TheraSense FreeStyle blood glucose meter
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Evaluation of TheraSense FreeStyle blood glucose meter

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The FreeStyle blood glucose system has the capability of performing blood glucose measurements on a capillary blood sample obtained from a conventional fingerstick, as well as from an alternative site such as the forearm, upper arm, palm, thigh or calf. The FreeStyle is a small meter intended for home use by diabetics. The system is easy to use, and is pre-calibrated to give “plasma-calibrated results”. It requires 0.3 µl of blood, which is drawn into the strip by a “capillary fill” mechanism. The measurement time is dependent on the glucose concentration and takes approximately 15 seconds on average. The system has few operator dependent steps, and does not require complex maintenance as the blood is applied to the test strip externally to the body of the instrument and does not come into contact with the meter’s internal components.

Although the FreeStyle uses whole blood samples, the results displayed are calibrated to give “plasma-equivalent” values. Results from the FreeStyle system were therefore compared with plasma adjusted YSI 2300 results (taking into account the haematocrit of the sample) and the plasma hexokinase method.

The fingerstick capillary blood results, when compared with those obtained using the YSI 2300 (plasma equivalent results) or the plasma hexokinase method, gave a correlation of 0.99. There was an overall bias of −0.44 mmol/L relative to YSI 2300 plasma equivalent results, and −1.0 mmol/L relative to plasma hexokinase results. In both cases the bias becomes increasingly negative as concentration increases, reaching −1.0 and −2.6 mmol/L at the higher concentration studied. Error grid analysis against the YSI 2300 plasma equivalent results and the plasma hexokinase results would classify the system as clinically acceptable. Against the YSI 2300 plasma equivalent results 99.5 and 100 % of results from meters A and B respectively fell in zone A, whilst with the hexokinase method 99 and 100 % of results fell in zone A for meters A and B respectively.

In the clinical study of fingerstick glucose measurements, imprecision was estimated to be approximately 5 % on average across the concentration range studied when compared with YSI 2300 plasma equivalent results. In the laboratory study, imprecision (CV) of the results at glucose concentrations of 3.9, 11.4, 21.1 and 26.2 mmol/L was 3.1, 2.7, 1.9 and 2.6 % respectively, which meets the criterion for acceptable imprecision of 5 % or less at all four concentration levels. The total error of 9.9, 9.9, 9.6 and 9.9 % respectively meets the criterion for acceptable total error of no more than 10 % at all four levels.

The manufacturer’s quoted haematocrit range is 0 to 60 %. In the laboratory study, at glucose concentrations of approximately 10 mmol/L variation of around 3 mmol/L was found across this haematocrit range in FreeStyle result compared with a change of 0.5 mmol/L in the YSI 2300 plasma result. Equivalently, at concentrations of around 18 mmol/L there was variation of approximately 5.5 mmol/L in result from the FreeStyle compared with a change of 0.7 mmol/L in the YSI 2300 plasma result. In the clinical study, there was evidence of haematocrit influence on the magnitude of the FreeStyle
bias at all concentrations studied. The minimum sample of blood required to obtain an accurate result was assessed to be consistent with the manufacturer’s claim of approximately 0.3 µl.

As it is difficult to obtain sufficient blood from the alternative site, glucose analysis using reference methods could not be performed. Results were therefore compared with those obtained from a capillary fingerstick. This comparison will be affected by a number of additional variables that are not present in the fingerstick comparison. The sample collection technique differs and there is some variation in sample time, although this was kept to a maximum of approximately five minutes. In addition, there may be a difference in the glucose result obtained compared with that from a fingerstick sample, caused by the delay in equilibration of glucose concentration between highly vascular tissues (such as the fingertips) and those with a more limited blood supply. This difference may be more apparent in situations when the glucose concentration is changing rapidly, such as after exercise, medication, stress, after a meal and in hypoglycaemic episodes. Published data highlights that this lag phase can vary from subject-to-subject and could also delay detection of hypoglycaemia\(^{(1,2)}\).

The 107 alternative site results obtained predominantly from the forearm, when compared with those obtained using the YSI 2300 plasma adjusted results or the plasma hexokinase methods from corresponding fingerstick samples gave a correlation of 0.98. There is a significant negative overall mean bias of –0.37 mmol/L, with standard error of 0.12 mmol/L \((t_{106} = -3.2, p = < 0.01)\). Relative to the FreeStyle fingerstick results the mean bias is 0.15 mmol/L with standard error 0.11 mmol/L. Actual differences between the alternative site and fingerstick sample results ranged from -3.4 mmol/L to +4.7 mmol/L. Imprecision from the FreeStyle alternative site results was slightly higher at 9 % compared with 5 % from the FreeStyle fingerstick samples, which may reflect the variables cited above. Error grid analysis of 104 alternative site results compared against the YSI 2300 plasma adjusted fingerstick results were clinically acceptable with 95 % within zone A and the remaining 5 % in zone B. The lower percentage of results in zone A compared with the fingerstick samples may be due to comparisons being made on separate samples from physiologically different sites.

In conclusion, the FreeStyle offers alternative site testing, providing clinically acceptable results. It was stated by 79 % of patients to be less painful than using conventional fingerstick sampling. This may be particularly beneficial to those who find testing painful, and could result in increased patient compliance.
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 \(^{(3, 4)}\). 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 encountered in using earlier systems \(^{(5 - 9)}\). In addition, the low volume of blood required minimises the operator-dependency involved in obtaining an adequate blood sample. Due to the possibility of obtaining misleading results, adversely affecting patient treatment, the Department of Health has issued a Hazard Notice \(^{(10)}\). A safety notice has also been issued by the Medical Devices Agency \(^{(11)}\) entitled *Extra-laboratory use of blood glucose meters and test strips: contra-indications, training and advice to the users*.

Recent development of glucose meters that are capable of performing glucose measurements on blood obtained from alternative sites such as the forearm, upper arm, thigh, calf or base of the thumb should help to improve compliance in patients required to monitor their blood glucose frequently. However, when performing blood glucose measurements from an alternative site the operator needs to be aware that there maybe a difference in the glucose result obtained compared with that from a fingerstick sample, caused by the delay in equilibration of glucose concentration between highly vascular tissues (such as the fingertips) and those with a more limited blood supply. This difference maybe more apparent in situations when the glucose concentration is changing rapidly, such as after exercise, medication, stress, after a meal and in hypoglycaemic episodes. Published data highlights that this lag phase can vary from subject-to-subject and could also delay detection of hypoglycaemia \(^{(1, 2)}\).

The FreeStyle is a small meter intended for home use by diabetics. The meter uses coulometric ‘non-wipe’ technology. The system is simple to operate, is pre-calibrated, and requires 0.3 µl of blood which is automatically drawn into the strip by a “capillary fill” mechanism. The measurement time is dependent on the glucose concentration, and approximately 15 seconds on average. The system is capable of storing 250 test results with a date/time stamp. It is also capable of calculating a 14 day average of the glucose results stored in the memory. This evaluation of the FreeStyle is part of a programme assessing the suitability of analytical systems for use in primary care and health screening locations \(^{(12 - 52)}\).

