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Determination of microglucosuria

El Hadji Malick Ndour[1,2], Khuthala Mnika[3], Fatou Gueye Tall[1,2], Moussa Seck[4], Indou Deme Ly[2], Victoria Nembaware[3], Gaston Kuzamunu Mazandu[3], Helene Ange Therese Sagna-Bassene[2], Rokhaya Dione[2], Aliou Abdoulaye Ndongo[5], Jean Pascal Demba Diop[6], Nene Oumou Kesso Barry[1], Moustapha Djite[1], Rokhaya Ndiaye Diallo[6], Papa Madieye Gueye[1], Saliou Diop[4], Ibrahima Diagne[7], Aynina Cisse[1], Ambroise Wonkam[3], Philomene Lopez Sall[1,2,2]

[1]Department of Pharmaceutical Biochemistry, Faculty of Medicine, Pharmacy and Dentistry, Cheikh Anta Diop University, Dakar, Senegal; [2]Albert Royer National Children's Hospital Center, Dakar, Senegal; [3]Division of Human Genetics, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; [4]National Blood Transfusion Center, Dakar, Senegal; [5]Pediatrics Department of Aristide Le Dantec Hospital, Dakar, Senegal; [6]Department of Human Genetics, Faculty of Medicine, Pharmacy and Dentistry, Cheikh Anta Diop University, Dakar, Senegal; [7]Health Sciences Training and Research Unit, Gaston Berger University, Saint-Louis, Senegal

El Hadji Malick Ndour

ABSTRACT

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.

MATERIALS

Spectrophotometer

Protocol status: Working
We use this protocol and it's working

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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.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxydase}} \text{Acide gluconique} + H_2O_2$$

$$H_2O_2 + \text{phénol} + \text{amino 4 phenazone} \xrightarrow{\text{Peroxydase}} \text{Quinone imine} + 2\,H_2O$$

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 mg/g, it is called physiological glucosuria.

If the RGCU ≥ 20 mg/g, with test strip results indicating an absence of glucose in the urine, it is called microglucosuria.

If RGCU ≥ 20 mg/g with test strip results indicating the presence of glucose in the urine, this is

called glucosuria.