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# The effect of adjusting the stroke value of jet dispenser on the performance of glucose biosensor with gold-plated electrode

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**Abstract**

This study investigates the effect of dispensing glucose oxidase enzyme onto the reaction zone of a golden electrode via a jetting dispenser. Each droplet of enzyme solution dispensed onto the reaction zone of golden electrode weighs exactly the same at around 0.4 mg. This study shows that the spring and needle assembly can be controlled by adjusting the stroke value of the stroke adjustment knob to generate different jet dispensing effects, thus affecting the change of droplets of glucose oxidase enzyme solution within the reaction zone of the golden electrode. This study performs experiments using three stroke values, which are 0.8, 1.2, and 1.6 mm. The experimental results show that adjusting the stroke value to change the droplet of the dispensing liquid will significantly affect the accuracy of the test strip reading value. When the stroke value is adjusted to 1.5 mm, the standard deviation of the test strip is 3.8 mg/dL and the coefficient of variation is 4.1%–6.1%. The study suggested that adjusting the stroke value can stabilize the dispensed droplet to increase the stability of test strip reading, improving the accuracy of the test strip reading. This adjustment method can also be applied to the process of other biochemical sensors.

**Keywords**

Biosensor, glucose oxidase, golden electrode, jetting dispenser, reaction

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## Introduction

A blood glucose meter is an electrochemical analysis tool, which is easy to operate, and only needs a very small amount of blood to obtain accurate blood sugar data quickly. Diabetic patients can monitor their physical conditions at home.[1,2] The most important issues in this technology are the process control of the jet dispensing driver on the medication, the establishment of the model and the control equation, and the computer simulation of the experiment. The dispensing simulation determines the nozzle variation and dynamic performance and then verifies the precision of the jet dispenser.[3] Glucose oxidase (GOx) is sprayed on the reaction zone of the test strip to enable the transfer of electrons between the oxidase and the electrode surface directly to the biosensor,[4–6] where the characteristics of the droplets from the nozzle depend on the spray dispensing technology. Parameters change the stability of the droplet ejection.[7,8] Spray the enzyme on reaction zone. The enzyme should be sprayed onto test strip electrode uniformly and accurately.[3] Therefore, researchers have tested various test strip substrates, such as graphene, carbon paste, and gold-plated test strip electrode.[4,9–13] Gold has the highest electric conductivity, and a stable manufacturing process. This study investigates the use of Nordson

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ASYMTEK Axiom™ X-1010 SMT Dispenser, a non-contact jetting dispenser, which has the advantages of fast dispensing, stable droplet size, and good repeatability, and it does not pollute the substrate surface. Adjusting the scale of the Stroke Adjustment Knob changes the stroke of the striker on the base, thus adjusting the injection effect. The stroke affects the glucose oxidase solution sprayed from the nozzle on the surface of the gold-plated printed circuit board (PCB) test strip, affecting the dimension of the reaction zone droplets, resulting in deviation of the number of droplets of the enzyme solution. The deviation of test strip should follow the ISO 15197 specification.[14] This study is to investigate the effect of the Stroke Adjustment Knob on the accuracy of blood glucose test strips and to determine the best dispensing parameters to improve accuracy.

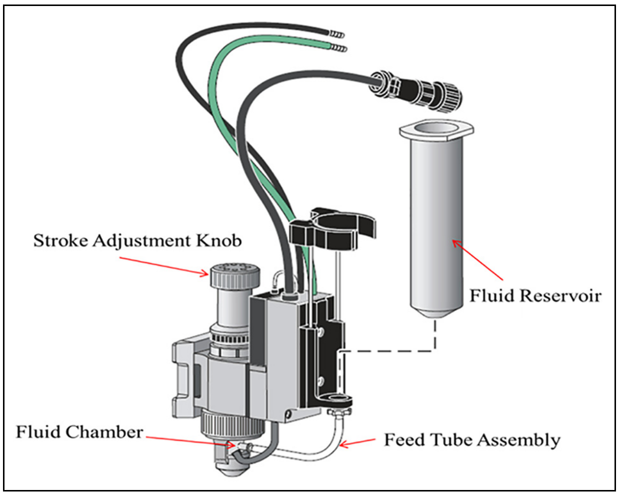


**Figure 1.** DJ-9000 dispensing valve (Nordson ASYMTEK DJ-9000, 2014, P18).[17]

## Materials and methods

### *Preparation of GOx*

GOx (260 U/mg, with formulation (1) and chemical equations (2) and (3)) was purchased from Amano Enzyme Inc. (Nagoya, Japan).[15] GOx was mixed uniformly with various reagents. The reagents were prepared by using de-ionized water, which is generated treating water with different filters to remove impurities. The use of de-ionized water can reduce the impact of impurities in water on the characteristics of the reagents.