The FreeStyle was tested in a clinical setting on 210 diabetic patients by obtaining capillary blood samples from a fingerstick. Accuracy and imprecision, meter-to-meter and strip batch-to-batch variation were assessed on two meters and two batches of test strips using conventional fingerstick capillary blood samples. Alternative site testing was performed on 107 of the above 210 patients. Imprecision, volume dependency, linearity, the influence of haematocrit and the effects of incorrect programme number were also assessed in separate laboratory experiments.
The FreeStyle is a small battery powered meter for blood glucose estimations using non-wipe coulometric technology. Glucose measurements can be performed using capillary blood samples obtained in a conventional manner from a fingerstick, or from an alternative site such as a forearm, upper arm, hand, thigh or calf. The front of the meter has a test port, visual display panel and two buttons labelled M and C. The button labelled M is used to set up the meter, enter the memory mode and turn the meter on and off. The C button is used to change the date and time, code number and units of measurement; mark the control solution tests as well as review test results while in the memory mode. The test strip port for test strip insertion is located at the bottom of the meter, and will turn the meter on automatically when a strip is inserted into the port. The battery compartment is located at the back of the meter, and uses two 1.5-volt AAAA batteries capable of measuring 1000 tests. To preserve battery life the meter automatically turns itself off after 2 minutes if a strip has been inserted into the port. Specifications of the FreeStyle glucose system are given in Table 1.
### Table 1: Details of the FreeStyle glucose meter

| **Manufacturer** | TheraSense  
| 1360 South Loop Road  
| Alameda  
| CA 94502  
| USA |
| **Suppliers** | TheraSense  
| Centaur House  
| Ancells Business Park  
| Ancells Road  
| Fleet  
| Hampshire  
| GU51 2UJ |
| **List price** | Not yet available. |
| **Dimensions** | L 9.7 x W 5.15 x D 2.5 cm. |
| **Weight** | 79 g. |
| **Essential accessories** | Lancing unit, lancet, two AAAA batteries. |
| **Reagents** | Pack of 50 test strips (price not available), control materials (1 level x 3 ml, price not available). |

The FreeStyle meter employs a coulometric technology that relies on the measurement of charge rather than current and utilises the enzyme glucose dehydrogenase. In coulometry all the glucose in the sample is reacted, and the total charge generated by this reaction is measured. The manufacturer states that “as the system is measuring charge rather than current, there is no interference from reducing substances such as paracetamol, uric acid, vitamin C and haematocrit”. In the FreeStyle system the enzyme and the mediator are coated onto the working electrode. This serves to transfer electrons from the glucose in the sample to the working electrode according to the following reaction scheme:

1. \( \text{Glucose} + \text{enzyme (oxidised)} \rightarrow \text{gluconolactone} + \text{enzyme (reduced)} \)
2. \( \text{enzyme (red)} + \text{mediator (ox)} \rightarrow \text{enzyme (ox)} + \text{mediator (red)} \)
3. \( \text{mediator (red)} - e^{-} \rightarrow \text{mediator (red)} \)

Steps i and ii occur in the bulk solution, whilst step iii occurs at the working electrode surface.
Reagents are available in packs of 50 (25 x 2) test strips, are stored at room temperature, have a shelf life of up to 18 months, and can be used until the expiry date displayed on the container or three months after opening. The temperature range for determination of glucose is between 5 and 40 °C. Humidity should be 5 to 90% relative humidity. The meter recognises temperatures outside the specified range as inadmissible, and a thermometer symbol is displayed when the meter is switched on. Glucose measurement can still be performed, although the result obtained will flash, warning the operator to treat the result with caution. The result is stored in the memory with the thermometer symbol.

### Calibration

The system is pre-calibrated by the manufacturers. The operator has to manually enter batch-specific information for the test strips into the meter’s memory. This is achieved by inserting a test strip into the test port to switch the meter on. As soon as the meter turns on, four screen messages appear briefly on the display in sequence. The initial display is a system check in which all segments of the display messages are shown (ie 88.8 in the centre, and directly above messages mem, a blood drop, check sign and thermometer and the battery sign, mmol/L and mg/dL immediately below). The display then shows the date and time, followed by the code number and then the message “apply sample” with a symbol of a sample drop directly above. The manufacturer states that to ensure accurate results the code number must be compared with the numerical code between 1 and 50 printed on the glucose test strip vial. The code number can be entered or altered by pressing the C button. Once the correct code has been programmed into the meter’s memory, the message “code set” will appear on the screen followed by the message “apply sample”, followed by the symbol of a sample drop. If for any reason the operator needs to access the code number after the message “apply sample” has come up, this can be done by pushing and holding down the C button.

### Operation of the meter

#### Quality control

The performance of the FreeStyle system can be assessed using the FreeStyle Control Solution, which is an aqueous, viscosity adjusted quality control material. It is stored at room temperature, and has an open vial stability of 90 days from first use. The control solution results can be tagged so that they are stored separately from blood sample results.

Everytime a control measurement is performed, the test has to be marked with the check symbol (”✓”) so that the result can be distinguished from a blood glucose test in the meter’s memory. The meter is switched on by inserting a test strip into the test port with the name “FreeStyle” facing up. The meter switches on automatically and briefly displays all the segments, followed by the date and time. The display message changes to “Code 10” (example), followed by a symbol of a drop of blood at the top of the screen, and “apply sample” at the bottom of the screen. A drop of the control solution is touched
to the test strip along the dark-blue sample target area. When sufficient sample has been detected, the meter beeps and the display shows a row of moving arrows (>>>>>>>) on the bottom of the screen. The glucose result in the units selected is displayed in an average of 15 seconds. To tag the value as a control solution, the “C” button is pressed and held for two seconds, until the check symbol “✓” is displayed. At the end of the measurement procedure the glucose result obtained for the control solution is checked against the acceptance ranges printed on the reagent test strip vial. The used test strip is removed and discarded.

**Fingerstick capillary blood glucose measurement**

Having analysed the quality control materials, the system is ready for blood glucose measurement. Prior to performing a test, and to reduce difficulties in obtaining a blood sample by improving circulation, the manufacturer recommends washing hands with soap in warm water.

The meter is switched on by inserting a test strip into the test port with the name “FreeStyle” facing up. The meter switches on automatically and briefly displays all the segments, followed by the date and time. The display message changes to “Code 10” (example), followed by a symbol of a drop of blood at the top of the screen, and “apply sample” at the bottom of the screen to indicate that a blood sample can be applied to the test area. The meter will remain ready to perform a measurement for two minutes, before switching itself off.

A fingerstick sample of whole blood is obtained by lancing, and gently squeezing a finger. The drop of blood is touched to the test strip along the dark-blue sample target area. When sufficient sample has been detected, the meter beeps and the display show a row of moving arrows (>>>>>>>) on the bottom of the screen. The glucose result in the units selected is displayed in an average of 15 seconds and automatically stored in the memory. If the test fails to start, a second drop of blood can be applied to the target area within 60 seconds of the first drop. After the measurement has been completed and to conserve battery power the meter switches off automatically after two minutes. The test strip is removed from the meter and discarded.

