REFLOTEST HYPOGLYCEMIE — A MORE SENSITIVE SCREENING METHOD FOR NEONATAL HYPOGlyCAEMIA

SYNOPSIS

Neonatal hypoglycaemia if detected early and treated adequately carries a good prognosis. Hence a rapid and reliable screening method for neonatal hypoglycaemia is essential. This study evaluates the accuracy of a new glucose-oxidase test strip, Reflotest-Hypoglycemie. It also compares the visual readings of both Reflotest and Dextrostix readings with laboratory glucose values. Their sensitivities in the detection of neonatal hypoglycaemia are 100% and 73% respectively. The errors inherent in the Dextrostix system are also discussed. It is concluded that the Reflotest-Hypoglycemie test strips are a better alternative to Dextrostix in screening neonates with suspected hypoglycaemia.

INTRODUCTION

A rapid and reliable method of measuring blood glucose levels in newborn babies is essential as it is important to detect neonatal hypoglycaemia early preferably before the onset of symptoms, and treat it adequately to prevent any untoward cerebral complications. (1) Ideally, blood glucose should be assayed in the laboratory. However, in a busy hospital practice, to obtain rapid results is often difficult, and most neonatal units resort to the use of glucose test strips for routine screening of neonatal hypoglycaemia.

Since 1967, the Dextrostix has been widely used for this purpose (2). However, from 1982, reports have questioned its accuracy (3). Lately, the use of a new test strip, the Reflotest-Hypoglycaemia (B.M.) has been introduced. This gives both a visual estimation as well as a quantitative reading when used together with the Reflotomat, which is a twin beam reflectance photometer. The range of blood glucose readings accurately measured by the meter is 10 — 150 mg/dl (0.55 — 8.3 mMol/L). The use of these strips has never been evaluated in Singapore before.

This study was done with the following aims: 1) To assess the accuracy of blood Reflotest glucose values measured quantitatively on a meter (Reflomat) with laboratory glucose values, and 2) To compare the visual readings of both Reflotest and Dextrostix with laboratory glucose measurements.
PATIENTS AND METHODS

Ninety-six high-risk neonates consecutively admitted to the neonatal ward of Alexandra Hospital for monitoring had their blood sampled. These patients had birth weights ranging from 800 — 5000 grams, and their gestational ages were from 30 — 42 weeks. 25 neonates had suspected sepsicaemia; 19 had pneumonia; 17 were low birth weight infants (birth weight 2270 gm); 15 were infants of diabetic mothers; 6 were products of multiple pregnancies, of which there were 5 pairs of twins and 1 set of triplets; 4 were big babies (birth weight 4000 gm); and 3 had birth asphyxia.

A total of 211 venepuncture blood specimens were taken. At the same time and at the same site, the following were analysed:

1) Laboratory plasma glucose levels.
2) Reflotest-Hypoglycemia readings done in the ward, both visually as well as quantitatively with a meter (Reflomat).
3) Dextrostix readings, read visually in the ward.

All readings in the ward were done by a team of 2 doctors and occasionally 1 experienced nurse. In patients where blood sampling was difficult, and the volume of blood obtained was small, one or both visual estimations was not done. The results of each estimation was recorded independently, and before laboratory results were known.

In the laboratory, the plasma glucose was measured using the Beckman glucose analyser via the glucose oxidase method. Blood was sent in a plain plastic tube, and all measurements were made within one hour from the time of blood sampling.

Dextrostix is a test strip containing glucose oxidase, peroxidase and a chromogen. One venepuncture blood drop large enough to cover the test pad was applied. Exactly 1 minute later, the blood was washed off with a controlled stream of water from a squeeze bottle. The colour generated was compared immediately with the colour charts on the label of the Dextrostix bottle. The label has colour ranges for blood sugar values of 25, 45, 90, 130, 175 and 250 mg/dl (1.4, 2.5, 5.0, 7.2, 9.7 and 13.4 mMol/L). In-between visual readings were also recorded. A meter was not used as it has not been found to improve the accuracy of estimation.

The Reflotest-Hypoglycemia test strip contains the same enzymes, but has a different chromogen. A venepuncture blood drop was applied to cover the whole test pad. Exactly 1 minute later, the blood was wiped off with fresh cotton wool. The test area was gently re-wiped 2 more times to ensure that all traces of blood and cotton-wool were removed. 2 minutes after application, (1 minute after wiping off blood), the test strip was read both visually, as well as quantitatively. Visual estimations were done using the colour charts on the label of the bottle. This showed colour ranges for blood sugar levels of 20, 40, 80, 120 and 180 mg/dl (1.1, 2.2, 4.4, 6.7 and 10 mMol/L). In-between visual readings were also recorded. The range of blood glucose readings accurately measured by the meter is 10 — 150 mg/dl (0.55 — 8.3 mMol/L).

RESULTS

The results were analysed in two stages. Firstly, Reflotest readings, determined quantitatively on the Reflomat meter, were compared with laboratory glucose values. Next, visual readings from both Reflotest and Dextrostix were also compared with laboratory glucose readings to assess their individual sensitivities.