Activity can be calculated using the following formula

$$\text{Glucose Oxidase activity } (U/mg) = \frac{(A5 - A2) - (Ab5 - Ab2)}{3} \times \frac{1}{12.88 \times 1/2} \times 3.2 \times \frac{Dm}{0.1} \times 1.339 \quad (1)$$

where "3" is the reaction time; "12.88" is the millimolar extinction coefficient of quinoneimine dye; "½" is the factor based on the fact that two moles of $H_2O_2$ form one mole of quinoneimine dye; "3.2" is the final volume of the reaction mixture; "0.1" is the volume of enzyme solution; Dm is the dilution multiple of enzyme solution; "1.339" is the exchange coefficient to the original Amano method using O-dianisidine which is found to be a carcinogenic substance; and (A5 – A2) is exactly at 2 and 5 min after the addition of enzyme solution (H), and measure the absorbances of the reaction mixture at 500 nm. As a blank, pipette 0.1 M phosphate buffer (E) into another cuvette (d = 10 nm) instead of the enzyme solution (H) and take the same procedure described above (Ab2 and Ab5)

$$\beta\text{-D-Glucose} + O_2 + \xrightarrow{\text{glucose oxidase}} \text{D-Glucono-}\delta\text{-lactone} + H_2O_2 \quad (2)$$

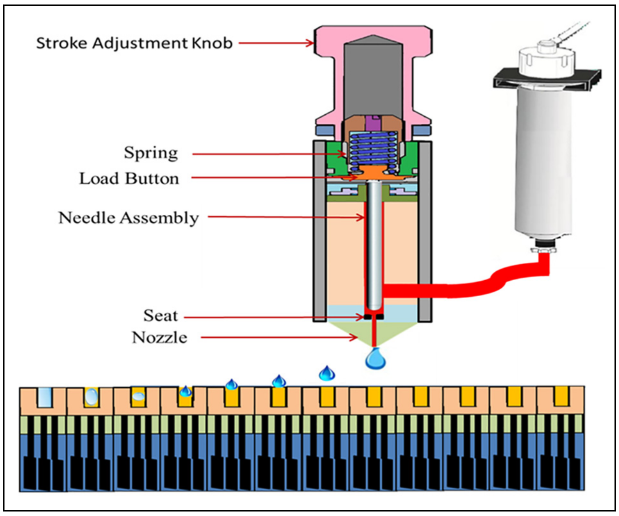


**Figure 2.** Jet dispensing using DJ-9000 dispensing valve.

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O \quad (3)$$

### *Volume of GOx droplet dispensed by the jet dispenser*

This study investigates the use of a Nordson ASYMTEK X-1020 dispense jet to dispense glucose oxidase solution onto the reaction zone of test strip made of gold. This dispense jet adopts a non-contact approach for high-efficiency dispensing.[16] This study investigated the dispensing on biological test strips. Figure 1 shows the jet nozzle of the DJ-9000 dispensing valve, which can dispense a droplet with diameter as small as 200 μm, and can dispense a 300-μm wide feature at the edge of the silicon crystal score.[17] It can separate the solution into fine spots, and rapidly and continuously form 50 μm droplets from the nozzle for dispensing.

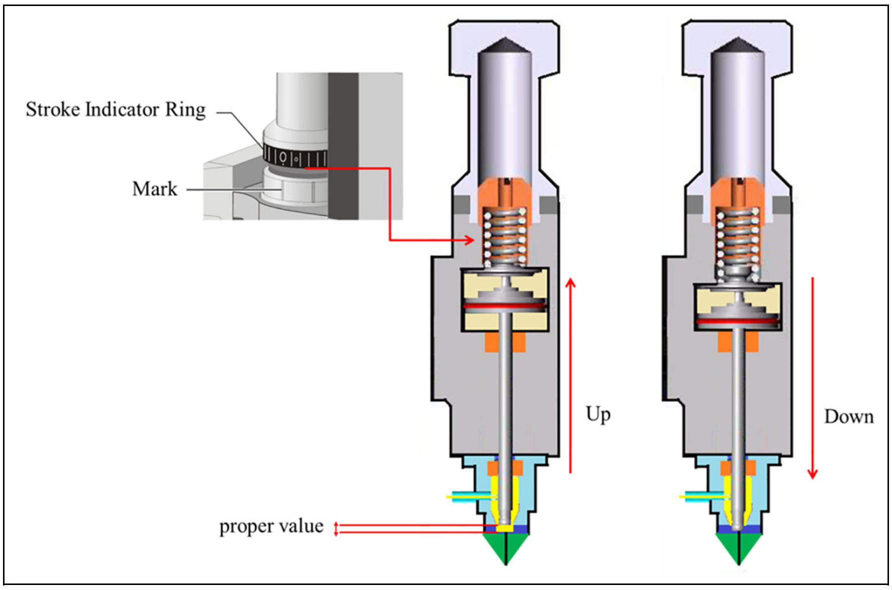


**Figure 3.** Stroke indicator ring on the stroke adjustment knob of the jet dispenser. Turning the stroke indicator ring can adjust the tightness of the internal spring, which affects the movement of the needle assembly. When the needle assembly moves up, droplet is dispensed out from the dispensing nozzle. When the needle assembly moves down, the dispensing of droplet is stopped.

