Glucoflex-R™ generic blood glucose test strips
(For use with Roche Reflolux blood glucose meters)
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Evaluation of the Glucoflex-R™
generic blood glucose test strips

(For use with Roche Reflolux blood
glucose meters)

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An evaluation has been made of the Glucoflex-R generic test strip for capillary blood glucose measurement using the Roche Reflolux S glucose meter. Generic strips/sensor electrodes employ another manufacturer’s meter, and are designed to be used in place of the existing manufacturer’s strips/electrodes. The Glucoflex-R blood glucose test strips are manufactured by NDP, and are generic test strips to replace the BM-TEST 1-44 test strips used with the Roche Reflolux II, IIM and S blood glucose meters (Roche, UK).

The 120 results obtained on capillary whole blood samples, when compared with those obtained using either a YSI 2300 or the hexokinase method gave a correlation coefficient of 0.99. There was a statistically insignificant overall average bias of 0.01 mmol/L against the YSI 2300. Results were on average < 1 % higher than those obtained using the YSI 2300, with 15 % of results showing an absolute bias of more than 10 % and 2 % of results a bias of more than 20 %. Against the hexokinase method, the mean bias was –0.70 mmol/L and the percentage bias -7 %, which is consistent with the observed difference between the YSI 2300 results and those of the hexokinase method.

Using error grid analysis the Glucoflex-R system was classified as clinically acceptable against either the YSI 2300 or the hexokinase reference method. Significant variation in the bias of the results relative to the YSI 2300 was found with concentration level, with the bias steadily increasing on average from –0.5 mmol/L to +1.4 mmol/L. Relative to the hexokinase method, the bias becomes increasingly negative with increasing concentration. Imprecision (CV) in laboratory studies at glucose concentrations of 3.3, 9.8, 16.8 and 21.8 mmol/L was 4.1, 4.5, 3.1 and 4.9 %, (compared with the usual requirement of no more than 5 %) and total error was 5.2, 5.8, 3.8 and 7.7 % (with a usual requirement of 10 % or less). There was significant variation in imprecision between two different batches of strip, with variability in the second batch of strips tested being approximately twice that seen in the first batch (meter-to-meter variation in bias was also significant).

There was a significant negative correlation between haematocrit and bias in results relative to the YSI 2300. The correlation coefficient was r = -0.19 (p < 0.05) and the effect was noticeable at glucose concentrations above 12 mmol/L where there was an estimated fall of 1.4 mmol/L across the range 35 – 55 % haematocrit.

If insufficient blood is applied to the test strip, it is possible to obtain an inappropriately low result. Results indicate that the minimum sample of blood required to obtain accurate results is dependent on the glucose concentration. At glucose concentrations of approximately 7 mmol/L a volume of 10 µl is required, but this becomes nearer 20 µl at a concentration of 17 mmol/L. At this concentration sample volumes of 5, 10 or 15 µl gave inappropriately low results, ranging from 7 to 15 mmol/L. As with the BM-TEST 1-44 test strips, the Glucoflex-R strips require a relatively large volume of blood in comparison with the newer generation systems (0.3 to 3.5 µl) to generate a result. In addition they also require timing and wiping of the red blood cells from the test area prior to glucose measurement.
Several issues concerning the use of generic strips/electrodes in place of those supplied specifically by the meter manufacturer remain unresolved. The question of legal liability in the event of a “system” failure with serious consequences for the user is potentially problematic. It should be noted that the performance figures quoted in this report have no bearing on the performance of the Reflolux system used with BM-TEST 1-44 test strips. They are applicable only to the combination of Glucoflex-R test strips and the Reflolux meter.
Introduction

Recent studies have shown that the use of self-monitoring devices by diabetic patients to achieve a tighter control of their glycaemic status can help reduce the onset of retinopathy, nephropathy and neuropathy\(^{(1-2)}\). Problems have been encountered in using some of the early blood glucose monitoring systems\(^{(3-7)}\). Problems were mainly due to difficulties in obtaining sufficient blood, inaccuracies in timing the application and removal of blood from the test strip, inappropriate wiping technique, lack of maintenance, and limited appreciation of quality control procedures. Due to the possibility of obtaining misleading results, adversely affecting patient treatment, the Department of Health has issued a Hazard Notice\(^{(8)}\). More recently a safety notice has been issued by the Medical Devices Agency \(^{(9)}\) entitled *Extra-laboratory use of blood glucose meters and test strips: contra-indications, training and advice to the users*. Recent developments in the manufacture of glucose test strips which use ‘non-wipe’ technology, reduction in the volume of blood required, and automatic timing sequences have helped in reducing the number of operator dependent steps. In addition, the low volume of blood required minimises the operator-dependency involved in obtaining an adequate blood sample.

A clinical and laboratory evaluation has been made of the Glucoflex-R generic test strip for capillary blood glucose measurement using the Roche Reflolux S glucose meter. The Glucoflex-R blood glucose test strips are manufactured by NDP, and are generic test strips for use with the Roche Reflolux II, IIM and S blood glucose meters (Roche, UK). The Reflolux is a small meter intended for professional use in point of care testing and for home use by diabetics. The Glucoflex-R strips like the BM-TEST 1-44 strips use reflectance ‘wipe’ technology, which requires the removal of the red blood cells from the reagent test strip prior to obtaining a result. The system is pre-calibrated, has important timing and wiping steps, requires approximately 20 µl of blood, and a result is obtained in 120 seconds. The meter is capable of storing 20 test results. This evaluation of the Glucoflex-R test strips is part of a programme assessing the suitability of analytical systems for use in primary care and health screening locations\(^{(10-47)}\).