**Alternative site blood glucose measurement**

The manufacturer’s suggestions for the most appropriate alternative sites for performing a blood glucose measurement are:

- the top or outer surface of the forearm
- the fleshy area located on the outside of the upper arm (not the shoulder)
- base of the thumb
- thigh
- calf
The manufacturer recommends that the site is washed with soap and warm water, ensuring that there is no cream or lotion where lancing will be carried out. To bring fresh blood to the surface of the skin, they also recommend that the test site be rubbed vigorously until it feels warm. This should help increase blood flow and also in obtaining an adequate sample of blood. Sites with obvious veins, or moles must be avoided to avoid excess blood. The test site is lanced using the FreeStyle-lancing device, with the clear cap. To help draw blood to the surface of the skin the lancing device is held to the test site and pressure gradually applied for several seconds. When sufficient blood sample is visible (size of a pinhead) the lancing device is removed carefully to avoid smearing the blood sample. The drop of blood is touched to the test strip along the dark-blue sample target area.

The analytical range quoted by the manufacturers for the determination of glucose concentration using the FreeStyle is 1.1 to 27.7 mmol/L. Error messages “LO” or “HI” indicates glucose concentrations outside this range.

The FreeStyle meter has a memory capacity for 250 blood glucose and control test results with time and date (if set). The meter is capable of recalling the last result, all the results, or calculating the average over 14 days. Having the meter switched off and pressing the “m” button accesses this function. The first memory screen is the 14 day average, followed by the most recent result. A value with the check symbol (“✓”) indicates a control result, excluded in the 14 day average. A value with the thermometer symbol indicates that the reading was taken outside the operating temperature range; these results are included in the 14-day average. Low and high glucose values are recorded as “LO” and “HI”.

Coded error messages (Er 1 to Er 5) are shown on the visual display panel to indicate procedural or meter errors. Error messages can be flagged for problems such as: not enough blood applied, strip error, incorrect test procedure; using a used strip; problem with meter; problem with strip; meter malfunction; calibration error; low battery power; replace battery; very high or low blood glucose.

The FreeStyle system requires some maintenance to keep the unit clean and ensure that blood has not accumulated. The manufacturer warns the operator to avoid getting dirt, dust, blood, control solution, water or any other liquid inside the meter through the test strip port and data port. The meter can be cleaned externally using a damp cloth with mild detergent and water, or 70 % isopropyl alcohol or 10 % bleach solution.
Methods and materials

The laboratory evaluation was carried out on instruments loaned by the manufacturer. The instruments were operated throughout according to the manufacturer's instructions. Reagents were obtained directly from the manufacturer.

Method comparison

Capillary blood

The performance of the glucose assay was evaluated by determining glucose concentration in capillary whole blood specimens from 210 patients. The results were compared with a routine laboratory method performed on a YSI 2300 analyser (YSI (UK) Ltd, Farnborough, Hants), and a method based on hexokinase/glucose-6-phosphate dehydrogenase following whole blood deproteinisation using perchloric acid.

To assess meter-to-meter and strip-to-strip variation, two FreeStyle meters and two different batches of test strips were tested. Capillary blood specimens from a finger were obtained using the Microtainer Safety Flow Lancet (Becton Dickinson, UK) from patients attending the diabetic out patient clinic at City Hospital, Birmingham. Samples were analysed immediately on two FreeStyle meters following the manufacturer'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 bloods were also collected for the hexokinase assay. Haematocrit measurements were performed on each patient sample in duplicate using a Microspin haematocrit centrifuge (Bayer Diagnostics, Basingstoke, UK) to assess the influence of haematocrit on the glucose value obtained.

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.

Plasma hexokinase assay

The same blood samples analysed on the YSI 2300 were used for the hexokinase assay. The microvette tubes were centrifuged within ten minutes of collection at 10,000 rpm for 10 minutes (Biofuge B, Heraeus Sepatech, Brentwood, Essex). The plasma was decanted using a pipette into 500 µl plastic tube, and the samples stored at 4 ºC and analysed the following day on a Cobas Bio using a Unimate 5 GLUC HK kit (Roche Diagnostics Ltd, Lewes, East Sussex).
Methods and materials

**Alternative site capillary blood**

Ethical approval was obtained for the study from the City Hospital NHS Trust Research Ethics Committee before recruiting subjects into the trial. Patients’ written consent was obtained prior to the alternative site testing. A total of 107 patient samples from an alternative site were used in the comparison of the glucose results against the FreeStyle fingerstick glucose results and results from the YSI2300 analyser. At the end of the procedure the patient was asked a simple question (see below) to assess the degree of pain from the alternative site in relation to that from a fingerstick.

The same two meters used for the fingerstick testing were used for alternative site testing. A fingerstick test was performed on each patient, followed no more than five minutes later by the alternative site test. A note was made of which particular meter (A or B) was used for each patient. To bring fresh blood to the surface of the skin, the test site was rubbed vigorously until it felt warm. This helps to increase blood flow. Sites with obvious veins or moles were avoided. The test site was lanced using the FreeStyle-lancing device, with the clear cap. To help draw blood to the surface of the skin the lancing device was held to the test site and pressure gradually applied for several seconds. When sufficient blood was visible (size of a pinhead) the lancing device was removed carefully to avoid smearing the blood sample. The drop of blood was touched to the test strip along the dark-blue sample target area.

**Question to determine the difference in pain felt between a finger prick and the alternative site**

Pain associated with using the FreeStyle on an alternative body site was:

- Much more painful than a finger prick  □ 1
- Slightly more painful than a finger prick □ 2
- Same as a finger prick □ 3
- Slightly less painful than a finger prick □ 4
- Much less painful than a finger prick □ 5
Plasma results comparison with the YSI 2300 using fingerstick blood

TheraSense state that the FreeStyle is calibrated to give “plasma-equivalent results” from whole blood capillary samples. In the following comparisons, the FreeStyle results were assumed to be plasma results and the YSI capillary whole blood results were adjusted to give plasma equivalent results, taking into account the haematocrit of the sample.

The correlation of the 210 FreeStyle fingerstick results against the YSI 2300 results (adjusted for plasma equivalence) from patients whole blood fingerstick capillary samples is illustrated in Figure 1 (correlation coefficient = 0.99). There was a significant negative overall bias in FreeStyle results, relative to the YSI 2300 plasma adjusted results, of –0.44 mmol/L (SEM = 0.04, t209 = -10.45, p < 0.001). As a percentage this represents an overall bias of –4%. The mean glucose levels obtained from the 210 patient specimens using the FreeStyle and the YSI 2300 are given in Table 2.

**Figure 1: Correlation from 210 patients’ fingerstick capillary blood samples using the FreeStyle (meter A) and the YSI 2300 plasma adjusted results**

*YSI 2300 whole blood glucose results adjusted to give plasma equivalent results*
Table 2: Summary statistics of the FreeStyle results (meter A) and the YSI 2300 adjusted results (n = 210)

<table>
<thead>
<tr>
<th></th>
<th>FreeStyle</th>
<th>YSI 2300</th>
<th>FreeStyle - YSI 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>11.56</td>
<td>11.99</td>
<td>-0.44</td>
</tr>
<tr>
<td>SD (mmol/L)</td>
<td>5.25</td>
<td>5.39</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Figure 2 shows the difference between the FreeStyle results and the adjusted YSI 2300 results plotted against the adjusted 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 a negative bias, which becomes increasingly negative with increasing concentrations.