### *Working principle of the jet dispenser*

Figure 2 shows the jet dispensing chamber when the oxidase solution in the tank is pressurized by fluidic gas and forced into the chamber via a plastic tube, during which the seat in the jet dispensing chamber is closed. The needle assembly is lifted when the compressed air drives the solenoid, thus opening the seat. The stroke adjustment knob controls the spring to press the load button when the needle assembly moves up and down, thus affecting the stroke speed of the up and down needle assembly movement. The oxidase solution then flows past the seat, and is discharged from the nozzle in the form of droplet, thus dispensing the glucose oxidase solution precisely onto the reaction zone of the test strip. However, the needle assembly pushes the seat to stop the jetting in the fluid channel when the air is depleted in the solenoid.

As shown in Figure 3, the lower part of Stroke Adjustment Knob has a stroke indicator ring to control the distance of the up and down stroke of the needle assembly. Rotating the stroke indicator ring counterclockwise increases the numerical stroke value, slows the speed of needle assembly closing position, and increases the stroke distance, thus leading to a higher dispensed volume. Conversely, clockwise rotation of the stroke indicator reduces the numerical stroke value and increases the closing speed of the needle assembly.

### *Test strip structure*

As shown in Figures 4 and 5, each test strip is composed a reaction zone with dimension of 2 mm × 2 mm. It is required to dispense sufficient amount of glucose oxidase on the reaction zone of the test strip. Each set of testing substrate contains 24 gold-plated testing strips and the dimension of each test strip after cutting is 5 mm (W) × 20 mm (L).

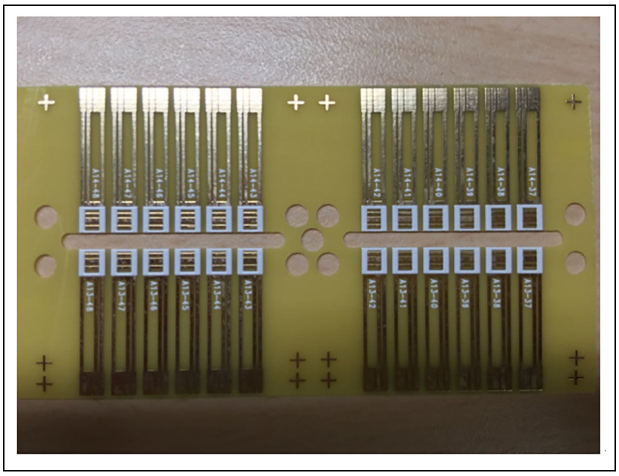

**Figure 4.** Blood glucose test strip substrate.

### *Blood glucose measurement*

The FDA-approved Joinsoon EON L blood glucose meter was used to measure test strips for the experiment. Three standard solutions with different glucose concentrations (58, 105, and 234 mg/dL) were used to carry out test strip measurement, and the standard solutions were calibrated using the YSI 2300 analyzer. Through cyclic voltammetric electrochemical reaction as shown in Figure 6, the test strip data measured by the blood glucose meter can be converted to blood glucose results. When glucose concentration is lower than

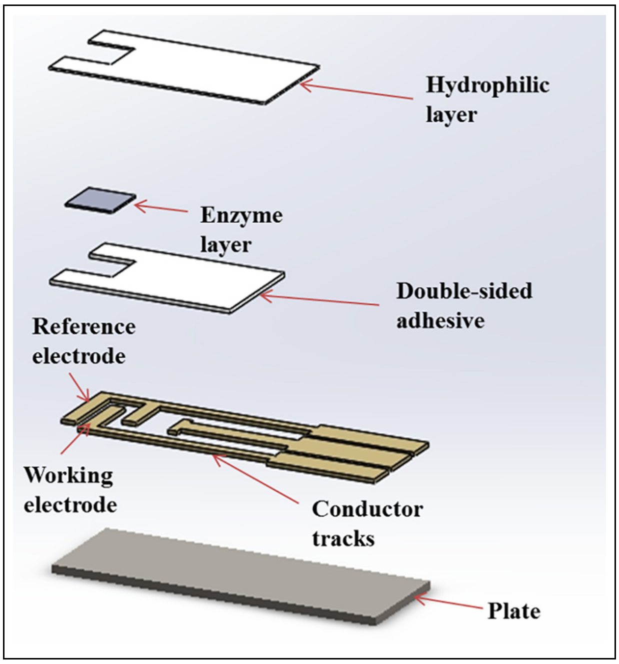


**Figure 5.** Schematic diagram illustrating the structure of a golden electrode test strip.

100 mg/dL, standard deviation (STD) of measurement results should be within the range of ±15 mg/dL. When glucose concentration is higher than 100 mg/dL, coefficient of variation (CV) of measurement results should be in the range of ±15%. In addition, the error of bias analysis for the measurement results should also be in the range of ±15%. Moreover, linear regression analysis was conducted for the measurement results; the closer the correlation coefficient ($R^2$) is to 1, the more accurate the test strip measurement is. Clarke Error Grid analysis was adopted to assess the significance of test strip measurement for clinical application:

A = No effect on clinical behavior.
B = altered clinical action: little or no impact on clinical outcomes.
C = altered clinical action: may have impact on clinical outcomes.
D = altered clinical action: may cause significant medical risks.
E = altered clinical action: will cause dangerous consequences.

