

Glucose estimation instruments

Hypoglycemia in neonates remains a common problem. Low blood glucose concentrations are associated with abnormal development. Therefore, recognizing and screening those neonates at the highest risk of hypoglycemia is an important skill for all pediatricians responsible for the care of newborns. This chapter aims to review the available techniques for rapid determination of blood/plasma glucose concentration. While various laboratory techniques are available, most require time to give results. This delay may not be acceptable in care of newborns, therefore, the need for rapid diagnostic techniques.

Principle for determining blood/plasma glucose concentrations

In the current era, the enzymatic methods have largely superseded the reductionometric methods in clinical practice, where precise determination of glucose concentrations is required.

Hexokinase method

A sequential reaction using glucose, ATP, hexokinase and glucose dehydrogenase yields NADPH/H⁺, which can be determined by using a suitable spectrophotometric indicator. This method is precise and more specific than glucose oxidase method due to G-6 phosphate which inhibits interfering substances except when sample is hemolysed.

Glucose oxidase method

Oxidation of glucose is catalyzed to yield glucuronic acid and hydrogen peroxide. The concentration of H₂O₂ can be measured by using peroxidase step coupled to a colored oxygen acceptor or an electrode. This method forms the basis of reagent strip and glucose electrode methods.

Reagent paper strips

These strips were initially developed for monitoring blood glucose concentration using glucose oxidase method in diabetic patients. These paper strips can be used alone where the blood glucose values are assessed after comparing with the color code available. The precision may be increased to some extent by coupling these with a reflectance metering system. Different meters are available in the market; these are meant for diabetic patients. There are none available exclusively for neonatal use.

The other technique uses glucose oxidase method and a electrode-based analyzer. This method is reportedly more precise. Recently, glucose oxidase based sensor has been used for blood glucose estimation. In this method, the enzyme oxidizes the glucose and H₂O₂ is produced. This leads to flow of electrons across the electrode, which is measured and reported as glucose

concentration in mg/dl(1mg/dl=18 mmol/l). This technique is reportedly superior to reflectance meters.

Problems with use of reagent strips for detection of neonatal hypoglycemia

One of the greatest problems with neonatal blood samples is that the hematocrit varies from 40-70%. Plasma glucose concentration is higher than that of the whole blood. All paper strip techniques are subject to intrinsic hematocrit bias-the higher the hematocrit lower the result. This may be due to discoloration of the test-pad and resistance to wiping or washing before reading. Higher sample viscosity may impede diffusion of plasma into the test-pad of the strip. Bilirubin and hemolysis interfere with the assay and produce falsely low values. Above-mentioned problems per se would produce low values. Also the values in lower range, that is the range we are interested in, are imprecise. Various studies have highlighted the inaccuracy and imprecision of these systems. However, in the absence of other, inexpensive, easily available technique, these strips, either alone or coupled with a reflectance meter, have to be relied upon in all neonatal setups. One should always repeat any low values obtained and also confirm the same by laboratory analysis. However, the therapy should not be delayed while waiting for the laboratory results.

Sources of error in the sample

1. The arterial blood glucose concentration is higher than those of the venous blood. Capillary sampling is unreliable if the peripheral perfusion is poor.
2. Sample should always be free flowing, squeezing the heel may cause hemolysis which may interfere with the assay.
3. Contamination of the test pad with alcohol can lead to falsely high values.

Sources of error in the technique

1. If the test pad is not covered completely with blood, the results may be erroneous – both by the visual method and by reflectance meters.
2. The time to wiping/washing the strip may be imprecise leading to variable reaction time and therefore, variable, imprecise results. However, some of the recent meters e.g. one-Touch, and glucose oxidase electrode based analyzers circumvent this problem by using a technique where the doctor does not have to wipe the test strip: Only a single drop of blood is placed on the test-site, after the strip is placed into the meter. The meter gives the result in 45 seconds.

Available literature suggests that reagent strip methods detect only 85% of true cases of neonatal hypoglycemia and 75% of normoglycemic neonates. This suggests that reagent strips are not suitable to diagnose neonatal hypoglycemia. These can be used only after taking the limitations into account and the results should always be confirmed by laboratory or ward-

based glucose-electrode method before giving a label of neonatal hypoglycemia.

How to take care of the instrument?

- i) Blood glucose meters must meet accuracy standards set by the International Organization for Standardization (ISO).
- ii) The meters should be calibrated regularly, as recommended by the manufacturers.
- iii) The instrument should not be exposed to excessive humidity, extreme heat or cold for prolonged periods.
- iv) A daily check of the strip guide, reflectance disc and optical window should be made. The strip guide can be cleaned with a brush and water or a mild detergent, after removing it from the instrument. The reflectance disc and optical window can be cleaned with a soft, lint free cloth or lens tissue soaked with water, surgical spirit or alcohol.
- v) The instrument should be handled gently.

How to store the strips properly?

The reagent strips contain enzymes glucose oxidase and peroxidase. Activity of these enzymes is affected by heat, humidity and excessive exposure to light. Most manufacturers recommend storage in a cool dark place at a temperature less than 25°C; but these should never be frozen. The bottles contain 'silica gel' to absorb the moisture. The color of the strip should be checked before using it.

In order to economize, many users cut the reagent strips into 2 or 3 strips, for visual reading. However, as the strips have more than one layer, this may alter the precision. As far as possible, cutting of strips should not be resorted to.

How to select a product for your unit?

This is a difficult question, as none of the manufacturers recommend use of their meters/reagent strips for use in neonates, because of the problems mentioned above. However, because of the requirement for rapid diagnostic method, the same have to be used, understanding the limitations well. The procedure of estimation should be simple. Visual techniques and most of the reflectance meters (Glucometer, etc.) require wiping/washing of the strip after a particular period. Any error here can lead to errors in results. 'One-TouchTM' meter does not require any wiping of the strip.

The meters should be preferably calibrated for plasma glucose this may improve the precision. The reagent strips should be freely available and the

cost should be reasonable. The strips should be stable for sufficient period of time in tropical climate.

Glucose estimation meters cost Rs. 3,000/- to 8,000/- each, while each strip cost varies from Rs. 8/- to Rs. 20/-.

Common brands of glucometers available in India

A) Glucose oxidase based reflectance meters

1. Ames Glucometer (Bayer Diagnostics)
2. One Touch (Johnson & Johnson)
3. Lifescan (Johnson & Johnson)
4. Glucosite (GDS Diagnostics)
5. Refcolux (Boehringer Mannheim)

B) Glucose oxidase & electrode based analyzers

1. Pulsatum (Pulsatum Health Care Pvt. Ltd.)
2. Glucometer Elite (Bayer)

C) Reagent strips for visual reading

1. Dextrostix (Bayer)
2. Glucostix (Bayer)
3. Haemoglukotest (Boehringer Mannheim)