GLUC2

Glucose HK - Hemolysate Application Gen.2

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20767131 322	Glucose HK (200 tests)	System-ID 07 6713 1	COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05067235 191	Glucose Hemolyzing Reagent Gen.2 (1000 mL)		

English

System information

Test GLUH2, test ID 0-613 (Hemolysate Application Gen.2); test GLU2P, test ID 0-001 (Hemolysate application Gen.2 plasma-level)

Intended use

In vitro test for the quantitative determination of the glucose concentration in hemolysate.

Summary[1,2,3]

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.

The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure, and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism, or insulin induced hypoglycemia.

Glucose measurement in hemolysate is a convenient method for routine diabetes monitoring and in cases where only small sample volumes are available.

Test principle

Enzymatic reference method with hexokinase.[4,5]

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH.

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{D-glucose-6-phosphate} + \text{ADP}$$

$$\text{D-glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate} + \text{NADPH} + H^+$$

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm.

Reagents - working solutions

R1 TRIS buffer: 100 mmol/L, pH 7.8; Mg^{2+}: 4 mmol/L; ATP: 1.7 mmol/L; $NADP^+$: 1 mmol/L

SR HEPES buffer: 30 mmol/L, pH 7.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 130 µkat/L; G6PDH (microbial): ≥ 250 µkat/L

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Glucose HK

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	8 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	8 weeks

Glucose Hemolyzing Reagent Gen.2

Shelf life at 15-25 °C	See expiration date on reagent label
Stability after opening	6 weeks
Storage after opening	15-25 °C

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable: Whole blood: The samples should be hemolyzed immediately after collection. Collect 20 µL of capillary blood. Take venous blood or blood from the earlobe or from the fingertip. Earlobe or fingertip should be well supplied with blood at the time of collection.

Hemolysate preparation

1. Place 0.5 mL of Glucose Hemolyzing Reagent Gen.2 in a test tube.
2. Add the filled 20 µL capillary and close the test tube.
3. Mix gently, avoiding the formation of foam.
4. Allow to stand for at least 5 minutes at room temperature prior to glucose determination. Do not centrifuge.

Stability in hemolysate[6]	8 days at 15-25 °C
	2 weeks at 2-8 °C

GLUC2

Glucose HK - Hemolysate Application Gen.2

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

Glucose Hemolyzing Reagent Gen.2, 1000 mL, Cat. No. 05067235191, for hemolysate preparation. See above for sample pretreatment procedure.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for hemolysate

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/378 nm
Calc. first/last	33/53
Unit	mmol/L
Laboratory correlation factor	26

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	20 µL	12 µL
SR	30 µL	
Total volume	212 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/378 nm
Calc. first/last	44/77
Unit	mmol/L
Laboratory correlation factor	26

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	20 µL	12 µL
SR	30 µL	
Total volume	212 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized against ID-MS[a)].

Note

Do not pretreat calibrator.

Calibrator f.a.s. is automatically diluted 1:26 (1 + 25) with water by the instrument. A conversion factor (laboratory correlation factor) of 26 is applied in order to achieve correct patient results. Enter the assigned lot-specific glucose value of the undiluted calibrator, indicated in the package insert of the Calibrator f.a.s.

a) Isotope Dilution Mass Spectrometry

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1 Dilute control serum 1:26 (1+25) in distilled/deionized water. Add control manually.
Pathological range	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2 Dilute control serum 1:26 (1+25) in distilled/deionized water. Add control manually.
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factors:	mmol/L × 18.02 = mg/dL
	mg/dL × 0.0555 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:[7] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Hemolysate (test ID 0-613):
1.5-45 mmol/L (27-811 mg/dL)

Hemolysate plasma-level (test ID 0-001):
1.7-50 mmol/L (31-901 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the tests (test IDs 0-613 and 0-001):
0.4 mmol/L (7.2 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Whole blood: 3.6-5.3 mmol/L (65-95 mg/dL)[11]

Whole blood plasma-level: 4.0-5.9 mmol/L (72-106 mg/dL)*

*calculated by a conversion factor of 1.11[12]

Hematocrit level may influence the difference between plasma and whole blood glucose levels due to lower glucose values in erythrocytes compared with plasma concentration. Higher hematocrit levels lead to an increased plasma glucose level compared to whole blood.[11,13]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Level 1	Level 2
Mean	3.43 mmol/L (61.8 mg/dL)	7.60 mmol/L (137 mg/dL)
CV	0.7 %	0.4 %

Intermediate precision	Level 1	Level 2
Mean	3.44 mmol/L (62.0 mg/dL)	7.60 mmol/L (137 mg/dL)
CV	1.0 %	1.1 %

Method comparison

Glucose values for samples hemolyzed with the Glucose Hemolyzing Reagent Gen.2 obtained on a COBAS INTEGRA 400 analyzer (GLUH2, test ID 0-613) using the COBAS INTEGRA Glucose HK reagent (GLUC2) (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 800 analyzer (GLUH2, test ID 0-613) (x). Single measurement was done.

		COBAS INTEGRA 800 analyzer
Pretreatment		Glucose Hemolyzing Reagent Gen.2 (1+25)
Sample size	(n)	57
Corr. coefficient	(r)	0.999
Linear regression		y = 0.992x + 0.013 mmol/L
Passing/Bablok[14]		y = 0.993x + 0.002 mmol/L

The sample concentrations were between 1.79 and 44.9 mmol/L (32.3 and 809 mg/dL).

Glucose values for samples hemolyzed with the Glucose Hemolyzing Reagent Gen.2 obtained on a COBAS INTEGRA 800 analyzer (GLUH2, test ID 0-613) using the COBAS INTEGRA Glucose HK reagent (GLUC2) (y) were compared with samples hemolyzed with the Hemolyzing Reagent "Fluid" determined on a Roche/Hitachi 917 analyzer (ACN 548) (x). Single measurement was done.

		Roche/Hitachi 917 analyzer
Pretreatment		Hemolyzing Reagent "Fluid" (1+50)
Sample size	(n)	58
Corr. coefficient	(r)	0.999
Linear regression		y = 1.018x + 0.120 mmol/L
Passing/Bablok[14]		y = 1.023x + 0.050 mmol/L

The sample concentrations were between 1.69 and 43.9 mmol/L (30.5 and 791 mg/dL).

References

1 Sacks DB. Carbohydrates. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 5th ed. Philadelphia: WB Saunders 2001;427-461.

2 Khan MI, Weinstock RS. Carbohydrates. In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 21st ed. Philadelphia: WB Saunders 2007:185-199.

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6 Data on file at Roche Diagnostics.

7 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

8 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

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10 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

11 Tietz NW, ed. Clinical Guide to Laboratory Tests, 4th ed. Philadelphia. WB Saunders 2006;444-455.

12 D'Orazio P, Burnett RW, Fogh-Andersen N, et al. Approved IFCC Recommendation on Reporting Results for Blood Glucose (Abbreviated). Clin Chem 2005;51:1573-1576.

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14 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

GLUC2

Glucose HK - Hemolysate Application Gen.2

CE

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