The Glucoflex-R strips were assessed in this evaluation for accuracy and imprecision and batch-to-batch variation using two meters and two batches of test strips. Separate laboratory experiments on imprecision, haematocrit influence, volume dependency, linearity, and operator dependent steps were also performed.
Specifications of the Glucoflex-R blood glucose test strips for glucose measurement are given in Table 1.

Table 1: Details of the Glucoflex-R blood glucose test strips

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>National Diagnostic Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7-9 Merriwa Street</td>
</tr>
<tr>
<td></td>
<td>Gordon NSW</td>
</tr>
<tr>
<td></td>
<td>2072 Sydney</td>
</tr>
<tr>
<td></td>
<td>Australia</td>
</tr>
<tr>
<td>Essential accessories</td>
<td>Reflolux II, IIM or S glucose meter, lancet, quality control material, cotton wool.</td>
</tr>
</tbody>
</table>

The generic blood glucose test strips are designed for use with the Roche Reffolux II, IIM and S glucose meters, and use dry reagent technology based on the glucose oxidase method. The Glucoflex-R test strip utilises the glucose oxidase and peroxidase reaction with dyes (tetra methyl benzidine, APAC and tolidine, which on oxidation produces blue colour on the lower pad and a brown to green colour on the top pad. The intensity of the colours formed is proportional to the concentration of glucose in the blood sample. The reaction utilises the oxygen in the blood sample.

Glucoflex-R strips are available in packs of 50, and are stored at temperatures between 4 and 30°C. NDP state “when kept in the original bottle these test strips are stable up to the expiry date specified on the label.”

The analytical range for glucose measurement quoted by NDP for the Glucoflex-R test strips is 0.5 to 27.7 mmol/L.
The Reflolux II, IIM, or S meters require the operator to initiate the timing sequence and wipe blood from the reaction zone manually. The twin pad test strip is composed of a plastic support, on top of which are laid two pieces of polymer membrane formulated with the reagents glucose oxidase, peroxidase and chromogens (tetra-methyl-benzidine on the lower test pad and apac ortho toolidine on the upper test pad), sealed with a plastic dispersive layer. The test area to which the blood sample has been applied must be wiped to remove red blood cells before the glucose concentration can be determined.

### Calibration

The Glucoflex-R strip, in similar manner to the BM-TEST 1-44 test strip, is designated a specific calibration code, corresponding to pre-calibration contained in the meter. The operator is however required to manually enter batch-specific information into the meter’s memory by means of a bar-coded calibrator strip supplied with each pack of test strips. The calibration procedure for the Relolux II and IIM is different to that for the Reflolux S. Once the meter is calibrated for the reagent test strip in use by means of the barcode strip, the information is retained in the meter's memory. The code is displayed each time the instrument is switched on, and must be checked against the code printed on the reagent container prior to each glucose measurement. This ensures that the calibration information is correct for the strips in use.

### Operation of the meter

To perform a blood glucose measurement, the meter is switched on and the lot code displayed by the meter checked against that on the test strip container (and altered if necessary). To reduce difficulties in obtaining a blood sample and improve circulation, NDP recommend washing the hands with soap in warm water. A fingerstick sample of whole blood is obtained by lancing a finger, and gently squeezing the finger to obtain a hanging drop of blood.

Sufficient blood is applied to completely cover the test area of the test strip and the timing sequence initiated manually by pressing the “TIME” button. The time, in seconds is displayed and exactly 60 seconds after sample application, the red blood cells are removed from the test area by wiping with cotton wool three times, ensuring that a clean area of the cotton wool is used each time. The test area is allowed to dry for a few seconds, and the test strip inserted into the aperture with the test area facing towards the ON/OFF button. At 120 seconds, the meter gives an audible bleep, and displays the result in the chosen units (mmol/L or mg/dl). Results are automatically stored within the memory (a total of 10 test results). The meter switches itself off automatically after 60 seconds.

Error messages are shown on the meter’s visual display panel to indicate procedural or meter errors such as: test strip inserted incorrectly, electronic problem, low battery power, glucose concentration above the limit of measurement.
The evaluation was carried out on two Reflolux S meters and Glucoflex-R test strips provided by NDP. The instruments were operated throughout according to NDP and Roche (meter) instructions.

### Method comparison

#### Capillary blood

The performance of the glucose assay was evaluated by determining glucose concentration in capillary whole blood specimens from 120 Patients. Results were compared with a routine laboratory method performed on a YSI 2300 analyser (YSI Ltd, Farnborough, Hants), and a method based on hexokinase/glucose-6-phosphate dehydrogenase following whole blood deproteinisation using perchloric acid.

To assess strip-to-strip and meter-to-meter variation, two Reflolux S meters and two different batches of Glucoflex-R test strips were tested. Capillary blood specimens from a finger were obtained using the Safe-T-Pro lancet (Roche, UK) from patients attending the diabetic outpatient clinic or the Diabetes Centre at City Hospital, Birmingham. Samples were analysed immediately on two Reflolux S meters with the Glucoflex-R test strips following NDP’s instructions. A further 200 µl of blood from the same fingerstick was collected into a microvette tube (Sarstedt, Leicester, UK) containing lithium heparin as anticoagulant, and blood glucose estimation carried out immediately on the YSI 2300. Duplicate blood samples were also collected for the hexokinase assay. The influence of haematocrit on the glucose value obtained was assessed by carrying out haematocrit measurements on each patient sample in duplicate using a Microspin haematocrit centrifuge (Bayer Diagnostics, Newbury, UK).