Figure 2: Differences between the FreeStyle capillary fingerstick (meter A) and the YSI 2300 adjusted results plotted against the YSI 2300 adjusted results

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results

Figure 3 gives a histogram of the FreeStyle bias, and confirms the presence of an overall negative bias. The corresponding plot of percentage bias shows 80% of results from the FreeStyle having a negative bias relative to the YSI 2300. There were 11% of the results with an absolute bias of more than 10% and 1% with an absolute bias of more than 20%. A conventional regression analysis of FreeStyle and YSI results (Table 3) confirms the trend in bias illustrated in Figure 2, where bias becomes increasingly negative with increasing concentration. The slope of the regression line is 0.97, which is significantly lower than the ideal value of 1.0.
Figure 3: Histogram of the bias and percentage bias in capillary fingerstick results on the FreeStyle (meter A)

Table 3: Regression statistics of capillary fingerstick FreeStyle (meter A) results against the YSI 2300 adjusted results

<table>
<thead>
<tr>
<th></th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate (standard error)</td>
<td>-0.06 (0.10)</td>
<td>0.97 (0.01)</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.26 to 0.14</td>
<td>0.95 to 0.98</td>
</tr>
</tbody>
</table>

Dividing the data into groups according to the YSI adjusted result and calculating the bias of all FreeStyle results relative to the YSI 2300 adjusted results gives the data shown in Table 4. Figure 4 shows a corresponding group scatter plot of the FreeStyle bias at each of the ten levels of YSI result used in Table 4. The mean bias varies between –0.1 and –1.1 mmol/L or –6% to -1% on average for most concentration levels, and the standard deviation of bias suggests imprecision of about 5% on average. The about line standard deviation in the regression analysis reported in Table 3, which should also provide an estimate of imprecision is 0.58 mmol/L or approximately 5%. Both of these statistics are in broad agreement with standard deviations seen in Table 4.
Table 4: The mean bias of the FreeStyle capillary fingerstick results (meter A) relative to the YSI 2300 adjusted results

<table>
<thead>
<tr>
<th>YSI 2300 adjusted results (mmol/L)</th>
<th>Number of results</th>
<th>Mean FreeStyle bias mmol/L (% bias)</th>
<th>SD of bias mmol/L (% SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>13</td>
<td>-0.17 (-4 %)</td>
<td>0.26 (6 %)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>33</td>
<td>-0.42 (-6 %)</td>
<td>0.38 (6 %)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>27</td>
<td>-0.36 (-5 %)</td>
<td>0.33 (4 %)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>27</td>
<td>-0.34 (-3 %)</td>
<td>0.44 (4 %)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>31</td>
<td>-0.27 (-2 %)</td>
<td>0.56 (4 %)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>20</td>
<td>-0.46 (-3 %)</td>
<td>0.65 (5 %)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>15</td>
<td>-0.11 (-1 %)</td>
<td>0.48 (3 %)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>19</td>
<td>-0.61 (-3 %)</td>
<td>0.82 (5 %)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>13</td>
<td>-1.08 (-5%)</td>
<td>0.90 (4 %)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>12</td>
<td>-1.00 (-4%)</td>
<td>0.81 (3 %)</td>
</tr>
</tbody>
</table>

Figure 4: FreeStyle (meter A) capillary fingerstick biases relative to the YSI 2300 at ten concentration levels as measured on the YSI 2300

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results
Figure 5a illustrates a significant negative correlation between the haematocrit and the bias in the FreeStyle result ($r = -0.29$, $p < 0.001$). A regression analysis of bias on haematocrit suggests, on average, falls of 0.04 mmol/L per unit increase in haematocrit. This would imply a difference of approximately 1.1 mmol/L in the readings of a given glucose level at 25 and 55% haematocrits, the limits of the range studied in this evaluation.

**Figure 5a: Scatterplot of bias versus haematocrit for the FreeStyle capillary fingerstick results (meter A)**

Figure 5b has been created by calculating the bias of each FreeStyle 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 FreeStyle results at different concentrations. The effect would appear to be evident at all concentrations above 6 mmol/L. At concentrations above 6 mmol/L, the difference in FreeStyle glucose result is on average 1.2 mmol/L, over the haematocrit range 25 – 55%, the limits of the range studied in this evaluation.
Comparison of two FreeStyle meters

Two FreeStyle systems referred to as meters A and B were used in parallel throughout the patient phase of this evaluation. In general, they gave similar performance with the bias for meter B almost identical on average at –0.39 mmol/L with standard error 0.04. This bias is significantly different from zero ($t_{209} = -9.61$, $p < 0.001$). The overall percentage biases from the two systems relative to the YSI 2300 were -4 % for meter A (already reported) and -3 % for meter B. Regression statistics for the FreeStyle meter B were similar to those already reported for meter A. A similar table to Table 4 for the second system suggests a mean bias changing from –1.2 to –0.1 mmol/L with varying concentrations. The mean difference overall between the two FreeStyle systems was 0.05 mmol/L or, expressed as a percentage, < 1 %. Actual differences range from –1.3 mmol/L to +1.2 mmol/L.

The association between the bias on the FreeStyle system and the haematocrit was also evident with meter B. For meter B the correlation between bias and haematocrit was $r = -0.30$, $p < 0.001$. 
## Comparison of two batches of test strips

Two batches of test strips were used during the patient stage of the evaluation: *Batch 1* lot number 0121904, expiry 02/2003, programme number 16; *Batch 2* lot number 0125306, expiry 03/2003, programme number 16. There was significant variation in bias between the results from the two batches of test strips. The mean bias for the two batches of test strips was -0.69 mmol/L and -0.12 mmol/L (t\textsubscript{208} = -7.73, p = < 0.001) using meter A, and -0.59 mmol/L and -0.13 mmol/L (t\textsubscript{208} = -6.42, p = < 0.001) for meter B. Table 5 gives the mean bias and standard error of the mean for each batch of test strips using both meters. On both meters, *Batch 1* test strips gave a larger bias than *Batch 2* on average. There was no significant variation from meter to meter. For each batch and with both meters the bias is significantly different from zero. The difference in bias between the two batches was greater for meter A (0.57 and 0.46 mmol/L for meters A and B respectively).

Table 5: Capillary fingerstick mean bias using two FreeStyle meters and two different batches of 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 0121904)</td>
<td>118</td>
<td>-0.69 (0.05)</td>
<td>-0.59 (0.05)</td>
</tr>
<tr>
<td>Batch 2 (lot 0125306)</td>
<td>92</td>
<td>-0.12 (0.05)</td>
<td>-0.13 (0.05)</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 similar greater imprecision associated with each batch on each meter.

Table 6: Standard deviation of bias using two FreeStyle meters and two different batches of 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 0121904)</td>
<td>0.56</td>
<td>0.59</td>
</tr>
<tr>
<td>Batch 2 (lot 0125306)</td>
<td>0.51</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 7 gives regression statistics for each batch of test strips using meter A and YSI 2300 plasma adjusted results. Perfect agreement between YSI 2300 and the FreeStyle would result in a slope of 1 and an intercept of 0. For *Batch 1* the slope is significantly less then unity, indicating a concentration dependent bias. For *Batch 2* the slope is less than unity, but not significantly so.
Table 7: Capillary fingerstick regression statistics from two different batches of test strips (meter A)

