

Wako

Autokit Glucose

Enzymatic Method (Mutarotase-GOD)

For Research Use Only. Not for use in diagnostic procedures.

Intended use

The Autokit Glucose is *in vitro* assay for the quantitative determination of glucose in serum, plasma or urine.

Summary and explanation of the test

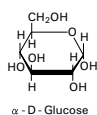
Muller first demonstrated that β -glucose oxidase promoted the oxidation of glucose by molecular oxygen to gluconic acid. Franke and Lorenz discovered that hydrogen peroxide is simultaneously produced in this reaction. In 1956, first Keston and then Teller introduced the enzymatic methods for the determination of glucose, combining glucose oxidase (GOD), peroxidase (POD), and an oxygen acceptor (chromogen); which had high specificity and simplicity.

There were drawbacks with this method, such as interferences from the reducing substances in samples and a potential for carcinogenesis (*o*-tolidine and *o*-dianisidine). Several modifications and improvements, including the use of 4-aminoantipyrine as chromogen introduced by Trinder in 1969, have been reported.

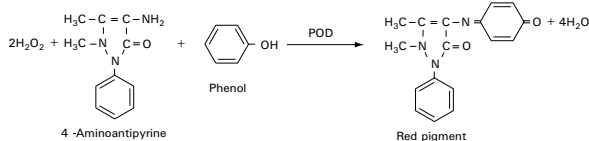
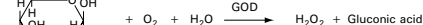
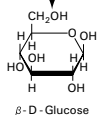
Principle of the method

The equilibrium of D-glucose in solution is maintained in the ratio of α -D-glucose 36.5% and β -D-glucose 63.5%. GOD reacts only with β -D-glucose. When a test sample is allowed to react with the reagent, α -D-glucose existing in the sample is converted rapidly to the β -isomer by the action of mutarotase and is then oxidized by GOD to produce hydrogen peroxide. In the absence of mutarotase, the reaction proceeds slowly because β -D-glucose is first consumed by GOD as α -D-glucose is gradually converted to β -D-glucose.

When mutarotase is added, α -D-glucose is rapidly converted to β -D-glucose so that GOD action is facilitated.



Mutarotase



Reagents

- Buffer Solution 2 × 150 mL
60 mmol/L Phosphate buffer (pH7.1) containing 5.3 mmol/L Phenol.
Store at 2-10°C.
- Color Reagent (When reconstituted) 2 × for 150 mL
Containing 0.13 U/mL Mutarotase, 9.0 U/mL Glucose oxidase, 0.65 U/mL Peroxidase, 0.50 mmol/L 4-Aminoantipyrine, 2.7 U/mL Ascorbate oxidase.
Store at 2-10°C.
- Standard Solution I 1 × 10 mL
Containing 200 mg/dL Glucose.
Store at 2-10°C.
- Standard Solution II 1 × 10 mL
Containing 500 mg/dL Glucose.
Store at 2-10°C.

Warnings and precautions

- For Research Use Only. Not for use in diagnostic procedures.
- Not to be used internally in humans or animals.
- Do not use reagents past the expiration date on each reagent container label.
- Do not use the preparations, test solutions and reagents for any other purpose than described herein.

Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

This reagent is designed to be used on commercially available spectrophotometer or on RA-1000 analyzer. Refer to the operating manual for a description of instrument operation and specifications.

Specimen collection and preparation

Serum and Plasma

- Sample can be stored for 1 day at 25°C or 3 days at 2-10°C without a significant effect on the measured values.
- The whole blood glucose is consumed by blood cells during blood coagulation and serum separation. Therefore, the separation of blood cells should be done as quickly as possible after the blood collection.
- Sodium fluoride, and inhibitor of glycolysis, does not affect the measurement of glucose.
- Anticoagulants such as heparin, oxalate, citrate and EDTA do not influence measurements when they are employed in their usual amounts.
- Ascorbic acid gives a slight negative error in the measurement.
- Bilirubin gives a slight negative error in the measurement.
- Hemolysis gives a slight positive error in the measurement.

Reagent preparation

Working solution

Dissolve the contents of one bottle (for 150 mL) of Color Reagent in one bottle 150 mL of Buffer Solution. This solution is stable for one month at 2-10°C.

Manual procedure

Materials supplied

Refer to the section entitled "Reagents."

Materials required but not supplied

Spectrophotometer, pipettes; water bath or heating block capable of maintaining 37°C.