**YSI 2300**  
The YSI 2300 analyser uses a thin membrane containing immobilised glucose oxidase placed over a platinum anode. The glucose from the sample diffuses into the membrane, producing hydrogen peroxide, which is then oxidised at the platinum anode producing electrons. The electron flow is linearly proportional to the steady state hydrogen peroxide concentration, and therefore to the concentration of the glucose. The YSI 2300 was calibrated and maintained according to the manufacturer's instructions.
Hexokinase assay
The blood sample for the hexokinase assay was collected into 40µl glass sodium heparin capillary tubes (Hirschmann Laborgerate GmbH, Eberstadt, Germany) in duplicate. The capillary tubes were then placed into two prefilled tubes containing 400 µl of 0.33M perchloric acid (Kabe Labortechnik, Nuenbrecht -Elsenroth, Germany) and mixed vigorously for 10 seconds to allow protein precipitation. The perchloric acid tubes were centrifuged for five minutes at 10,000 rpm (Biofuge B, Heraeus Sepatech, Brentwood, Essex), and the precipitate decanted using a positive displacement pipette. The samples were recentrifuged to ensure all traces of the precipitate were eliminated. The samples were stored at 4°C and analysed the following day on a Cobas Bio using a Unimate 5 GLUC HK kit (Roche Diagnostics Ltd, Lewes, East Sussex).
Results comparison with the YSI 2300

Capillary whole blood samples were used for evaluating the measurement of glucose with the Glucoflex-R test strips and the Reflolux S meters. The correlation of the 120 results from patient specimens using the Glucoflex-R test strips and the YSI 2300 is illustrated in Figure 1 (correlation coefficient = 0.99). The line shown in Figure 1 is the 45° line that would be seen if the Glucoflex-R test strips and the YSI 2300 gave identical results. Results were on average < 1 % higher using the Glucoflex-R test strips than those obtained for the YSI 2300. Table 2 gives the mean glucose level obtained for the 120 patient specimens using the Glucoflex-R test strips and the YSI 2300. There is a non-significant positive overall mean bias of 0.02 mmol/L, with standard error of 0.07 mmol/L (t_{119} = 0.24, p = 0.81).

Figure 1: Correlation obtained for glucose results from 120 patients’ capillary blood samples using the Glucoflex-R test strips (Reflolux S meter A)

<table>
<thead>
<tr>
<th></th>
<th>Glucoflex-R</th>
<th>YSI 2300</th>
<th>Glucoflex-R - YSI 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (mmol/L)</strong></td>
<td>10.87</td>
<td>10.86</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>SD (mmol/L)</strong></td>
<td>5.44</td>
<td>5.13</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 2: Summary statistics of glucose results obtained by the Glucoflex-R test strips (Reflolux S meter A) and YSI 2300 (n = 120)
Figure 2 shows the differences between the Glucoflex-R test strips and the YSI 2300 results plotted against the YSI 2300 result. Perfect agreement between the two sets of results would give a horizontal line of points passing through zero on the y axis and a persistent trend away from that general pattern would indicate some pattern of bias. The pattern evident in Figure 2 suggests an upward trend in bias with concentration with imprecision increasing with concentration.

**Figure 2: Differences between the Glucoflex-R test strips (Reflolux S meter A) and the YSI 2300 results plotted against the YSI 2300 result**

Figure 3 gives a histogram of the Glucoflex-R test strips bias, and confirms the absence of overall bias. The corresponding plot of percentage bias shows 4.2 % of results from the Glucoflex-R test strips having a positive bias relative to the YSI 2300. There were 15 % of the results with an absolute bias of more than 10 % and 2 % of results with an absolute bias of more than 20 %. A conventional regression analysis of Glucoflex-R test strips and YSI results (Table 3) confirms the presence of a bias, increasing with concentration. The slope of 1.05 is significantly different from unity.
Figure 3: Histogram of the bias and percentage bias in results using the Glucoflex-R (Reflolux S meter A)

Table 3: Regression statistics of the Glucoflex-R test strips (Reflolux S meter A) against the YSI 2300 glucose results

<table>
<thead>
<tr>
<th></th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate (standard error)</td>
<td>0.54 (0.15)</td>
<td>1.05 (0.01)</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.83 to –0.25</td>
<td>1.03 to 1.07</td>
</tr>
</tbody>
</table>

Dividing the data into groups according to the YSI result and calculating the bias of all Glucoflex-R test strips results relative to the YSI 2300 gives the data shown in Table 4. Figure 4 shows a corresponding group scatter plot of the Glucoflex-R test strips bias at each of the ten levels of YSI result used in Table 4. The mean bias varies between –0.5 and +1.4 mmol/L or -8 and +7 % on average for most concentration levels for which a reasonable number of results were available, and the standard deviation of bias suggests imprecision of between 3 and 8 % on average. The about line standard deviation in the regression analysis reported in Table 3, which should also provide an estimate of imprecision is 0.7 mmol/L or approximately 6 %. Both of these statistics are in broad agreement with standard deviations seen in Table 4.
Table 4: The mean bias of the Glucoflex-R results (Reflolux S meter A) relative to the YSI 2300 results