A) COMPARISON OF QUANTITATIVE REFLOTEST GLUCOSE LEVELS WITH LABORATORY GLUCOSE LEVELS:

Since blood glucose was assessed using the Reflomat and plasma glucose assayed by laboratory procedures, a correlation factor of 10% was used (4). Figure 1 gives the scatter diagram which shows the

![Fig 1 Comparison of Reflotest & Lab Glucose Values](image-url)
bivariate relationship between Reflotreat glucose levels measured quantitatively, with their corresponding laboratory-assayed levels. A sample correlation coefficient of 0.87 (p < 0.001) was obtained.

Of the 211 samples that were analysed, 16 were in the hypoglycaemic range. These were neonates with plasma sugar levels of 35 mg/dl or less. Of the 16, the Reflotreat correctly diagnosed 14, giving it a sensitivity of 87.5%. The sensitivity of a test measures its ability to identify as positive those patients who have the disease. A test which can correctly identify all those patients who have the disease is said to have a sensitivity of 100%. The two specimens missed actually gave readings of 36 and 38 respectively. These two specimens were detected on the visual scale of 20 — 40 mg/dl.

B) COMPARISON OF VISUAL DEXTROSTIX AND VISUAL REFLOTEST WITH LABORATORY GLUCOSE RESULTS

As the readings from the visual Reflotest and Dextrostix methods were ordered qualitative values (scores), the non-parametric Spearman’s rank correlation coefficients were computed in the comparison of each of these two methods in turn with laboratory glucose values. Figures 2 and 3 show these relationships. For the visual Reflotest, a Spearman’s rank
correlation coefficient of 0.92 was obtained. Out of a
total sample size of 178, 10 were found to be hypo-
glycemic using laboratory assay of blood glucose.
Taking the recommended visual reading of 40 or less
as the criterion for hypoglycaemia NO case of hypo-
glycaemia was missed. The sensitivity of the test, as
far as this sample of 178 is concerned is therefore
100%.

For the case of Dextrostix, a rank correlation coeffi-
cient of 0.62 was obtained for a sample size of 165.
There were 15 hypoglycaemic samples, of which 11
were detected by the visual Dextrostix procedure. This
gave a sensitivity of 73%.

Hence, the conclusions drawn from this study are:
1) Blood glucose results measured on the Reflomat
correlate well with laboratory glucose levels, and
2) Visual Reflomtest strips can detect neonatal hypo-
glycaemia more accurately than Dextrostix; their
sensitivities being 100% and 73% respectively.

DISCUSSION

Both Reflomtest and Dextrostix contain the enzymes
glucose oxidase and peroxidase, but they have dif-
f erent chromogens. The mechanism of action is as
follows:

Blood Glucose + Glucose Oxidase = Gluconic acid + H₂O₂
H₂O₂ + Chromogen  → Peroxidase → Colour

The errors obtained with the Dextrostix readings will
now be discussed. Firstly, (refer to Figure 4) as

and if the blood is not washed off completely, it may
obscure the actual colour reaction.

Thirdly, use of isopropyl alcohol in cleaning the skin
of the neonate prior to sampling may also cause
eroneously high Dextrostix values.

In the case of Reflomtest, the colour reaction is read
lose to the end-point. Also, the procedure is such that
only one function is done at one time i.e. at one
minute, wipe off the blood, and at two minutes, read
the colour. It has been found that even if the colour is
read at three minutes, there is hardly any change.

Hence this allows more room for error with regards to
timing for reading of the colour reaction.

The other advantages of the Reflomtest-Hypogly-
cemie test strips are:
1) The test pad area on the Reflomtest strip is two-
thirds that of Dextrostix and hence less blood is
needed.
2) There is not necessity for a squeeze-bottle of
water, as only fresh cotton wool is required.
3) In terms of costs, the two test-strips are

It is the policy of the nursery to use the visual Dext-
rostix procedure, as use of a meter does not improve
its sensitivity. Also, more errors with timing and

This study was initially done with the Reflomtest meter to ascertain the absolute
accuracy of the Reflomtest strips. However, from this
study, the visual Reflomtest procedure is as good as if
not better than the concomitant use of the Reflomtest

Fig 4 Calibration of the Dextrostix System

Table: Calibration Results of the Dextrostix System

<table>
<thead>
<tr>
<th>Blood Glucose (mg/dl)</th>
<th>Time of Exposure of Blood on Dextrostix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
</tr>
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described by Spellacy et al, the Dextrostix system is
not end-point in nature, and is read on the steep part
of the curve. This means that the colour continues
to develop even after the blood is washed off and any
error in timing would lead to a falsely high reading.
The washing of blood and the interpreting of the
colour also makes this a source of error, as it entails
the need for two procedures to be done at the same
time. It has been reported that the reading can change
by 1mgidl per second delay (8).

Secondly, neonatal blood has a high haematocrit,

meter. Hence we would recommend that in inex-
perienced hands, a visual reading would be sufficient
for screening purposes.

This study therefore supports the findings of
Wilkins et al (3) that the Reflomtest-Hypoglycemia strips
are a better alternative than Dextrostix for the routine
screening of neonates with suspected hypoglycaemia.

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REFERENCES