Test procedure

Wavelength : 505 nm*1 Light path : 1 cm
Temperature : 37°C

		Sample (S)	Standard (Std)	Blank (BL)
Pipette into a cuvette				
Sample	(mL)	0.02	—	*2
Standard 1 or 2	(mL)	—	0.02	—
Working solution	(mL)	3.0	3.0	3.0

Mix well, incubate for 5 minutes and measure the absorbance of S (A_S) and Std (A_{Std}) against Bl (A_{BL}) at 505 nm.

- Accurately pipette 0.02 mL of sample or standard into the cuvettes (test tubes)
- Add 3.0 mL of Working solution.
- Mix, incubate for 5 minutes and measure the absorbance of Sample (A_S) and Standard (A_{Std}) against Blank (A_{BL}) at 505 nm.

*1 When measuring two wavelengths : $\lambda_1 / \lambda_2 = 505/600$ nm

*2 The omission of 0.02 mL of water does not significantly affect the absorbance measured

Concentration in the test (Manual procedure)

60 mmol/L Phosphate buffer, 5.3 mmol/L Phenol, 0.13 U/mL Mutarotase, 9.0 U/mL GOD, 0.65 U/mL POD, 0.50 mmol/L 4-Aminoantipyrene and 2.7 U/mL AOD.

Results (Manual procedure)

Calculation

$$\text{Glucose (mg/dL)} = \frac{A_s}{A_{\text{Std}}} \times C_{\text{Std}}$$

A_s = Absorbance of sample

A_{Std} = Absorbance of Standard I or II

C_{Std} = Concentration of Standard I or II in mg/dL

Limitation of the procedure (Manual procedure)

When glucose value exceeds 700 mg/dL, dilute sample 1 : 1 with saline or distilled water. Repeat assay and multiply result by 2.

Automated procedure (RA-1000)

Materials supplied

Refer to the section entitled "Reagents."

Materials required but not supplied

RA-1000 analyzer

Test procedure (RA-1000)

CHEM#	*	DECIMAL	0
NAME	1 GLC	RBL LOW	0.0
IMMUNOASSAY	0	RBL HI	0.1
TYPE	2	RANGE LO	0
INVERSE	0	RANGE HI	500
% SMP VOL	6 (3 μ L)	CAL FACT	—
FILTER P	4 (500 nm)	NORMAL L	70
DELAY	5:00	NORMAL H	110
% RGT VOL	70 (350 μ L)	SLOPE	1.0
2ND RGT	0	INTERCEPT	0.0
UNIT	2 (MG/DL)	1 = ACCEPT	1
UNIT FAC	1.0		

Concentration in the test (RA-1000)

60 mmol/L Phosphate buffer, 5.3 mmol/L Phenol, 0.13 U/mL Mutarotase, 9.0 U/mL GOD, 0.65 U/mL POD, 0.50 mmol/L 4-Aminoantipyrene, and 2.7 U/mL AOD.

Results (RA-1000)

The final results are automatically calculated and printed in concentration.

Limitation of the procedure (RA-1000)

When glucose value exceeds 700 mg/dL, dilute sample 1 : 1 with saline or distilled water, Repeat assay and multiply result by 2.

Quality control

A quality control program is recommended for all laboratories. The analysis of control sera is recommended for monitoring the performance of the procedure. The values obtained for the controls should fall within the manufacturer's acceptable ranges.

If values are to be established for unassayed control sera, the laboratory should assay each serum a sufficient number of times to generate a valid mean and acceptable range.

References

- (1) Miwa, i., Okuda, J., Maeda, K and Okuda, : Clin. Chim. Acta, **37** 538-540 (1972).
- (2) Okuda, J. and Miwa, i.: Protein, Nucleic acid and Enzyme, **17**, 216-244 (1972).
- (3) Okuda, J. and Miwa, i., and Maeda K. : Japanese Journal of Clinical Chemistry, **2**, 289-296 (1973).
- (4) Trinder, P., Ann. Clin. Biochem., **6**, 24 (1969).
- (5) Okuda, J., Miwa, i., Maeda K. and Tokui, KJ. : Carbohydrate Research, **58**, 267 (1977).
- (6) Miwa, I., Toyoda, Y. and Okuda, J : Journal of Medical Technology, **22**, 1232 (1977).

Ordering information

Code No.	Product	Package
997-03001	Autokit Glucose (<ul style="list-style-type: none">• Buffer Solution 2 \times 150 mL• Color Reagent 2 \times for 150 mL(When reconstituted)• Standard Solution I 1 \times 10 mL• Standard Solution II 1 \times 10 mL)	100 Tests

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997-03001F

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