## Results and discussion

### *STD and CV*

Blood glucose reading test strips were tested with glucose standard solutions at concentrations of 58 mg/dL (low), 105 mg/dL (medium), and high (234 mg/dL). Tables 1 and 2 show the test results at the three stroke scales values 1, 1.5, and 2. As shown in Table 1, the average blood glucose reading with stroke scale value reading 1 in the low-glucose concentration standard solution was 44.8 mg/dL, which is obviously low. However, the average blood glucose reading at stroke scale value 1.5 was 55.9 mg/dL, which is the closest to the standard solution reading of 58 mg/dL. The average blood glucose reading at stroke scale value 2 was 50.6 mg/dL, which is also lower than the standard solution reading.

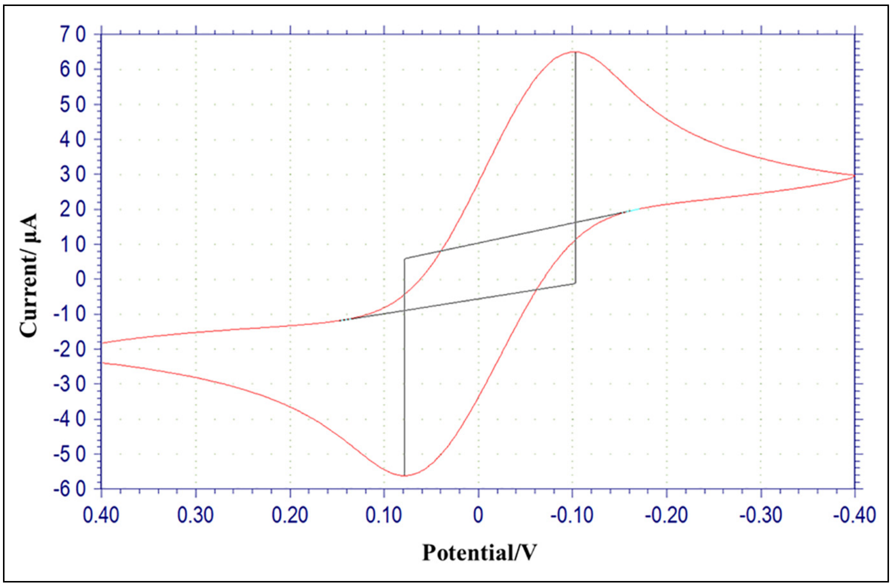


**Figure 6.** The oxidative and reductive peaks of test strip containing standard solution measured by the CHI1221 cyclic voltammetric electrochemical analyzer under the potential range of ±0.4 V. Similarly, blood glucose meter also uses cyclic voltammetry to convert the electrochemical reaction occurred on the test strip containing standard solution to blood glucose reading.

**Table 1.** Readings of test strips of glucose standard solutions of different concentrations at the three stroke scale values.

| YSI reading (mg/dL) | 1 (0.8 mm) (mg/dL) | 1.5 (1.2 mm) (mg/dL) | 2 (1.6 mm) (mg/dL) |
|---|---|---|---|
| 58 | 44.8 | 55.9 | 50.6 |
| 105 | 87.8 | 106.3 | 92.1 |
| 234 | 187.1 | 230.9 | 208.6 |

YSI: YSI 2300 STAT Plus™.

**Table 2.** Readings of STD and CV of test strips of glucose standard solutions of different concentrations at the three stroke scale values.

| YSI reading (mg/dL) | 1 (0.8 mm) STD/CV | 1.5 (1.2 mm) STD/CV | 2 (1.6 mm) STD/CV |
|---|---|---|---|
| 58 | 9.4/– | 3.8/– | 8.5/– |
| 105 | –/13.1 | –/6.1 | –/14.7 |
| 234 | –/15.9 | –/4.1 | –/12.0 |

STD: standard deviation; CV: coefficient of variation.

As shown in Table 1, the average blood glucose reading at stroke scale value 1 in the medium glucose concentration standard solution was 87.8 mg/dL, which is obviously lower than the medium-concentration standard solution reading of 105 mg/dL. However, the average blood glucose reading at stroke scale value 1.5 was 106.3 mg/dL, which is the closest to the standard solution reading. The average blood glucose reading of 92.1 mg/dL at stroke scale value 2 was much lower than the reading of the medium standard solution. As shown in Table 1, the average blood glucose reading at stroke scale value of 1 in the high glucose concentration standard solution was 187.1 mg/dL, which is obviously lower than the standard solution reading of 234 mg/dL. However, the average blood glucose reading at scale value 1.5 was 230.9 mg/dL, which was closest to the reading of the standard solution. The average blood glucose reading of 208.6 mg/dL at stroke scale value of 2 was far lower than the reading of the high-concentration standard solution.