<table>
<thead>
<tr>
<th>YSI 2300 results (mmol/L)</th>
<th>Number of results</th>
<th>Mean Glucoflex-R test strips bias mmol/L (% bias)</th>
<th>SD of bias mmol/L (% SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>18</td>
<td>-0.03 (-1%)</td>
<td>0.35 (8%)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>17</td>
<td>-0.51 (-8%)</td>
<td>0.46 (7%)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>21</td>
<td>-0.16 (-2%)</td>
<td>0.34 (4%)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>10</td>
<td>-0.09 (-1%)</td>
<td>0.40 (4%)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>15</td>
<td>0.02 (1%)</td>
<td>0.55 (5%)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>15</td>
<td>0.13 (1%)</td>
<td>0.74 (5%)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>7</td>
<td>0.17 (1%)</td>
<td>0.43 (3%)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>8</td>
<td>0.74 (4%)</td>
<td>1.08 (6%)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>5</td>
<td>1.44 (7%)</td>
<td>0.92 (5%)</td>
</tr>
<tr>
<td>&gt; 21</td>
<td>4</td>
<td>-0.28 (-1%)</td>
<td>1.88 (8%)</td>
</tr>
</tbody>
</table>

Figure 4: Glucoflex-R test strips (Reflolux S meter A) biases relative to the YSI 2300 at ten concentration levels as measured on the YSI 2300
Results

Figure 5a illustrates a significant negative correlation between the haematocrit and the bias in the Glucoflex-R test strips result \( (r = -0.19, p < 0.05) \).

**Figure 5a: Scatterplot of bias versus haematocrit for the Glucoflex-R test strips (Reflolux S meter A)**

Figure 5b has been formed by calculating the bias of each Glucoflex-R test strips result and then displaying it at a level of 3, 9, 15 and 21 mmol/L depending on whether the corresponding YSI 2300 result fell in the intervals 0 to 6, > 6 to 12, > 12 to 18, or >18 mmol/L. This representation of bias at different concentration levels then gives an indication of how haematocrit has affected the accuracy of Glucoflex-R test strips results at different concentration levels. An effect of haematocrit would appear to occur at concentrations above 12 mmol/L. There is an estimated reduction of 0.07 mmol/L per unit increase in haematocrit, which equates to a fall of 1.4 mmol/L across the haematocrit range 35 – 55 %, the limits of the range studied in this evaluation.
Comparison of Glucoflex-R test strips with two Reflolux S meters

Two Reflolux S meters designated meters A and B were used in parallel throughout the patient phase of this evaluation. The bias for meter B was more pronounced than with meter A, being 0.24 mmol/L with standard error 0.06. This bias is highly significantly different from zero, $t_{119} = -3.92$, $p < 0.001$. The overall percentage biases from the two systems relative to the YSI 2300 were -1% for meter A and -3% for meter B. Regression statistics for the Glucoflex-R test strips using meter B suggested a less pronounced increase in bias with concentration than that reported for meter A. A similar table to Table 4 for the second system suggests a mean bias increasing from -0.6 to +0.1 mmol/L with increasing concentrations. The mean difference overall between the two Glucoflex-R systems was 0.26 mmol/L or, expressed as a percentage, 3%. Actual differences range from -1.4 mmol/L to +2.3 mmol/L.

An association between the bias with the Glucoflex-R test strips on the Reflolux system and the haematocrit was evident with meter B. For meter B the correlation between bias and haematocrit was $r = -0.23$, $p < 0.05$. 

![Figure 5b: Bias versus haematocrit at four glucose concentrations for the Glucoflex-R test strips (Reflolux S meter A)](image-url)
Comparison of two batches of Glucoflex-R test strips

Two batches of test strips were used during the patient stage of the evaluation: Batch 1 lot number 60199, programme number 311; Batch 2 lot number 118014, programme number 311. There was no significant difference in bias between the results from the two batches of strips. The mean bias for the two batches of strips was −0.02 mmol/L and +0.05 mmol/L ($t_{118} = -0.57$, $p = 0.57$) using meter A, and −0.23 mmol/L and −0.25 mmol/L ($t_{118} = 0.13$, $p = 0.90$) for meter B. Table 5 gives the mean bias and standard error of the mean for each batch of strips using both meters. There was significant variation from meter to meter. For each batch with meter B the bias is significantly different from zero.

Table 5: Mean bias using two Reflolux S meters and two different batches of Glucoflex-R test strips

<table>
<thead>
<tr>
<th>Batch</th>
<th>Number of results</th>
<th>Meter A mean bias (SEM)</th>
<th>Meter B mean bias (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1 (lot 60199)</td>
<td>55</td>
<td>-0.02 (0.06)</td>
<td>-0.23 (0.05)</td>
</tr>
<tr>
<td>Batch 2 (lot 118014)</td>
<td>65</td>
<td>0.05 (0.11)</td>
<td>-0.25 (0.10)</td>
</tr>
</tbody>
</table>

Note: SEM = standard error of the mean

Table 6 gives the standard deviation of the bias of results in each batch for each meter. These figures give some idea of the imprecision associated with each batch used with each meter and suggest that there is variation in imprecision from batch to batch, but that results from meters A and B are equally imprecise.

