

REFLOTEST HYPOGLYCEMIE — A MORE SENSITIVE SCREENING METHOD FOR NEONATAL HYPOGLYCAEMIA

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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 Reflomat, 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.

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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 septicaemia; 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-Hypoglycemie 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 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 Dex-

trostix 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-Hypoglycemie 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).

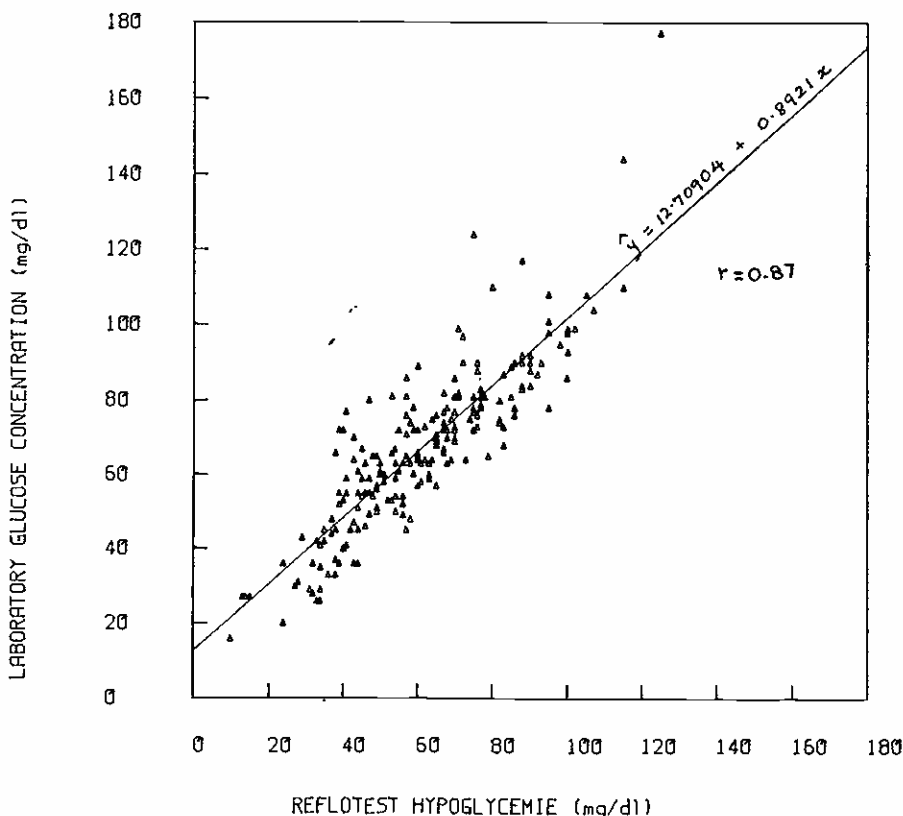
RESULTS

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 REFLATEST 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



bivariate relationship between Refloat 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 Refloat 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 (5). 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

Fig 2 Comparison of Visual Reflotest & Blood Glucose Values

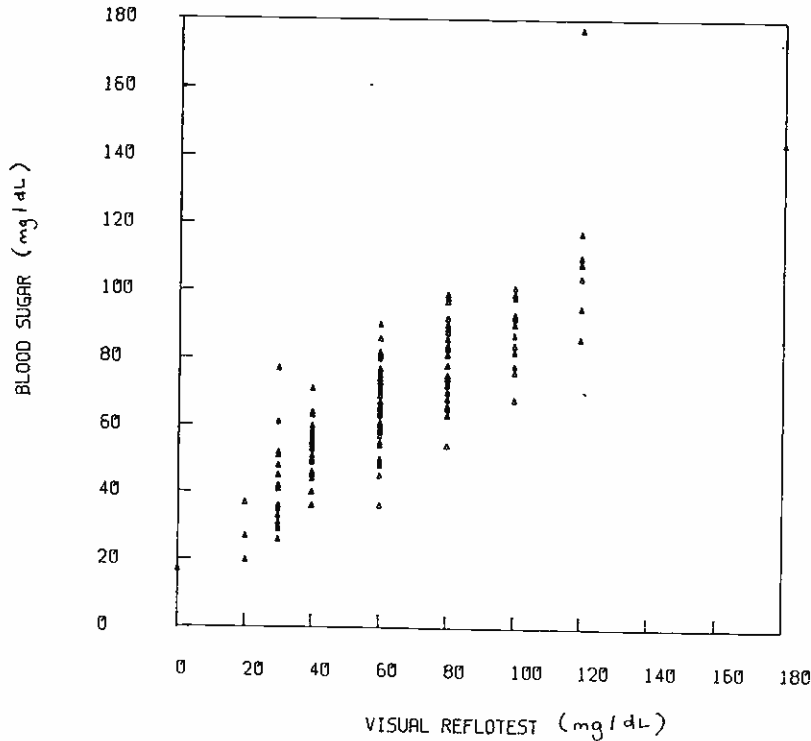
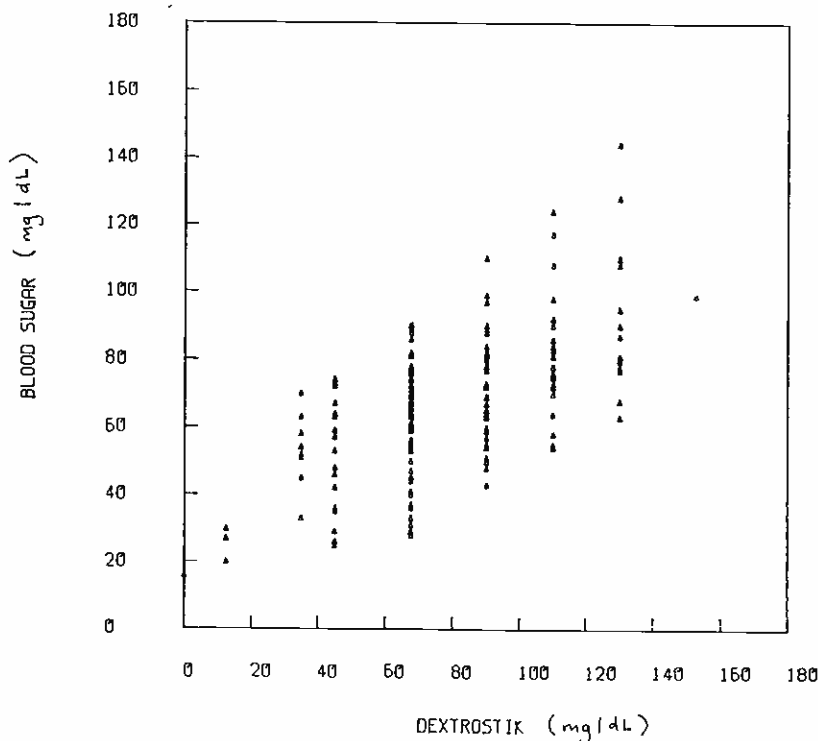


