GLUCOSE AND FRUCTOSE IN URINE

UV method for determination of D-glucose and D-fructose in urine

TEST SUMMARY

D-glucose, in presence of ATP, is transformed from Esochinase (EK) in glucose-6-phosphate, which at the same time is transformed into 6-phosphogluconate from G6P-DH with formation of NADPH. NADPH formed in this reaction causes an increase of absorbance at 340 nm.
D-fructose, in presence of ATP, is transformed from Esochinase (EK) in Fructose-6-phosphate. The Fructose-6-phosphate is transformed from Phospho-Gluco-Isomerase (PGI) in glucose-6-phosphate, that in its turn is transformed in 6-phosphogluconate from G6P-DH with formation of NADPH. NADPH formed in this reaction causes absorbance's increase at 340 nm.

SAMPLES

Fresh urine.

REAGENTS

Reagent A:	Good's buffers > 10 mM ATP > 2 mM pH 7.8
Reagent B:	NAD > 0.2 mM, pH 4.5.
Starter 1:	HK > 300 U/l; G6PDH > 700 U/l.
Starter 2:	PGI > 100 U/l.
Standard Glucose	Glucose 25 mg/dl.
Standard Fructose	Fructose 25 mg/dl.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Reagents, if not contaminated, are stable stored at 2-8°C until the expiration date indicated on the package.

Working Reagent preparation
Mix 4 parts of Reagent A + 1 part of Reagent B (8 ml RA + 2 ml RB).
The working reagent is stable for 8 days at 2-8°C or 2 months at -20°C.
Freeze only one time. Do not repeat freezing. It's advisable to fractionate quantities to freeze in accordance with the number of daily tests.

Gently mix Starter 1 and Starter 2 before use to resuspend enzymes in solution.

SAMPLE PREPARATION

Centrifuge or filter turbid samples.

PROCEDURE

Kind of analysis:	End Point
Reading time:	3, 10, 20 minutes
Wavelength:	340 nm (334-365)
Temperature:	37°C
Zero:	Blank reagent

Reagents	Blank	Standard glucose	Standard fructose	Sample
Working Reagent	1000 µl	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	--	--	--
Standard glucose	--	20 µl	--	--
Standard fructose	--	--	20 µl	--
Sample	--	--	--	20 µl
Mix, wait for 3 minutes and measure absorbance of solutions (A_0) bring to zero against blank.				
Starter 1	25 µl	25 µl	25 µl	25 µl
Mix, wait the end of the reaction (10 minutes) and measure the absorbance of solutions (A_1) bring to zero against blank.				
Starter 2	25 µl	25 µl	25 µl	25 µl
Mix, wait the end of the reaction (10 minutes) and measure the absorbance of solutions (A_2) bring to zero against blank.				

CALCULATION

Glucose (mg/dl)

$$\frac{A_1 \text{ (sample)} - A_0 \text{ (sample)}}{A_1 \text{ (standard glucose)} - A_0 \text{ (standard glucose)}} \times 25$$

Fructose (mg/dl)

$$\frac{A_2 \text{ (sample)} - A_1 \text{ (sample)}}{A_2 \text{ (standard fructose)} - A_1 \text{ (standard fructose)}} \times 25$$

EXPECTED VALUES

GLUCOSE

Urine (fasting patients)	
Spontaneous Urine	< 300 mg/dl
24h urine	< 500 mg/24h

FRUCTOSE 0 - 4 mg/dl

Since the normal values depend on age, sex, diet, geographic area and other factors, each laboratory should establish its own normal values for this procedure.

NOTES

- If the results are incompatibles with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control using control sera with well-know concentration of Fructose and Glucose.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

TEST PERFORMANCE

Precision (glucose determination)

Intra-assay (n = 10)	Media (mg/dl)	SD (mg/dl)	CV%
Sample 1	14.845	0.464	3.12
Sample 2	89.156	1.703	1.91

Inter-assay (n = 10)	Media (mg/dl)	SD (mg/dl)	CV%
Sample 1	15.152	0.529	3.49
Sample 2	89.376	2.121	2.37

Methods comparison (glucose determination)
A comparison with an available commercial method gave following results on 25 samples compared:

Glucose / Fructose LTA = x
Glucose competitor = y
n = 25

y = 0,9863x + 0,0162 mg/dl r = 0,99312

Precision (fructose determination)

Intra-assay (n = 10)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	7.966	0.301	3.78
Sample 2	49.691	1.031	2.08

Inter-assay (n = 10)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	8.045	0.341	4.24
Sample 2	49.549	1.299	2.62

Methods comparison (fructose determination)
A comparison with an available commercial method gave following results on 25 samples compared:

Glucose / Fructose LTA = x
Fructose competitor = y
n = 25

y = 0,9894x - 0,008 mg/dl r = 0,99659

Linearity
The method is linear up to 100 mg/dl (glucose + fructose)

PACKAGING

CODE CC01720	(100 TESTS)	
Reagent A	1 x 80 ml	(liquid)
Reagent B	1 x 20 ml	(liquid)
Starter 1	1 x 2.5 ml	(liquid)
Starter 2	1 x 2.5 ml	(liquid)
Standard glucose	1 x 5 ml	(liquid)
Standard fructose	1 x 5 ml	(liquid)

REFERENCES

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Beutler, H.O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., Vol. VI, pp. 356-362, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel.

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SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

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