**Final Glucose Working STANDARD concentration (mg/dL)**
Blank	0	1000	0
Std 1	12.5	987.5	12.5
Std 2	25	975	25
Std 3	50	950	50
Std 4	75	925	75
Std 5	100	900	100
Std 6	150	850	150
Std 7	200	800	200

Assay: Infinity Glucose Hexokinase					Date/Tech: 10/29/20 MR			
Project: Kendall and Macie Cows - Plasma								
In-house pooled control made from bovine serum 2020 ??Date??								
Average	74.80							
1SD	6.09		In-house range:					
2SD	12.18		62.62	to	86.98	mg/dL		
3SD	18.27							
						Linear regression		
Date	Running Plate #	Control Glucose (mg/dL)	Average	%CV		Slope A	Intercept B	
10/29/2020	1	72.665	71.956	1.393		0.00465	-0.00640	
	1	71.247						
10/29/2020	2	74.003	73.709	0.564		0.00476	-0.00537	
	2	73.415						
10/29/2020	3	73.030	71.999	2.026		0.00465	0.00482	
	3	70.967						
10/29/2020	4	72.828	73.838	1.934		0.00465	0.00332	
	4	74.848						
10/29/2020	5	93.548	84.124	15.844		0.00459	0.00805	
	5	74.699						
10/29/2020	6	70.962	73.190	4.304		0.00492	-0.01990	
	6	75.417						
average			74.802		*average*	0.00470	-0.00258	
stdev			6.091		*stdev*	0.00012	0.01025	
%CV			8.14	4.34	*%CV*	2.54	-397.14	
			%CV inter (between) assay	**%CV intra (within) assay**				

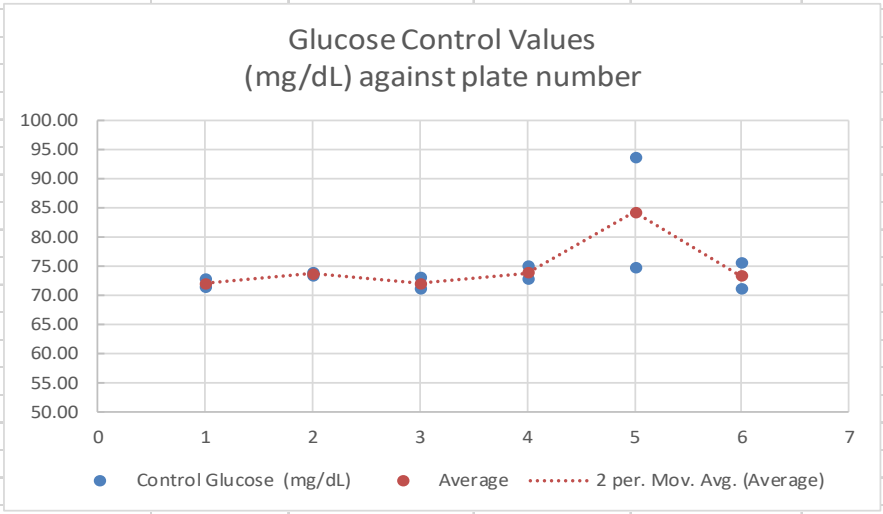

Figure 2. Example of QC Control Sheet for the Infinity Glucose assay.

Infinity™
Glucose Hexokinase Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	0 - 45 mmol/L (0 - 810 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Enzymatic Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult Instructions for use		

INTENDED USE
This reagent is intended for the in vitro quantitative determination of glucose in human serum, plasma or urine.

CLINICAL SIGNIFICANCE
The accurate estimation of glucose is important in the diagnosis and management of hyperglycaemia and hypoglycaemia. Hyperglycaemia may occur as a result of diabetes mellitus, in patients receiving glucose containing fluids intravenously, during severe stress and cerebrovascular accidents. Hypoglycaemia may be the result of an insulinoma, insulin administration, inborn errors of carbohydrate metabolism or fasting.[1] Often in the investigation of these disorders glucose determinations are performed in conjunction with various tolerance tests or stimulation tests. For a more detailed discussion of glucose metabolism the user should refer to a standard text book such as Kaplan.[2]