Fig 3 Comparison of Dextrostix Readings & Blood Glucose Values



correlation coefficient of 8.2 was obtained. Out of a total sample size of 178, 10 were found to be hypoglycemic using laboratory assay of blood glucose. Taking the recommended visual reading of 40 or less as the criterion for hypoglycaemia **NO** case of hypoglycaemia 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 coefficient 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 Reflotest strips can detect neonatal hypoglycaemia more accurately than Dextrostix; their sensitivities being 100% and 73% respectively.

DISCUSSION

Both Reflotest and Dextrostix contain the enzymes glucose oxidase and peroxidase, but they have different chromogens. The mechanism of action is as follows:

Blood Glucose + Glucose Oxidase = Gluconic acid + H₂O₂

H₂O₂ + Chromogen $\xrightarrow{\text{Peroxidase}}$ 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 erroneously high Dextrostix values.

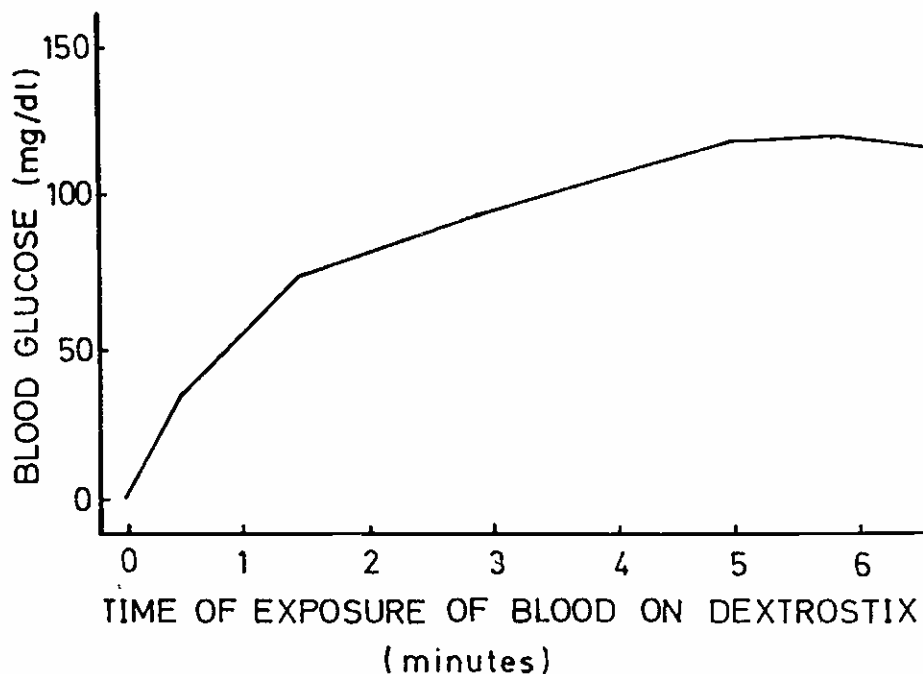
In the case of Reflotest, the colour reaction is read close to the end-point. Also, the procedure is such that only one function is done at one time i.e. at one minute, wipe off the blood, and at two minutes, read the colour. It has been found that even if the colour is read at three minutes, there is 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 Reflotest-Hypoglycemia test strips are:

- 1) The test pad area on the Reflotest 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 equivalent.

It is the policy of the nursery to use the visual Dextrostix procedure, as use of a meter does not improve its sensitivity. Also, more errors with timing and calibration may occur. This study was initially done with the Reflomat meter to ascertain the absolute accuracy of the Reflotest strips. However, from this study, the visual Reflotest procedure is as good as if not better than the concomitant use of the Reflomat

Fig 4 Calibration of the Dextrostix System



described by Spellacy et al, the Dextrostix system is not end-point in nature, and is read on the steep part of the curve (6, 7). 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 1mg/dl per second delay (8).

Secondly, neonatal blood has a high haematocrit,

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

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

ACKNOWLEDGEMENTS

The following people are acknowledged:

- 1) Prof. Victor Yu and Dr Tan Keng Wee for their advice and guidance.
- 2) Dr S K Lam for his keen participation in the study.
- 3) All medical and nursing staff of Neonatal Ward, Alexandra Hospital for their patience and tolerance during the study period.
- 5) Boehringer Mannheim for the supply of the Reflotest Hypoglycemia test strips and the Reflomat meter.

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