<table>
<thead>
<tr>
<th></th>
<th>Intercept (SE)</th>
<th>Slope (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1 (lot 0121904)</td>
<td>-0.09 (0.11)</td>
<td>0.95 (0.01)</td>
</tr>
<tr>
<td>Batch 2 (lot 0125306)</td>
<td>0.03 (0.13)</td>
<td>0.99 (0.01)</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\(^{(53-56)}\). Clarke \textit{et al.\(^{(53)}\)} 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 4.3 to 11.0 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\(^{(55)}\) and specifies 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 meters A and B are shown in Figures 6a and 6b. Using these criteria on the 201 results within the range 0 to 25.0 mmol/L from the FreeStyle, 99.5 % of results from meter A fell into zone A, with the remainder in zone B, whilst 100 % of the results from meter B fell into zone A. No results for either meter fell within zones C - E. The FreeStyle is therefore classified as clinically acceptable with fingerstick capillary samples when compared against the YSI plasma equivalent results plotted on a plasma error grid.
Figure 6a: Clinical evaluation of the FreeStyle capillary fingerstick results (meter A) by error grid analysis

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results

Figure 6b: Clinical evaluation of the FreeStyle capillary fingerstick results (meter B) by error grid analysis

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results
Results comparison with the plasma hexokinase assay

The correlation of 203 results from patient specimens using the FreeStyle and the plasma hexokinase method (Figure 7) is similar to that between the FreeStyle and the YSI 2300 at 0.99. However, when the FreeStyle results on whole blood specimens (displayed as plasma equivalent results) were compared with the plasma hexokinase method, results were on average 8% lower using the FreeStyle than those by the hexokinase method. Previous in-house studies have demonstrated that the YSI 2300 results based on glucose oxidase to be approximately 5.6% below a hexokinase method on the same sample. The difference in the study of 5% is in line with this trend. Table 8 gives the mean glucose level obtained on the 203 patient specimens using the FreeStyle and the hexokinase method. There is a significant negative overall mean bias of $-1.0 \text{ mmol/L}$, with a standard error of 0.06 mmol/L ($t_{202} = -17.56, p < 0.001$).

Figure 7: Correlation obtained for glucose results from 203 patients' capillary fingerstick blood samples using the FreeStyle (meter A) and plasma hexokinase reference method

<table>
<thead>
<tr>
<th></th>
<th>FreeStyle</th>
<th>Hexokinase</th>
<th>FreeStyle - Hexokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (mmol/L)</strong></td>
<td>11.51</td>
<td>12.55</td>
<td>-1.04</td>
</tr>
<tr>
<td><strong>SD (mmol/L)</strong></td>
<td>5.20</td>
<td>5.74</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Table 9 shows regression statistics from a linear regression of FreeStyle result on plasma hexokinase result and gives strong evidence of a concentration dependent bias with the slope at 0.89, highly significantly different from unity. This is evident in Table 10, which gives the mean bias in the FreeStyle result relative to the hexokinase assay. The bias shows a marked decline with increasing concentrations and as a percentage, varies between –11 and -6 %. Correlation between bias and haematocrit is –0.17, slightly lower than when the YSI 2300 was used as the reference method.

**Table 9: Regression statistics of the FreeStyle capillary fingerstick results (meter A) against the plasma hexokinase glucose results**

<table>
<thead>
<tr>
<th>Estimate (standard error)</th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.30 (0.10)</td>
<td>0.89 (0.01)</td>
</tr>
</tbody>
</table>

**Table 10: The mean bias of the FreeStyle capillary fingerstick results (meter A) relative to the plasma hexokinase results**

<table>
<thead>
<tr>
<th>Hexokinase results (mmol/L)</th>
<th>Number of results</th>
<th>Mean FreeStyle bias mmol/L (% bias)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>11</td>
<td>-0.18 (-4 %)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>29</td>
<td>-0.60 (-9 %)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>27</td>
<td>-0.56 (-7 %)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>24</td>
<td>-0.60 (-6 %)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>27</td>
<td>-0.85 (-7 %)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>20</td>
<td>-1.23 (-9 %)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>19</td>
<td>-1.18 (-7 %)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>16</td>
<td>-1.41 (-8 %)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>12</td>
<td>-1.82 (-9 %)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>18</td>
<td>-2.61 (-11 %)</td>
</tr>
</tbody>
</table>

In error grid analysis, 99 and 100 % of results for meters A and B respectively fell within zone A, with the remaining 1 % in zone B in the case of meter A. Thus using the plasma hexokinase method as the reference method and a plasma error grid, the FreeStyle would be classified as clinically acceptable for use with capillary fingerstick samples.
Comparison of FreeStyle alternative site results with plasma adjusted fingerstick results from the YSI 2300

As it is difficult to obtain sufficient blood from the alternative site, glucose analysis using reference methods could not be performed. Results were therefore compared with those obtained from a capillary fingerstick. This comparison will be affected by a number of additional variables that are not present in the fingerstick comparison. The sample collection technique differs; and there is some variation in sample time, although this was kept to a maximum of approximately five minutes. In addition, there maybe a difference in the glucose result obtained compared with that from a fingerstick sample, caused by the delay in equilibration of glucose concentration between highly vascular tissues (such as the fingertips) and those with a limited blood supply. This difference maybe more apparent in situations when the glucose concentration is changing rapidly, such as after exercise, medication, stress, after a meal and in hypoglycaemic episodes. Published data highlight that this lag phase can vary from subject-to-subject and could delay detection of hypoglycaemia \(^{(1, 2, 57, 58)}\).

Results obtained on fingerstick capillary whole blood by the YSI 2300 were adjusted to give plasma equivalent results taking into account the haematocrit of the sample. The correlation of the 107 results from patient specimens using the FreeStyle alternative site and the fingerstick YSI 2300 plasma adjusted results is illustrated in Figure 8 (correlation coefficient = 0.98). The line shown in Figure 8 is the 45° line that would be seen if the FreeStyle and the YSI 2300 gave identical results. Results were on average 2.4 % lower using the FreeStyle alternative site sample than the YSI 2300 plasma adjusted fingerstick samples. Table 11 gives the mean glucose level obtained for the 107 patient specimens using the FreeStyle and the YSI 2300. There is a significant negative overall mean bias of –0.37 mmol/L, with standard error of 0.12 mmol/L (t\(_{106} = -3.2\), p = < 0.01).
Figure 8: Correlation obtained for glucose results from 107 alternative site capillary blood samples using the FreeStyle (meter A) and the YSI 2300 fingerstick plasma adjusted results

Table 11: Summary statistics for glucose results obtained from the FreeStyle alternative site (meter A) and the YSI 2300 fingerstick plasma adjusted results (n = 107)

<table>
<thead>
<tr>
<th></th>
<th>FreeStyle</th>
<th>YSI 2300</th>
<th>FreeStyle – YSI 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>13.48</td>
<td>13.85</td>
<td>-0.37</td>
</tr>
<tr>
<td>SD (mmol/L)</td>
<td>5.62</td>
<td>5.76</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Table 12: Regression statistics of FreeStyle (meter A) alternative site results against the YSI 2300 fingerstick plasma adjusted results

<table>
<thead>
<tr>
<th></th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate (standard error)</td>
<td>0.27 (0.30)</td>
<td>0.95 (0.02)</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.03 to 0.87</td>
<td>0.91 to 0.99</td>
</tr>
</tbody>
</table>

The slope of the regression line of 0.95, reported in Table 12, indicates concentration dependence in the bias. Dividing the data into groups according to the YSI adjusted result, and calculating the bias of all FreeStyle results relative to the YSI 2300 adjusted results gives the data shown in Table 13.
The mean bias varies between +0.1 and –1.3 mmol/L or +1 to –5 % on average for most concentration levels for which a reasonable number of results were available, and the standard deviation of bias suggests imprecision between 4 and 13 % on average. The about line standard deviation in the regression analysis reported in Table 12, which should also provide an estimate of imprecision is 1.2 mmol/L or approximately 9 %, considerably greater than that seen with the fingerstick FreeStyle results. Both of these statistics are in broad agreement with standard deviations seen in Table 13.