METHODOLOGY[3]
The Hexokinase / glucose-6-phosphate dehydrogenase method developed by the American Association of Clinical Chemistry and Centres for Disease Control has been accepted as the reference method for glucose determination. In this procedure protein free filtrates prepared by the Somogyi technique using $ZnSO_4$ / $BaSO_4$ precipitation are used. For routine laboratory use however serum or plasma without protein removal is the preferred method. The Glucose Hexokinase reagent is based on this reference method.
The series of reactions involved in the assay system is as follows:

1. Glucose + ATP $\xrightarrow{Hexokinase}$ G-6-P + ADP
2. G-6-P + NAD^+ $\xrightarrow{G\text{-}6\text{-}PDH}$ 6-PG + NADH + H^+

1. Hexokinase catalyses the phosphorylation of glucose by ATP producing ADP and glucose-6-phosphate.
2. Glucose-6-phosphate is oxidised to 6-phosphogluconate with the reduction of NAD^+ to NADH by G-6-PDH. The amount of NADH formed is proportional to the concentration of glucose in the sample and can be measured by the increase in absorbance at 340 nm.

Abbreviations

ATP	=	Adenosine-5'-triphosphate
ADP	=	Adenosine-5'-diphosphate
G-6-PDH	=	Glucose-6-phosphate dehydrogenase
G-6-P	=	Glucose-6-phosphate
6-PG	=	6-phosphogluconate
NAD^+	=	Nicotinamide Adenine Dinucleotide
NADH	=	Reduced NAD

REAGENT COMPOSITION

Active Ingredients	Concentration
Buffer	37.6 mmol/L
ATP	2.1 mmol/L
NAD	2.5 mmol/L
Hexokinase (Recombinant Yeast)	> 1500 U/L
G-6-PDH (Recombinant Leuconostoc)	> 2500 U/L

pH 7.7 ± 0.1 at 20°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity Glucose Hexokinase Liquid Stable Reagent Material Safety Data Sheet.

CAUTION: This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
Prior to use:
When stored refrigerated at 2-8°C the reagent is stable until the expiry date stated on the bottle and kit box label.

Once opened:
Once opened, the reagent is stable until the expiry date stated on the bottle and kit box label when stored refrigerated at 2-8°C.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent absorbance >0.5 (340 nm, 1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Collection: The stability of glucose specimens is reduced by bacterial contamination and glycolysis. In order to inhibit glycolysis samples should be collected into tubes containing Sodium Fluoride. As soon as possible serum or plasma should be separated from the cells.
Serum: Use non-haemolysed serum.
Plasma: Use heparin.
Urine: If a delay in transport to the laboratory is expected the use of a chemical preservative such as merthiolate (0.23 mmol/L) is recommended.[4]
Storage: In separated, non-haemolysed serum or plasma, glucose is stable for up to 72 hours at 4°C or as long as 8 hours at 25°C.[2,5] In the presence of sodium fluoride, glucose is stabilized for as long as 3 days at room temperature.[6] For long term storage samples should be placed in sealed containers and frozen at -10°C.[7] Urine samples are stable for 1 day at 4°C.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm (334-365 nm).
- Analyzer specific consumables, e.g.: sample cups.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous glucose standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	340 nm (334 - 365 nm)
Secondary Wavelength	380 nm (380 - 410 nm)
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:150
e.g. Sample vol	3 µL
Reagent vol	450 µL
Incubation Time	3 minutes
Reagent Blank Limits	Low 0.00 AU
(340 nm, 1cm lightpath)	High 0.50 AU
Linearity	0-45 mmol/L (0-810 mg/dL)
Analytical Sensitivity	0.038 ΔAbs per mmol/L
(340 nm, 1cm lightpath)	(0.002 ΔAbs per mg/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Glucose} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:
Absorbance of Calibrator = 0.30
Absorbance of unknown = 0.10
Value of Calibrator = 13.2 mmol/L (238 mg/dL)

$$\text{Glucose} = \frac{0.10}{0.30} \times 13.2 = 4.4 \text{ mmol/L}$$

$$\text{Glucose} = \frac{0.10}{0.30} \times 238 = 79 \text{ mg/dL}$$

Thermo SCIENTIFIC

Addendum Figure. Protocol is written based on Product Insert dated 2012.