These experimental results reveal that among the three tested stroke scale values, the stroke scale value of 1.5 gave the closest reading to the actual standard concentration standard solution in all three glucose standard solutions. According to the requirement of ISO 15197:2013, the STD should be within $\pm$15 mg/dL when the glucose concentration is lower than 100 mg/dL, and the CV should be at $\pm$15% when the glucose concentration is higher than 100 mg/dL. Therefore, only STD value is considered for the low-concentration glucose standard solution at 58 mg/dL, which is lower than 100 mg/dL, while only the CV value is considered for the glucose standard solutions at concentrations of 105 and 234 mg/dL, both of which are higher than 100 mg/dL. Therefore, the reading for each concentration in Table 2 only shows the STD or CV as appropriate.

As shown in Table 2, the STDs of test strips with low-concentration glucose standard solution and the stroke scale values of 1 and 2 were 9.4 and 8.5 mg/dL, respectively. Both values were obviously higher than the STD of stroke value at scale 1.5, which was 3.8 mg/dL.

The CV value of the test strip using the medium-concentration glucose standard solution and stroke scale value 1.5 was 6.1%, which is obviously lower than the CV values of the test strips with stroke scale values 1 and 2, which were 13.1% and 14.7%, respectively. The CV value of the test strip with the high-concentration glucose standard solution at 234 mg/dL and stroke scale value 1.5 was 4.1%, which is obviously lower than the CV values of test strips with stroke values 1 and 2, which were 15.9% and 12%, respectively. The results in Tables 1 and 2 show the importance of the proper stroke value on the STD and CV.

**Table 3.** Ranges of test strip readings with test results of glucose concentration standard solution readings $<$ 100 mg/dL among jet-dispensed test strips with stroke values of scale 1, 1.5, and 2.

| Stroke indicator ring | Within 5 mg/dL | Within 10 mg/dL | Within 15 mg/dL |
|---|---|---|---|
| 1 (0.8 mm) | 12/50 (24%) | 22/50 (44%) | 32/50 (64%) |
| 1.5 (1.2 mm) | 40/50 (80%) | 50/50 (100%) | 50/50 (100%) |
| 2 (1.6 mm) | 19/50 (38%) | 28/50 (56%) | 39/50 (78%) |

## *Accuracy of the control solution tests*

The test strip statistics were further analyzed by measuring to compare the reading accuracy of glucose standard solutions with concentration $<$ 100 and $\geqslant$ 100 mg/dL at stroke scale values 1, 1.5, and 2, with 100 readings taken for each concentration range and stroke scale value. Tables 3 and 4 summarize the analytical results for concentrations $<$ 100 and $\geqslant$ 100 mg/dL, respectively.

Table 3 shows the test strip readings for the $<$ 100 mg/dL solutions. With the stroke scale value of 1, 24% of the test results were within 5 mg/dL, 44% were within 10 mg/dL, and 64% of the test results were within 15 mg/dL. With the stroke scale value 1.5, 80% of the test results were within 5 mg/dL and 100% of the test results were within 10 mg/dL. However, at the stroke scale value of 2, 38% of the test results were within 5 mg/dL, 56% of the test results were within 10 mg/dL, and 78% of the test results were within 15 mg/dL. Thus, these findings show that the stroke value of scale 1.5 leads to extremely accurate reading.

Table 4 shows the test results of reading accuracy of glucose concentration standard solutions at

**Table 4.** Ranges of test strip readings with test results of glucose concentration standard solution readings ⩾ 100 mg/dL among jet-dispensed test strips with stroke values of scale 1, 1.5, and 2.

| Stroke indicator ring | Within 5% | Within 10% | Within 15% |
|---|---|---|---|
| 1 (0.8 mm) | 17/100 (17%) | 29/100 (29%) | 41/100 (41%) |
| 1.5 (1.2 mm) | 61/100 (61%) | 98/100 (98%) | 100/100 (100%) |
| 2 (1.6 mm) | 28/100 (28%) | 41/100 (41%) | 50/100 (50%) |

**Table 5.** Linear regression.

| Stroke indicator ring | N | Slope | Y-intercept | Correlation coefficient ($R^2$) |
|---|---|---|---|---|
| 1 (0.8 mm) | 150 | 0.7963 | 0.9781 | 0.9759 |
| 1.5 (1.2 mm) | 150 | 0.9898 | 0.1316 | 0.998 |
| 2 (1.6 mm) | 150 | 0.8874 | –0.9349 | 0.9876 |

concentrations ⩾ 100 mg/dL, where 100 test strip readings were taken for each of the three stroke values for the analysis. At a stroke value of 1, 17% of the test results of test strip in the zone of 5%, 29% of those for the zone of 10%, and 41% of the test results of the zone of 15% fell within this range. However, with the stroke value of 1.5, 61% of the test results of the zone of 5%, 98% of those of the zone of 10%, and 100% of the test results of the zone of 15% fell within this range. However, with the stroke value is at scale 2, 28% of the test results in the zone of 5%, 41% of those of the zone of 10%, and 50% of the test results of the zone of 15% fell within this range. These findings also reveal the excellent stability when the stroke value is at scale 1.5.