Table 6: Standard deviation of bias using two Reflolux S meters and two different batches of Glucoflex-R test strips

<table>
<thead>
<tr>
<th>Batch</th>
<th>Meter A SD of bias (mmol/L)</th>
<th>Meter B SD of bias (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1 (lot 60199)</td>
<td>0.45</td>
<td>0.39</td>
</tr>
<tr>
<td>Batch 2 (lot 118014)</td>
<td>0.92</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Table 7 gives regression statistics for each batch of strips using meter A and YSI 2300 results. Perfect agreement between YSI 2300 and the Glucoflex-R test strips would result in a slope of 1 and an intercept of 0. For both batches the slope is significantly greater than unity, indicating a concentration dependent bias with strips from either batch. However, the about line standard deviation is roughly twice as large for Batch 2 test strips at 0.86 mmol/L, but with Batch 1 at 0.42 mmol/L.
Results

Table 7: Regression statistics from two different batches of Glucoflex-R test strips

<table>
<thead>
<tr>
<th></th>
<th>Intercept (SE)</th>
<th>Slope (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1 (lot 60199)</td>
<td>-0.39 (0.13)</td>
<td>1.04 (0.01)</td>
</tr>
<tr>
<td>Batch 2 (lot 118014)</td>
<td>-0.66 (0.26)</td>
<td>1.06 (0.02)</td>
</tr>
</tbody>
</table>

Error grid analysis

Evaluations of devices for self-monitoring of blood glucose have been criticised for determining accuracy of the systems in ways which are not clinically useful\(^{48-51}\). Clarke et al\(^{48}\) have developed an error grid analysis (Figures 6a and 6b) of data to indicate if the results obtained by glucose systems used for self monitoring are clinically accurate and acceptable. This is based on trying to maintain a patient's glucose within the range 3.9 to 10.0 mmol/L, and the consequences of inappropriate treatment due to obtaining an incorrect result. Zone A represents glucose values which differ by < 20% from the reference. Zone B represents values that differ from the reference by >20%, but would lead to "benign or no treatment". Results in zone C would lead to "inappropriate intervention to alter an acceptable glucose concentration". Zone D depicts "dangerous failure to detect and treat errors", whilst zone E indicates an "erroneous treatment". The interpretation of error grid analysis has been extended\(^{50}\) to specify that "an SMBG device can be considered acceptable if at least 95% of tests fall into the A zones and 0% fall in the C-E zones".

Results for Glucoflex-R test strips with meters A and B are shown in Figures 6a and 6b. Using these criteria on the 116 results from the Glucoflex-R test strips, 99% of results from both meters A and B fell into zone A, with the remaining 1% in zone B. No results for either meter fell within zones C - E. The Glucoflex-R test strip with the Reflolux system is clinically acceptable for use with capillary blood.
Results

Figure 6a: Clinical evaluation of the Glucoflex-R (Reflolux S meter A) by error grid analysis

Figure 6b: Clinical evaluation of the Glucoflex-R test strip (Reflolux S meter B) by error grid analysis
Results comparison with the hexokinase assay

The correlation of 120 results from patient specimens using the Glucoflex-R test strips and the hexokinase method is similar to that between the Glucoflex-R and the YSI 2300 at 0.99. Results were on average 7% lower using the Glucoflex-R test strips than those obtained by the hexokinase method. Figure 7 records the pattern of results produced by the two methods. Previous in-house studies have demonstrated that the YSI 2300 results based on glucose oxidase are approximately 5 to 6% below a hexokinase method on the same sample. Table 8 gives the mean glucose level obtained on the 120 patient specimens using the Glucoflex-R test strips and the hexokinase method. There is a significant negative overall mean bias of -0.70 mmol/L, with a standard error of 0.07 mmol/L ($t_{119} = -10.18$, $p < 0.001$).

Figure 7: Correlation obtained for glucose results from 120 patients' capillary blood samples using the Glucoflex-R (Reflolux S meter A) and hexokinase reference method

Table 9 shows regression statistics from a linear regression of Glucoflex-R test strips result on hexokinase result and gives evidence of a concentration dependent bias with the slope at 0.97, significantly different from unity, $p < 0.05$. This is evident in Table 10, which gives the mean bias in the Glucoflex-R test strips relative to the hexokinase assay. As a general trend, the bias becomes increasingly negative with increasing concentrations. As a percentage, the bias varies between -11 and -2%.
Table 8: Summary statistics for glucose results obtained by the Glucoflex-R test strips (Reflolux S meter A) and hexokinase assay (n = 120)

<table>
<thead>
<tr>
<th></th>
<th>Glucoflex-R</th>
<th>Hexokinase</th>
<th>Glucoflex-R - Hexokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>10.87</td>
<td>11.57</td>
<td>-0.70</td>
</tr>
<tr>
<td>SD (mmol/L)</td>
<td>5.44</td>
<td>5.53</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Table 9: Regression statistics of the Glucoflex-R test strips (Reflolux S meter A) against the hexokinase glucose results

<table>
<thead>
<tr>
<th></th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate (standard error)</td>
<td>-0.40 (0.16)</td>
<td>0.97 (0.01)</td>
</tr>
</tbody>
</table>

Table 10: The mean bias of the Glucoflex-R test strips (meter A) relative to the hexokinase glucose results

<table>
<thead>
<tr>
<th>Hexokinase results (mmol/L)</th>
<th>Number of results</th>
<th>Mean Glucoflex-R test strips bias mmol/L (% bias)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>13</td>
<td>-0.18 (-4%)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>17</td>
<td>-0.64 (-11%)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>16</td>
<td>-0.78 (-10%)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>16</td>
<td>-0.80 (-8%)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>14</td>
<td>-0.71 (-6%)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>12</td>
<td>-1.04 (-7%)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>12</td>
<td>-0.67 (-4%)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>6</td>
<td>-0.57 (-3%)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>8</td>
<td>-0.41 (-2%)</td>
</tr>
<tr>
<td>21 - 27</td>
<td>6</td>
<td>-1.33 (-5%)</td>
</tr>
</tbody>
</table>