For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in liters.

Urine Glucose (mmol/24 hours) = Glucose Result (mmol/L) x Dilution Factor x Volume (L)

Example:
Glucose result = 0.7 mmol/L (12.6 mg/dL)
Dilution of Urine = Neat
24 Hour volume of urine = 0.95 Liters

Urine Glucose = 0.7 x 1 x 0.95 = 0.67 mmol/24 hours
Urine Glucose = 12.6 x 1 x 0.95 = 11.97 mg/24 hours

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. May also be run at 334 or 365 nm.
3. Specimens with glucose values above 45 mmol/L (810 mg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
4. Unit Conversion: mmol/L x 18 = mg/dL.

CALIBRATION
Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (e.g. NIST or IFMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-
- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values for this methodology should be run as unknown samples:-
- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the established limits indicate the assay may be out of control. The following corrective actions are recommended in such situations:-
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control contact Technical Services or your local distributor

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin (free and conjugated) and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 470 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to 281 µmol/L (16.4 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to 298 µmol/L (17.4 mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 23 mmol/L (2000 mg/dL).
2. Young DS[8] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

Fasting serum:[9]	4.11 - 5.56 mmol/L (74 - 100 mg/dL)
Urine:[9]	0.06 - 0.83 mmol/L (1 - 15 mg/dL)

For the diagnosis of diabetes, Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT) the W.H.O. recommend the following criteria:[10]

Diabetes

Fasting plasma glucose	≥7.0 mmol/L (≥126 mg/dL)
2 hrs after glucose load	≥11.1 mmol/L (≥200 mg/dL)

IFG

Fasting plasma glucose	6.1-6.9 mmol/L (110-125 mg/dL)

IGT

Fasting plasma glucose	≤7.0 mmol/L (≤126 mg/dL)
2 hrs after glucose load	7.8-11.0 mmol/L (140-199 mg/dL)

PERFORMANCE DATA
The following data was obtained with the Infinity Glucose Hexokinase Liquid Stable Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on the specific analyzer used.

IMPRECISION
Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.[11]

Within run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.09 / 91.6	19.27 / 346.9
SD (mmol/L / mg/dL)	0.09 / 1.44	0.26 / 4.68
C.V. (%)	1.8	1.4

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.09 / 91.6	19.27 / 346.9
SD (mmol/L / mg/dL)	0.20 / 3.6	0.85 / 15.3
C.V. (%)	3.9	4.4

METHOD COMPARISON
Comparison studies were done using another commercially available glucose hexokinase reagent as a reference. Normal and abnormal patient serum and urine samples were assayed in parallel. The results were compared by least squares regression and the following statistics were obtained.

Serum/plasma:	
Number of sample pairs	60
Range of sample results	2.3 - 26.7 mmol/L (41.4 - 480.6 mg/dL)
Mean of reference method results	6.25 mmol/L (112.5 mg/dL)
Mean of Infinity Glucose HK results	6.27 mmol/L (112.9 mg/dL)
Slope	1.021
Intercept	-0.13 mmol/L (-2.34 mg/dL)
Correlation coefficient	0.9993

Urine:	
Number of sample pairs	60
Range of sample results	0.0 - 44.0 mmol/L (0.0 - 792.0 mg/dL)
Mean of reference method results	9.8 mmol/L (176 mg/dL)
Mean of infinity Glucose HK results	10.4 mmol/L (187 mg/dL)
Slope	1.096
Intercept	-0.29 mmol/L (-5.22 mg/dL)
Correlation coefficient	0.9962

LINEARITY
When run as recommended the assay is linear between 0 and 45 mmol/L (0 - 810 mg/dL).

ANALYTICAL SENSITIVITY
When run as recommended the sensitivity of the assay is 0.038ΔAbs per mmol/L or 0.002 ΔAbs per mg/dL (1cm light path, 340 nm).

REFERENCES

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JL840715-en (R1)

CE

REF **Reorder Information**

Catalogue No.	Configuration
TR15421	2 x 125 mL

Addendum Figure (cont). Protocol is written based on Product Insert dated 2012.