### *Linear regression analysis*

Table 5 shows the linear regression analysis of experimental statistics with stroke scale values 1, 1.5, and 2. Figures 7–9 show the respective correlation graphs of each scale value, based on 150 measurement results, and the test results of the YSI comparison standard solutions. Figure 7 show the comparison results for the 150 measurement results with the stroke scale value of 1, which show that the points are rather decentralized, especially within the zone of high-concentration glucose standard solution. The graph has a slope of 0.7963, Y-intercept of 0.9781, and correlation coefficient ($R^2$) of 0.9759. Figure 8 shows the comparison results with the stroke value at 1.5, showing rather centralized points of low-, medium- and high-concentration glucose standard solutions. The graph has a slope of 0.9898, Y-intercept of 0.1316, and correlation coefficient ($R^2$) of 0.998. Figure 9 shows the comparison results with the

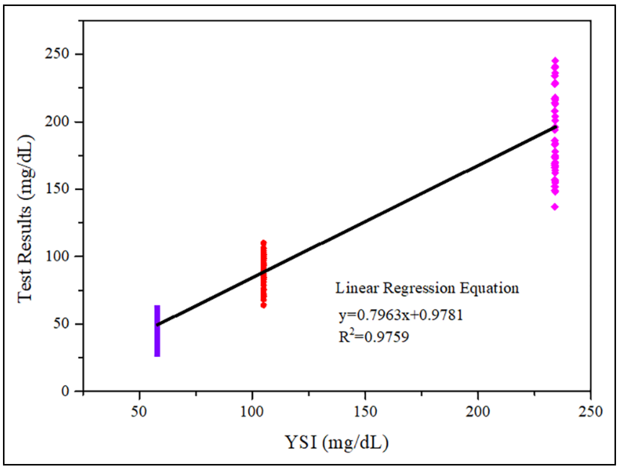


**Figure 7.** Correlation graph of measured results corresponding to the comparison between test strip with stroke value of scale 1 and YSI standard solution.

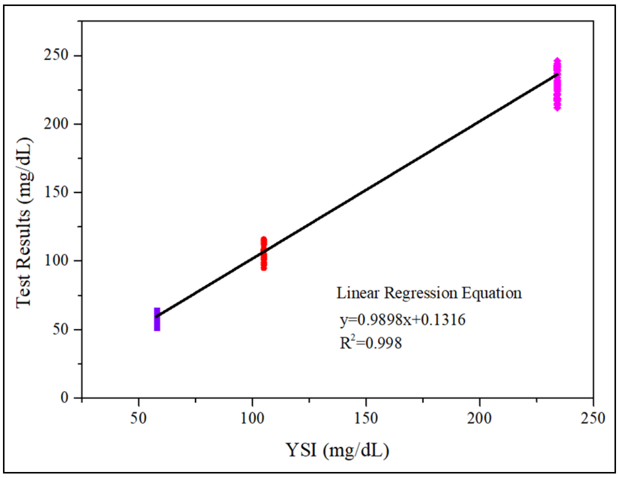


**Figure 8.** Correlation graph of measured results corresponding to the comparison between test strip with stroke value of scale 1.5 and YSI standard solution.

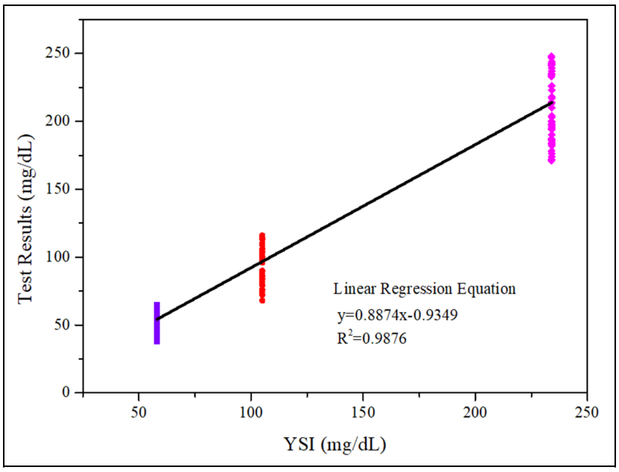


**Figure 9.** Correlation graph of measured results corresponding to the comparison between test strip with stroke value of scale 2 and YSI standard solution.