### Table 13: The mean bias of the FreeStyle alternative site results (meter A) relative to the YSI 2300 adjusted results

<table>
<thead>
<tr>
<th>YSI 2300 adjusted results (mmol/L)</th>
<th>Number of results</th>
<th>Mean FreeStyle bias mmol/L (% bias)</th>
<th>SD of bias mmol/L (% SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>12</td>
<td>-0.14 (-3 %)</td>
<td>0.63 (13 %)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>11</td>
<td>-0.07 (-1 %)</td>
<td>0.71 (11 %)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>6</td>
<td>-0.65 (-7 %)</td>
<td>1.04 (12 %)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>11</td>
<td>0.03 (&lt;1 %)</td>
<td>0.93 (8 %)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>13</td>
<td>-0.42 (-3 %)</td>
<td>0.60 (4 %)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>16</td>
<td>0.12 (1 %)</td>
<td>1.33 (9 %)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>16</td>
<td>-0.55 (-3 %)</td>
<td>1.42 (8 %)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>12</td>
<td>-0.71 (-4 %)</td>
<td>1.14 (6 %)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>4</td>
<td>-1.21 (-6 %)</td>
<td>1.56 (7 %)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>6</td>
<td>-1.34 (-5 %)</td>
<td>2.50 (10 %)</td>
</tr>
</tbody>
</table>
Error grid analysis

Error grid analysis of the 104 alternative site results within the range 0 to 25 mmol/L are shown in Figure 9, and gave results that are clinically acceptable with 95% within zone A with the remaining 5% in zone B. This lower percentage of results in zone A compared with the fingerstick samples may be due to comparisons being made on samples from two different physiologically sites (1, 2, 57, 58). Differences might also arise from variation in the sample collection technique or variation in sample time.

Figure 9: Clinical evaluation of the FreeStyle alternative site results relative to the YSI 2300 plasma adjusted results by error grid analysis

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results
Comparison of FreeStyle alternative site results with FreeStyle fingerstick results

Comparing the 107 fingerstick FreeStyle results from meter A with the FreeStyle alternative site results, using 2 meters, shows a relatively consistent difference in results, on average 0.15 mmol/L with standard error 0.11 mmol/L. Actual differences between the alternative site and fingerstick sample results ranged from -3.4 mmol/L to +4.7 mmol/L. Using the fingerstick sample as reference, the slope of the regression line is 0.98 indicating a similar discrepancy at most concentrations. This is evident from Figure 10, which shows the pairs of results for each patient and the superimposed line of equality. The correlation between the two sets of results is \( r = 0.98 \).

**Figure 10:** Correlation obtained using the FreeStyle (meter A) for glucose results from 107 fingerstick capillary blood samples and the corresponding alternative site samples

![Figure 10](image)

**Table 14:** Regression statistics of FreeStyle (meter A) alternative site results against the FreeStyle fingerstick results

<table>
<thead>
<tr>
<th></th>
<th>Intercept (mmol/L)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estimate (standard error)</strong></td>
<td>0.39 (0.28)</td>
<td>0.98 (0.02)</td>
</tr>
<tr>
<td><strong>95% confidence interval</strong></td>
<td>-0.17 to 0.95</td>
<td>0.94 to 1.02</td>
</tr>
</tbody>
</table>
Dividing the data into groups according to the FreeStyle fingerstick result and calculating the bias of all FreeStyle alternative site results relative to the FreeStyle Fingerstick results gives the data shown in Table 15. The mean bias varies between -0.1 and +0.4 mmol/L or -1 to +8 % on average for most concentration levels for which a reasonable number of results were available. The standard deviation of bias suggests imprecision between 5 and 12 % on average. The about line standard deviation in the regression analysis reported in Table 14, which should also provide an estimate of imprecision is 1.13 mmol/L or approximately 9 %. Both of these statistics are in broad agreement with standard deviations seen in Table 15.

Table 15: The mean bias of the FreeStyle alternative site results relative to the FreeStyle fingerstick (meter A) results

<table>
<thead>
<tr>
<th>FreeStyle fingerstick results (mmol/L)</th>
<th>Number of results</th>
<th>Mean FreeStyle alternative site bias mmol/L (% bias)</th>
<th>SD of bias mmol/L (% SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>14</td>
<td>0.34 (8 %)</td>
<td>0.48 (11 %)</td>
</tr>
<tr>
<td>5 - 7</td>
<td>8</td>
<td>0.41 (7 %)</td>
<td>0.57 (10 %)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>6</td>
<td>0.23 (3 %)</td>
<td>0.98 (12 %)</td>
</tr>
<tr>
<td>9 - 11</td>
<td>9</td>
<td>0.20 (2 %)</td>
<td>1.28 (12 %)</td>
</tr>
<tr>
<td>11 - 13</td>
<td>8</td>
<td>-0.01 (negligible)</td>
<td>0.94 (8 %)</td>
</tr>
<tr>
<td>13 - 15</td>
<td>14</td>
<td>0.07 (negligible)</td>
<td>0.88 (6 %)</td>
</tr>
<tr>
<td>15 - 17</td>
<td>18</td>
<td>-0.21 (-1 %)</td>
<td>1.70 (11 %)</td>
</tr>
<tr>
<td>17 - 19</td>
<td>14</td>
<td>-0.04 (negligible)</td>
<td>0.86 (5 %)</td>
</tr>
<tr>
<td>19 - 21</td>
<td>8</td>
<td>0.28 (1 %)</td>
<td>1.00 (5 %)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>8</td>
<td>-0.13 (negligible)</td>
<td>2.01 (9 %)</td>
</tr>
</tbody>
</table>
Alternative site and fingerstick pain comparisons

All the patients who had volunteered for alternative site testing were asked to complete a questionnaire relating to the degree of pain felt between the fingerprick and the FreeStyle alternative measurement procedure (page 14).

For the alternative site testing, the FreeStyle-lancing device, which has five depth settings, was used. Initial experience highlighted that the deeper lancet penetration setting was required to avoid repeat lancing. Of the 107 patients (100 %) who replied to the question 79 % stated that the FreeStyle was less painful than a fingerstick, 10 % indicated that it was just as painful and the remaining 11 % that it was more painful. The latter group who stated it was more painful indicated that they did not find the lancing of the alternative site more painful, but found the pressure applied to the test site to increase the blood flow to the skin painful.
Imprecision

The imprecision of the FreeStyle was determined on four 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 to a \( pO_2 \) equivalent to that of capillary blood, and aliquoted into 20 x 0.5 ml plastic tubes. An aliquot was selected randomly and used once only for glucose measurements on four FreeStyle meters and the YSI 2300. Blood glucose measurements were performed on the YSI 2300 throughout 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 four meters. Results are summarised in Table 16. At plasma equivalent glucose concentrations of 3.9, 11.4*, 21.1* and 26.2* mmol/L (* samples spiked with glucose), coefficients of variation (CVs) of 3.1, 2.7, 1.9 and 2.6 % were obtained respectively. These CVs represent the variation between results performed on randomly selected FreeStyle meters. They do not include variations that might occur between batches of test strips. Total error (%), which includes imprecision and bias as outlined in Table 16, was estimated at 9.9, 9.9, 9.6 and 9.9 % relative to the YSI plasma adjusted results at the four concentration levels quoted.

