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Determination of microglucosuria

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protocol .

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El Hadji Malick Ndour

This protocol describes how to determine microglucosuria in patients living with sickle cell disease in order to diagnose a nephropathy as a result of proximal convoluted tubule damaged by heme catabolites.

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protocols.io

<https://protocols.io/view/determination-of-microglucosuria-b6u2reya>



Determination of microglucosuria, El Hadji Malick Ndour

Microglucosuria

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Spectrophotometer

DETERMINATION OF MICROGLUCOSURIA

1 . OBJECTIVE

The aim is to describe how microglucosuria is determined.

2 SAMPLING

Urine

A meadstream voiding urine sample at random at any time between 8 a.m. and 2 p.m. is collected. The urine sample is centrifuged before performing the test on the same day of collection with the undiluted supernatant.

3 PRINCIPLE

Several methods can be used, including hexokinase, glucose oxidase or glucose dehydrogenase.

The method used in this work is that of glucose oxidase / peroxidase whose principle is as follows:

Glucose, under the action of glucose-oxidase, is oxidized to gluconic acid with formation of hydrogen peroxide.

In the presence of peroxidase, the hydrogen peroxide produced transforms a colorless reduced chromogen into an oxidized chromogen colored pink whose intensity of coloration measurable by spectrophotometry at 505 nm is proportional to the glucose concentration of the sample.

Glucose oxydase

Glucose + O₂ + H₂O -----> Acide gluconique + H₂O₂

Peroxydase

H₂O₂ + phénol + amino 4 phenazone -----> Quinone imine + 2 H₂O

4 PROCEDURE

-Place the reagents at room temperature for 5 minutes.

-Pipette in test tubes according to the following table:

	Blanc	Standard	Control	Sample
Blanc	-	-	-	-
Standard	-	10 µl	-	-
Control	-	-	10 µl	-
Sample	-	-	-	10 µl
Reagent	1 ml	1 ml	1 ml	1 ml

-Shake well and incubate the tubes for 10 minutes at room temperature or for 5 minutes 37°C. A pink coloration is obtained (stable at least 1 hour).

-Read the concentrations by spectrophotometer at 505 nm.

5 RESULTS

The urine of a healthy subject does not contain glucose.

6 INTERPRETATION

To interpret glucosuria, the RGCU (urinary glucose/creatinine ratio) which is the ratio of glucosuria (mg/l) to creatininuria (g/l) is first calculated.

If $RGCU < 20 \text{ mg/g}$, it is called physiological glucosuria.

If the $RGCU \geq 20 \text{ mg/g}$, with test strip results indicating an absence of glucose in the urine, it is called microglucosuria.

If $RGCU \geq 20 \text{ mg/g}$ with test strip results indicating the presence of glucose in the urine, this is called glucosuria.