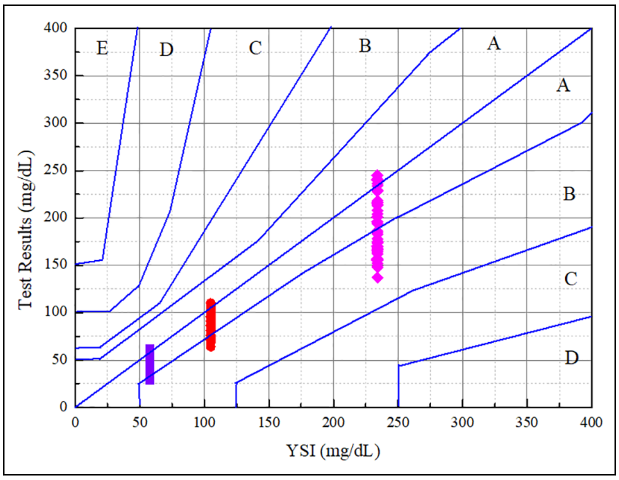


**Figure 10.** Diagram of Clarke Error Grid analysis of dispensed oxidase test strip with stroke scale value 1.

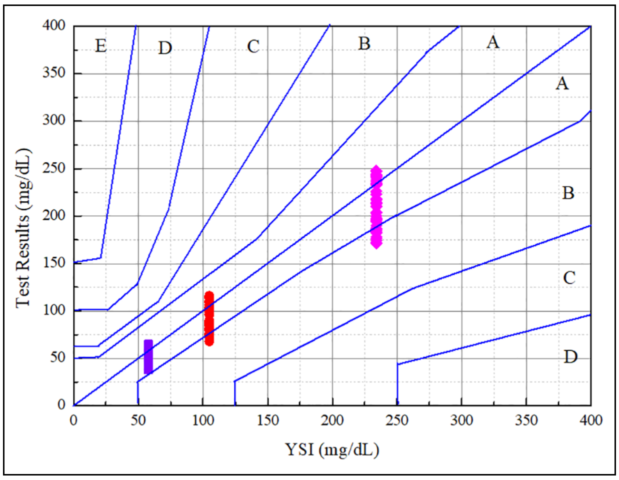


**Figure 12.** Diagram of Clarke Error Grid analysis of dispensed oxidase test strip with stroke scale value 2.

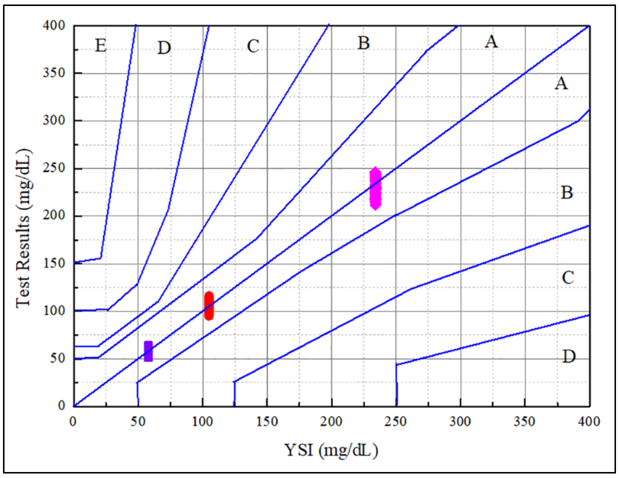


**Figure 11.** Diagram of Clarke Error Grid analysis of dispensed oxidase test strip with stroke scale value 1.5.

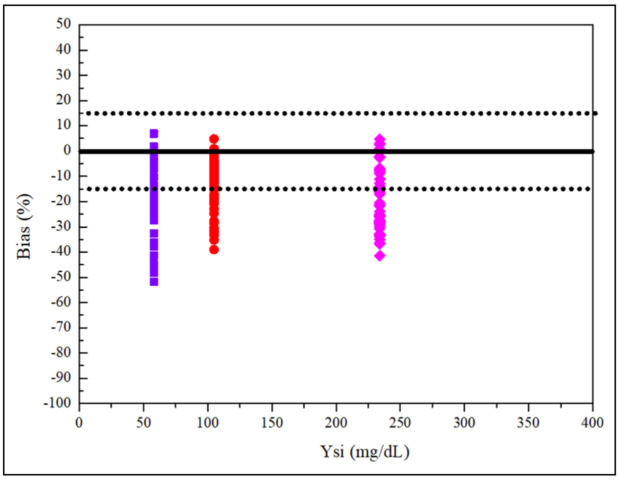


**Figure 13.** Bias analysis of test strip with dispensed oxidase solution and stroke scale value 1.

stroke scale value of 2, showing rather decentralized points of test statistics. The graph has a slope of 0.8874, Y-intercept of −0.9349, and correlation coefficient ($R^2$) of 0.9876.

### Clarke Error Grid analysis

Figures 10–12 in this section show the diagrams of the Clarke Error Grid analysis of measured results corresponding to the comparison between test strips of stroke scale values 1, 1.5, and 2, respectively, with the YSI standard solution. The results in Figure 10 for the stroke scale value 1 show that all 150 measured readings of test strips fell within either zones A or B. The results in Figure 11 for the stroke value at scale 1.5 show that all 150 measured readings of test strips fell within zone A. The results in Figure 12 for the stroke value at scale 2 show that the 150 measured readings of test strips fell within either zones A or B, with many obviously deviated from zone A. The comparison results clearly indicate that the stroke value scale of 1.5 had obviously superior readings to the other two stroke values.

### Bias analysis

Figures 13–15 show the bias analysis diagrams based on the measured results from comparing test strips of stroke scale values 1, 1.5, and 2 with YSI measurement standard solution, respectively.