The recommendations \((59, 60)\) 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 achieved by an experienced laboratory worker with the FreeStyle system met these criteria at all four concentrations studied here.
Table 16: Imprecision of the FreeStyle at four different glucose concentrations using YSI plasma equivalent reference concentrations

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>*YSI 2300 plasma results (mmol/L)</td>
<td>3.9</td>
<td>11.4</td>
<td>21.1</td>
<td>26.2</td>
</tr>
<tr>
<td><strong>FreeStyle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean (mmol/L)</strong></td>
<td>3.6</td>
<td>10.3</td>
<td>19.1</td>
<td>23.7</td>
</tr>
<tr>
<td><strong>SD₃ (mmol/L)</strong></td>
<td>0.12</td>
<td>0.31</td>
<td>0.40</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>CV₃ (%)</strong></td>
<td>3.1</td>
<td>2.7</td>
<td>1.9</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>SD₄ (mmol/L)</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>CV₄ (%)</strong></td>
<td>3.1</td>
<td>2.7</td>
<td>1.9</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Total error (%)</strong></td>
<td>9.9</td>
<td>9.9</td>
<td>9.6</td>
<td>9.9</td>
</tr>
</tbody>
</table>

**Notes:**

* whole blood results adjusted to be plasma equivalent
SD₃ = replicate standard deviation (n = 20 on each of 4 meters)
CV₃ = replicate coefficient of variation
SD₄ = meter to meter standard deviation (4 meters)
CV₄ = total (duplicate and meter) coefficient of variation
Total variance = (SD₃)² + (SD₄)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 (e.g., diabetic pregnant females with low haematocrit, producing falsely high glucose values).

The manufacturer states that “glucose results on the FreeStyle are not affected for haematocrit values in the range 0 to 60%”. 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 FreeStyle meter. 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 FreeStyle in quadruplicate using meter A and strip batch number 0125306. The haematocrit estimations were also performed in triplicate.

YSI whole blood results were adjusted using haematocrit levels to give plasma equivalent values. Results are shown in Figures 11a and 11b. At the lower glucose level of approximately 10 mmol/L variation with haematocrit is much greater than that of the reference method over most of the haematocrit range of 0 to 60% quoted by the manufacturer. There is a change in result of 3.4 mmol/L across this range with the FreeStyle compared with a change of 0.5 mmol/L in YSI plasma adjusted result. At the higher glucose concentration studied of approximately 18 mmol/L there is a variation of approximately 5.5 mmol/L in results from the FreeStyle across the recommended haematocrit range 0 to 60% compared with 0.7 mmol/L in YSI 2300 plasma adjusted result. The clinical study with capillary samples also highlighted a statistically significant negative correlation between bias and haematocrit of $r = -0.29$. 

MDA Evaluation Report: TheraSense FreeStyle blood glucose meter
Figure 11a: The effect of haematocrit on the measurement of glucose concentrations using the FreeStyle at level 1

Figure 11b: The effect of haematocrit on the measurement of glucose concentrations using the FreeStyle at level 2
Volume dependency

The volume of blood required for accurate measurement of glucose with the FreeStyle 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 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 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 µl, by applying the blood sample to the reagent test strip using a calibrated positive displacement pipette.

The experiments were performed on samples of blood obtained from ‘normal’ individuals with haematocrit in the range 30 to 60%.

The manufacturer states that the minimum volume of blood required for glucose measurement is 0.3 µl, which is consistent with results shown in Figures 12a and 12b. A sample volume of 0.1 µl was insufficient and no results were obtained. At the higher glucose concentration, sample volumes of 0.2 µl were not detected, whilst at level 1 results that were comparable were obtained on two occasions.
Figure 12a: The volume of blood required for accurate glucose measurement at glucose level 1

Figure 12b: The volume of blood required for accurate glucose measurement at glucose level 2
Linearity assessment

The linearity of the glucose results obtained using the FreeStyle 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\(_2\) 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 FreeStyle.

YSI whole blood results were adjusted using haematocrit levels to give plasma equivalent values. The results are displayed in Figure 13. The line superimposed on the graph is a 45° line of equality. The manufacturer’s quoted analytical range for glucose measurements is 1.1 to 27.7 mmol/L. Results above 27.7 mmol/L are flagged with a warning message “HI”. Glucose concentrations of approximately 32, 43, 57, 65 and 70 mmol/L gave an error message on all occasions.

**Figure 13: Linearity assessment**

* YSI 2300 whole blood glucose results adjusted to give plasma equivalent results
Influence of incorrect programme number

The influence of having an incorrect code in the FreeStyle meter’s memory was assessed by deliberately calibrating the meter with the incorrect programme number for the test strips in use. The experiment was performed using test strips batch number 0125306, expiry date 03/2003, code 16. Separate calibration test strips are produced with each new batch of test strips. Incorrect programme number 1, 6, 11, 20, 27, 32, 39, 45 and 50 were used to determine the effect on the glucose result obtained if the operator failed to recalibrate the system.

Results are shown in Figure 14 where it is evident that use of the incorrect programme number could produce an error of up to 4 mmol/L in reported glucose at a concentration of 8.8 mmol/L.

Figure 14: Influence of incorrect programme number on the glucose results
Analytical systems for use by non-laboratory operators should have a minimal number of complex manoeuvres, to reduce the risk of obtaining incorrect results. The newer blood glucose systems have either reduced the number of complex manoeuvres or have integrated automated procedures to make the systems easier to use, thus reducing the potential errors in glucose measurements. A major operator dependent step inherent to all analytical systems using capillary whole blood is in obtaining an adequate volume of free flowing blood. Introducing systems that require small sample volumes of 2 to 3 µl has reduced this potential error. The FreeStyle has made further progress by introducing a system that also allows glucose measurement from an alternative site. The system can also be used in the conventional manner to perform capillary glucose measurements on a pre-lanced finger.

Correct storage conditions as stated by the manufacturer should be maintained for all dry reagent strips. Additional operator dependent steps identified for the FreeStyle were:

- that the meter is calibrated for the correct batch number of test strips. Our experiment showed (Figure 14) that variation in results of up to 4.4 mmol/L in reported glucose at a concentration of 8.8 mmol/L could be obtained according to the programme number selected.

- ensuring that before performing a glucose measurement on a blood sample obtained from an alternative site that the area is rubbed vigorously to improve the circulation. This not only improves the chances of obtaining sufficient blood, but also minimises the discrepancy in the result relative to the fingerstick. This procedure was rigorously adhered to throughout this evaluation when testing from the alternative site (predominantly the forearm). Published data \(^{(1)}\) also highlight the importance of this procedure.

- the manufacturer states that the “peritoneal dialysis solutions containing icodextrin cause overestimation of the glucose result”. Galactose above 13 mg/dL and Maltose above 20 mg/dL may also produce elevated glucose results.

- FreeStyle alternative site testing may not be suitable for all types of patients. Patients with very hairy arms may find it easier to use the upper arm or the base of the thumb.