In error grid analysis, 98 % of results fell within zone A for meter A and 96 % for meter B; the remainder fell in zone B. Thus using the hexokinase method as the reference method, the Glucoflex-R test strips would be classified as clinically acceptable with capillary blood.
Imprecision

The imprecision of the Glucoflex-R was determined on two meters at four different glucose concentrations. Blood was collected into lithium heparin vacutainer tubes (Becton Dickinson), and spiked with a 0.5 Molar glucose solution to the required glucose concentration. The spiked blood sample was allowed to equilibrate for 30 minutes at room temperature on a rotary mixer. Blood glucose measurements were performed in duplicate on the YSI 2300, all through the experiment to ensure that the glucose level had not fallen due to glycolysis.

Twenty replicate glucose measurements were carried out at each level on the two meters. Results are summarised in Table 11. At glucose concentrations of 3.3, 9.8*, 16.8* and 21.8* mmol/L (*samples spiked with glucose), coefficients of variation (CVs) of 4.1, 4.5, 3.1 and 4.9 % were obtained respectively. These CVs do not include variations that might occur between batches of strips. Total error, which includes both bias and imprecision, was 5.2, 5.8, 3.8 and 7.7 % respectively.

The recommendations (52, 53) for all extra-laboratory blood glucose analyses quote a total allowable error of no more than 10 % and an imprecision CV of no more than 5 %. The imprecision and total error achieved by an experienced laboratory worker with the Reflolux S meter using the Glucoflex-R test strips meet these criteria at all four concentrations studied here.
Table 11: Imprecision of the Glucoflex-R test strips at four different glucose concentrations on two Reflolux S meters

<table>
<thead>
<tr>
<th></th>
<th>YSI 2300 (mmol/L)</th>
<th>Glucoflex-R Mean (mmol/L)</th>
<th>Glucoflex-R SDd (mmol/L)</th>
<th>Glucoflex-R CVd (%)</th>
<th>Glucoflex-R SDm (mmol/L)</th>
<th>Glucoflex-R CVt (%)</th>
<th>Total error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>3.3</td>
<td>3.4</td>
<td>0.10</td>
<td>3.1</td>
<td>0.09</td>
<td>4.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Level 2</td>
<td>9.8</td>
<td>9.4</td>
<td>0.36</td>
<td>3.7</td>
<td>0.26</td>
<td>4.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Level 3</td>
<td>16.8</td>
<td>16.5</td>
<td>0.46</td>
<td>2.7</td>
<td>0.24</td>
<td>3.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Level 4</td>
<td>21.8</td>
<td>20.5</td>
<td>1.06</td>
<td>4.9</td>
<td>NS</td>
<td>4.9</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Notes:

SDd = replicate standard deviation  
CVd = replicate coefficient of variation  
SDm = meter to meter standard deviation  
CVt = total (duplicate and meter) coefficient of variation  
Total variance = (SDd)^2 + (SDm)^2 + (bias)^2  
Total error (%) = 100 x (total variance)^1/2 / mean YSI glucose  
NS = not significant
Effect of haematocrit on glucose results

Anomalies in glucose results for whole blood specimens obtained from patients with abnormal haematocrit have been widely reported when comparing plasma glucose results obtained by the laboratory with those obtained using glucose meters. This has led to concern at errors in the measurements made by these meters on patients with polycythaemia (increased haematocrit giving falsely low glucose values generally), and anaemia (eg diabetic pregnant females with low haematocrit, producing falsely high glucose values).

NDP states in their current product insert leaflet that “at extremely high haematocrit values (> 55 %) and glucose values above 11 mmol/L, the value obtained may be up to 15 % too low. At extremely low haematocrit (<35 %), readings obtained may be up to 10 % too high”. This claim was further investigated.

Venous blood samples were collected from healthy individuals in tubes containing lithium heparin as anticoagulant. Blood glucose estimations were carried out in duplicate on the YSI 2300 and the Reflolux S meter with the Glucoflex-R test strips. Haematocrit estimations were performed using a Microspin centrifuge (Bayer Diagnostics). To assess the influence of haematocrit at various glucose concentrations, the blood samples were spiked with a 0.5M glucose solution made up the previous day. The blood samples were allowed to equilibrate for 30 minutes at room temperature which also allows oxygenation of the sample to a pO₂ equivalent to that of capillary blood. The glucose concentration of each spiked blood sample was measured by the reference method, and the samples centrifuged for 10 minutes at 3000 rpm. The plasma was separated from the cells, and haematocrit measured in triplicate. Cells and plasma were recombined to give a range of haematocrit values. The recombined aliquots of blood were allowed to equilibrate for ten minutes prior to use. Simultaneous blood glucose measurements were carried out on the YSI 2300 and the Reflolux S with the Glucoflex-R test strip in quadruplicate using meter A. Haematocrit estimations were performed in triplicate.