- ensuring that results from the control solution are tagged, so that the result is not entered into the blood glucose result memory and therefore used in the 14 day average calculated by the meter.
the FreeStyle test strip has dual sample target areas to make it easier to apply the sample from either the right or left hand side. Care must be taken to ensure that the blood sample is applied to one edge of the test strip only.

performing the recommended maintenance procedures and ensuring that the meter is kept clean
Instructions

Instructions for use of the meter included a 64-page fully illustrated user’s manual. The instructions are clear, concise and understandable for non-technical users. However, the instructions for obtaining a blood sample from an alternative site or fingerstick, and the diagram illustrating the alternative site testing positions do not appear in sequential order and may be confusing to non-professional operators. A “getting started guide” is provided in the form of a double-sided fully illustrated A3 sheet in colour. An instruction sheet is also provided in the reagent pack, and gives details for carrying out glucose measurements on the meter.
The major advantage of the FreeStyle blood glucose system is that it is capable of performing blood glucose measurements on samples obtained from a conventional fingerstick and from an alternative site such as the forearm, upper arm, palm, thigh or calf. The FreeStyle is a small meter intended for home use by diabetics. The system is easy to use, and is pre-calibrated to give “plasma-calibrated results”. It requires 0.3 µl of blood, which is drawn into the strip by a “capillary fill” mechanism. The measurement time is dependent on the glucose concentration, and is approximately 15 seconds on average. It has few operator dependent steps, and does not require complex maintenance as the blood is applied to the test strip externally to the body of the instrument and does not come into contact with the meter’s internal components.

Glucose estimations carried out using dry reagent strips, read visually or with a reflectance meter, may 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. The FreeStyle system helps minimise such factors.

Although the FreeStyle uses whole blood capillary samples, the results are calibrated to give “plasma-equivalent values”. The FreeStyle results, when compared with those obtained using the YSI 2300 plasma equivalent results or the plasma hexokinase method gave a correlation of 0.99 in both cases.

There was an overall bias of –0.44 mmol/L relative to YSI 2300 plasma adjusted results, and –1.0 mmol/L relative to the plasma hexokinase results. In both cases the bias becomes increasingly negative as concentration increases, reaching –1.0 and –2.6 mmol/L at the higher concentration studied. There was significant batch-to-batch variation in mean bias of test strips for the FreeStyle system. Meter-to-meter variation in bias was not significant and conclusions about the performance of the FreeStyle system were on the whole consistent from one meter to the other.

Error grid analysis of the YSI 2300 plasma equivalent results would classify the system as clinically acceptable, with 99.5 % of the results for meter A and 100 % for meter B falling in zone A. Relative to the hexokinase method, both meters A and B gave clinically acceptable results, with 99 and 100 % of the results in zone A.

In the clinical study imprecision was estimated to be approximately 5 % on average across the concentration range studied when compared with YSI 2300 plasma equivalent results. In the laboratory study, imprecision (CV) of the results at glucose concentrations of 3.9, 11.4, 21.1 and 26.2 mmol/L was 3.1, 2.7, 1.9 and 2.6 % respectively. The criterion for acceptable imprecision is no more than 5% and the FreeStyle meets this criterion at all concentration levels. The total error of 9.9, 9.9, 9.6 and 9.9 % respectively meets the criterion for acceptable total error of no more than 10 % at all four levels.
Haematocrit within the manufacturer’s quoted ranges of 0 to 60% was found to have a more noticeable effect on the glucose results obtained with the FreeStyle than on those obtained with the YSI 2300. This would appear to be evident at all concentrations studied.

The 107 alternative site capillary whole blood results collected predominantly from the forearm, when compared with those obtained using the YSI 2300 plasma adjusted results or the plasma hexokinase methods from corresponding fingerstick samples gave a correlation of 0.98. There is a significant negative overall mean bias of −0.37 mmol/L, with standard error of 0.12 mmol/L ($t_{106} = -3.2, p = < 0.01$). The bias varies with concentration from mean level of −0.14 mmol/L at concentrations of 0 to 5 mmol/L, down to −1.3 mmol/L at concentrations above 21 mmol/L. Relative to the FreeStyle fingerstick results the mean bias is 0.15 mmol/L with standard error 0.11 mmol/L. Actual differences between the alternative site and fingerstick sample results ranged from −3.4 mmol/L to +4.7 mmol/L. Imprecision from the FreeStyle alternative site was higher at 9 % compared with 5 % from the FreeStyle fingerstick samples.

Error grid analysis of 104 alternative site results gave 95 % within zone A, with the remaining 5 % in zone B which is clinically acceptable. This lower percentage of results in zone A compared with the fingerstick sample may be due to comparisons being made on samples from two physiologically different sites. Published data have highlighted that there is a time lag in the glucose concentration result obtained in the forearm compared to the fingerstick. This difference is more apparent in situations when the glucose concentration is changing rapidly, such as after exercise, medication, stress, after a meal and during hypoglycaemic episodes. This lag phase can vary from subject-to-subject and could also delay detection of hypoglycaemia (1, 2, 57, 58). In subjects who are prone to hypoglycaemic unawareness, it is recommended that glucose measurements be performed from a fingerstick sample.

In conclusion, the FreeStyle meter using the plasma calibrated test strips was easy to operate, and the use of non-wipe technology, very small sample volume and automatic timing sequence contributed to relatively few operator dependent steps. Error grid analysis classified the system as clinically acceptable. The FreeStyle also provides the operator with the option for glucose measurement from an alternative site, which in our study 79 % of patients found to be less painful than using conventional fingerstick sampling. This may be particularly beneficial to those who find testing painful, and could result in increased patient compliance.
The authors wish to thank: the representatives of TheraSense 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

Dr. Gary Thorpe
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Dear Dr. Thorpe:

Thank you for the time spent in the evaluation of the TheraSense FreeStyle Blood Glucose Meter and for the opportunity to review the evaluation report.

The FreeStyle system was developed utilizing differentiating electrochemical technology to bring improved product performance to glucose monitoring patients. The use of a coulometric, low redox potential detection technique offers not only minimization of interference from many endogenous constituents but also allows for the extremely low sample size of 1/3 μl of blood used in the test. The very low sample volume requirement allows patients to test not only with a conventional finger stick, but also from an alternate site such as the forearm, upper arm, palm, thigh or calf. By using the very low sample volume and with the option of alternate site testing, the vast majority of patients, as confirmed in your study report little or no pain using the product.

The Evaluation Report notes a more noticeable effect from hematocrit on the glucose results obtained with the FreeStyle versus that obtained with the YSI 2300. This effect is in general similar to that observed in studies conducted at TheraSense. Although there is a small effect of hematocrit, we have determined the differences from YSI in the range of 0-60% to be not clinically significant. In a study performed at TheraSense using samples with hematocrits of 0-60% at 10% intervals we evaluated at glucose concentrations of 2.8, 8.3, 13.9 and 22.2 mmol/L on three different lots of strips. In this study it was observed that 98.3% of the FreeStyle readings were in the A region of the Clarke Error Grid and 1.7% in the B region. It is from these results that we have concluded that this variation is not clinically significant over the 0-60% hematocrit range.

The results obtained with the YSI correlation, bias determination, imprecision, Error Grid analysis and correlation to the hexokinase methodology are consistent with internal and other external clinical testing of the FreeStyle system.

Once again we thank you for your time in the evaluation of the product and for the opportunity to review the resulting evaluation report.

Sincerely,

[Signature]

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