Results are shown in Figures 8a and 8b. At the lower glucose level of approximately 9 mmol/L variation with haematocrit is small but slightly larger than that for the reference method. There is a change in result of 1.1 mmol/L across this range with the Glucoflex-R compared with a change of 0.7 mmol/L for the YSI 2300 result. At the higher glucose concentration studied of approximately 17 mmol/L there is a variation of approximately 2.1 mmol/L in results from the Glucoflex-R across the evaluated haematocrit range of 35 to 55 % compared with 0.7 mmol/L for the YSI 2300. The clinical study with capillary samples also highlighted a statistically significant negative correlation between bias and haematocrit (r = -0.19).
Figure 8a: The effect of haematocrit on the measurement of glucose concentrations using the Glucoflex-R at level 1

Figure 8b: The effect of haematocrit on the measurement of glucose concentrations using the Glucoflex-R at level 2
Results

**Volume dependency**

The volume of blood required for accurate measurement of glucose with the Glucoflex-R system was investigated at two glucose concentrations. A venous blood sample was obtained in a lithium heparin vacutainer tube (Becton Dickinson), and spiked with a 0.5M glucose solution to obtain the required glucose concentration. The sample was allowed to equilibrate for 30 minutes by gently mixing on a rotary mixer which also allows oxygenation of the sample to a $\text{pO}_2$ equivalent to that of capillary blood. The glucose concentration of the blood was monitored throughout the experiment on the YSI 2300 to ensure glycolysis was not distorting the results obtained. Blood glucose measurements were carried out randomly in quadruplicate at 5, 10, 15, 20, 30, 40 and 50 µl, by applying the blood sample to the reagent test strips using a calibrated positive displacement pipette.

The experiments were performed on samples of blood obtained from ‘normal’ individuals with haematocrit in the manufacturer’s quoted range of 35 to 55%.

The minimum volume of blood required for glucose measurement with the Glucoflex-R test strip is not stated by NDP. Results shown in Figures 9a and 9b indicate that the minimum volume of blood required to obtain accurate results is dependent on the glucose concentration. At glucose concentrations of approximately 7 mmol/L a volume of 10 µl is required, but this becomes nearer 20 µl at a concentration of 17 mmol/L. The discrepancy in the glucose value obtained at low sample volume was dependent on the location at which the blood sample was applied. At the higher glucose concentration of approximately 17 mmol/L, sample volumes of 5, 10 or 15 µl gave inappropriately low results ranging from 7 to 15 mmol/L. An error message “Err” was displayed only on one occasion at 5 µl volume. At a glucose concentration of 7 mmol/L an error message “Err” was shown on one occasion with a 10µl sample, and on all four occasions with 5 µl samples. As with the BM-TEST 1-44 test strips, the Glucoflex-R strips require a relatively large volume of blood in comparison with the newer generation systems (0.3 to 3.5 µl).
Figure 9a: The volume of blood required for accurate glucose measurement at glucose level 1

Figure 9b: The volume of blood required for accurate glucose measurement at glucose level 2
Results

- **Linearity assessment**

The linearity of the glucose results obtained using the Glucoflex-R test strips with the Reflolux S meter was assessed in the laboratory. A 20 ml venous blood sample was obtained in lithium heparin vacutainer tubes, and combined into a single tube and mixed at room temperature on a rotary mixer to allow oxygenation of the sample to a pO\textsubscript{2} approximately equivalent to that of capillary blood. The blood was aliquoted into 1-ml samples for the experiments. Individual aliquots were analysed on the YSI 2300 in duplicate, and spiked to the required glucose concentration using a 0.5 Molar glucose solution (made up in 0.9% saline the previous day and allowed to mutarotate overnight). The sample was allowed to equilibrate for 10 minutes at room temperature. The glucose measurements were made in quadruplicate on the YSI 2300 and the Glucoflex-R.

The results are displayed in Figure 10. The line superimposed on the figure is a 45° line of equality which would be seen if the Glucoflex-R and the YSI 2300 gave identical results. NDP quoted analytical range for Glucoflex-R glucose measurement is 0.5 to 27.7 mmol/L. Results above 27.7 mmol/L are flagged with a warning message “HI”. Glucose concentrations of approximately 28, 39, 52, 59 and 64 mmol/L gave an appropriate error message on all occasions.

**Figure 10: Linearity assessment**
Operator dependency

Analytical systems for use by non-laboratory operators should have a minimal number of complex manoeuvres, to reduce the risk of obtaining incorrect results. A major operator dependent step inherent to all analytical systems using capillary whole blood is in obtaining an adequate volume of free flowing blood. Excessive "milking" of the finger can dilute the sample with tissue fluid or cause haemolysis. Other operator dependent steps involved in glucose measurement are the accurate timing of sample application, and the wiping of blood from the strips before analysis.

Correct storage conditions as stated by the manufacturer should be maintained for all dry reagent strips. The cap must be replaced on the container after each use, and the strips not subjected to extremes of temperature or humidity.

Additional operator dependent steps identified for the Glucoflex-R test strip system were:

- checking that the reagents have not passed their expiry date, and the meter is calibrated for the correct batch number of strips.

- taking care to ensure full coverage of the test area with the sample. If insufficient sample is applied an inappropriately low result can be obtained. In the assessment of the volume dependency of blood, at glucose concentrations of approximately 7 mmol/L (Figure 9a), sample volumes of 10 µl or less were not always detected. At the higher glucose concentration of approximately 17 mmol/L (Figure 9b), sample volumes of 5, 10 or 15 µl gave inappropriately low results, with an error message “ERR” on only one occasion with 5µl of blood.

- ensuring that all correct timing procedures are adhered to.

- ensuring that the correct wiping technique is adopted. This is an important and variable step. Results can be affected by too gentle or too aggressive wiping.

- ensuring that the colour on the strip is also used to visually check a result by comparison against the colour chart on the container label. This acts as a backup for the meter result, and can highlight instances of discrepant results.

- ensuring that maintenance procedures are followed to keep the meter and the test strip adapter clean and free from lint that may be left behind from the cotton wool swab used for wiping the red blood cells.
As generic strips are produced to be used in other manufacturer’s meter systems, instructions need to be in exact agreement so there is absolutely no ambiguity or confusion. An instruction sheet is provided in the Glucoflex-R reagent pack. This gives information on the meters the test strips can be used with, and the test strips from the original manufacturer it replaces. It also has sections on “preparing for and performing the measurement with the meter; performance checks; comparison with other whole blood methods; and common problems”. The small instruction leaflet is not illustrated and is printed in blue ink on a white background. This may prove difficult to read for diabetics suffering with poor visual acuity arising from their illness.
A clinical and laboratory evaluation has been made of the Glucoflex-R generic test strip for capillary blood glucose measurement using the Roche Reflolux S glucose meter. Generic strips/sensor electrodes employ another manufacturer’s meter, and are designed to be used in place of the existing manufacturer’s strips/electrodes. The Glucoflex-R blood glucose test strips are manufactured by NDP, and are generic test strips for use with the Roche Reflolux II, IIM or S blood glucose meters (Roche, UK).

Glucose estimations carried out using dry reagent strips, read visually or with a reflectance meter, often involve several operator dependent steps, increasing the possibility of error particularly when the system is used by non-technical operators. The problems are due to: skin contamination, difficulties in obtaining an adequate sample of blood, poor application of sample to the reagent strip, inaccurate timing, inadequate or aggressive wiping technique and results that are influenced by the haematocrit of the sample (37, 54-58). As with the BM-TEST 1-44 test strips, the Glucoflex-R glucose test strips have many of these operator dependent steps inherent within the system. The system requires timing and removal of the red blood cells from the test strip prior to glucose measurement. The test strips require a relatively large sample of blood and it takes 120 seconds for a result to become available. The meter requires maintenance procedures to ensure that the strip reader is kept clean and free from lint that may be left behind from the cotton wool swab used for wiping the red blood cells.

The Glucoflex-R results, when compared with those obtained using either a YSI 2300 or the hexokinase method, gave a correlation coefficient of 0.99. There was a statistically insignificant average bias of 0.01 mmol/L against the YSI 2300 but a statistically significant mean bias of −0.70 mmol/L against the hexokinase method (p < 0.001). Results were on average <1% higher than those obtained using the YSI 2300, with 15% of results showing an absolute bias of more than 10% and 2% of results a bias of more than 20%. Against the hexokinase method, the mean percentage bias was approximately −7%. Previous in-house studies have demonstrated that the YSI 2300 results based on glucose oxidase are approximately 5 to 6% below a hexokinase method on the same sample. This is in near agreement with the difference observed in this study (7%). The Glucoflex-R was classified as clinically acceptable using error grid analysis against either reference method.

Significant variation in the bias of the results was found with concentration level, with the average bias increasing from around −0.5 mmol/L to a maximum of +1.4 mmol/L relative to the YSI 2300. Relative to the hexokinase method the bias became increasingly negative with increasing concentration. There was significant batch-to-batch variation in imprecision of the Glucoflex-R test strips. Meter-to-meter variation in bias was also significant.
Discussion and conclusion

There was a significant negative correlation between haematocrit and bias in results relative to the YSI 2300. The correlation coefficient was $r = -0.19$ ($p<0.05$) and the effect was noticeable at glucose concentrations above 12 mmol/L where there was an estimated fall of 1.4 mmol/L across the range 35 – 55 % haematocrit.

Imprecision (CV) at glucose concentrations of 3.3, 9.8, 16.8 and 21.8 mmol/L was 4.1, 4.5, 3.1 and 4.9 %, (compared with the usual requirement of no more than 5 %) and total error was 5.2, 5.8, 3.8 and 7.7 % (with a usual requirement of no more than 10 %).

If insufficient blood is applied to the test strip, it is possible to obtain an inappropriately low result. In the assessment of volume dependency, at a glucose concentration of approximately 7 mmol/L sample volumes of 10 µl or less were not always detected and no result produced. At a higher glucose concentration of approximately 17 mmol/L, sample volumes of 5, 10 or 15 µl gave inappropriately low result ranging from 7 to 15 mmol/L, with an error message “Err” occurring only on one occasion.

In conclusion, the Glucoflex-R test strips used in conjunction with the Reflolux S by laboratory personnel gave results that were classified as clinically acceptable with acceptable levels of imprecision and total error. As with the BM-TEST 1-44 test strips their use involves operator dependent steps and results are dependent on the volume of blood added to the test strip. These strips require a large volume of blood in comparison to the newer generation systems (0.3 to 3.5 µl). The Glucoflex-R requires a minimum sample volume of approximately 20 µl and inappropriately low results may be obtained if insufficient blood is applied.
Acknowledgements

The authors wish to thank: the representatives of NDP for their assistance and helpfulness with the evaluation; Mr Alex Bignell, Consultant Clinical Scientist, and the staff of the Department of Clinical Biochemistry at City Hospital, Birmingham; Dr KT Taylor, Dr REJ Ryder and Dr SL Jones Consultant Diabetologists; staff at the Diabetic Centre, City Hospital, Birmingham.


Manufacturer’s comments

The manufacturers were given the opportunity to comment on this report, but made a statement to the effect that “NDP won’t be making any formal comment”.
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