The results from stroke scale value 1, shown in Figure 13, show that all 150 points of test strip statistics were obviously beyond ±15%, with some of them even reaching the range of −50%. However, the results from stroke value scale 1.5, shown in Figure 14, show that all 150 points of test strip statistics on the bias analysis

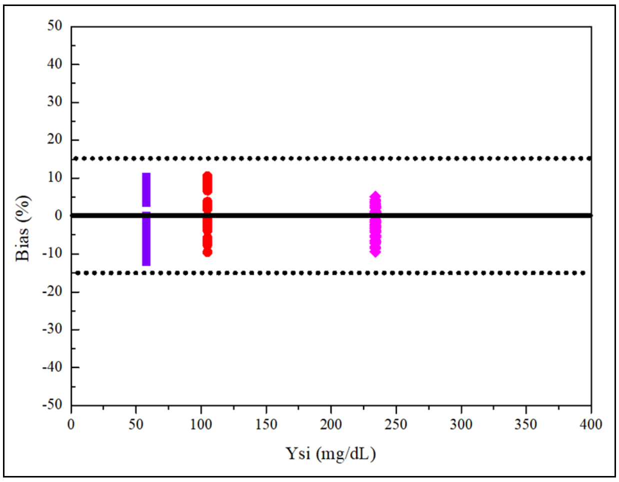


**Figure 14.** Bias analysis of test strip with dispensed oxidase solution and stroke scale value 1.5.

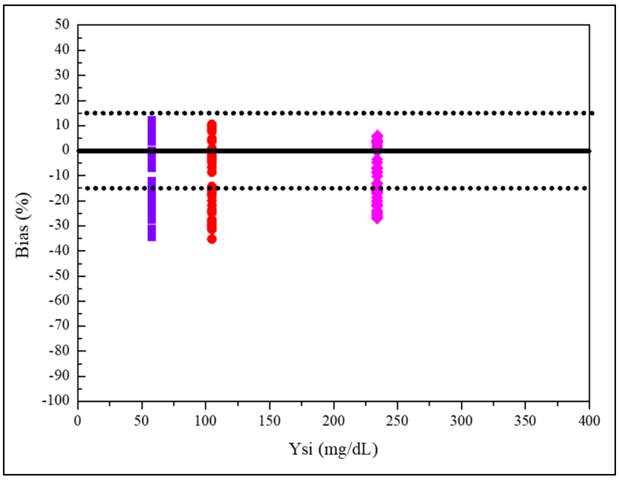


**Figure 15.** Bias analysis of test strip with dispensed oxidase solution and stroke scale value 2.

diagram were within the range ±15%. The result with stroke value at scale 2, shown in Figure 15, show that all 150 points of test strip statistics on the bias analysis diagram were beyond the range of ±15%, with some reaching –35%.

After verification using three standard solutions, the jet dispensing test strips with different stroke values were investigated to identify the causes of differences in test results. Analytical results show the needle assembly would slide and hit the seat during the dispensing action, resulting in the jet dispensing at the nozzle. The stroke adjustment knob can be turned to adjust the stroke value. The spring modifies the sliding of the needle assembly depending on the stroke value, thus changing the jet dispensing effect. The high pressure inside the tank can force the glucose oxidase solution into the fluid chamber, and the stroke adjustment knob can be adjusted to change the action of the needle assembly hitting on the seat, thus altering the jet dispensing effect.

Setting the stroke scale value to 1 tightens the spring such that it cannot provide sufficient force for the needle assembly to hit on the seat. The closing position is reached too quickly, resulting in inconsistent sizes of the droplets generated by the jet dispensing at the nozzle. Consequently, the glucose oxidase solution droplets may be unstable, affecting the coverage of electrode in the reaction zone of blood glucose test strip and reducing the accuracy of reading of the blood glucose test strip.

With the stroke scale value set to 1.5, the spring controlled by the stroke adjustment knob provides sufficient force to allow the needle assembly to hit on the seat to generate a stable volume of droplet from the nozzle. The droplets of the jet-dispensed glucose oxidase solution completely cover the electrode in the reaction zone of the blood glucose test strip. The glucose oxidase solution in the reaction zone of blood glucose test strip is in the shape of an arched bead, leading to accurate blood glucose test strip readings. With the stroke scale value set to 2, the spring controlled by the stroke adjustment knob is too loose to control the needle assembly to hit on the seat, and the closing position is reached too slowly. The excessive pressure from the jet dispenser results in rather unstable volumes of jet-dispensed droplets. Droplets of glucose oxidase solution dispensed onto the reaction zone of the blood glucose test strip may be scattered into the non-reaction zone and so do not have the arched bead shape, reducing the accuracy of blood glucose test strip readings. Thus, the stroke adjustment knob changes the stroke of the needle assembly and varies the dispensing effect, thus affecting the shape of droplets of glucose oxidase solution dispensed onto the reaction zone of test strip. Stable droplets completely cover the golden electrode within the reaction zone of test strip, optimizing the golden electrode test strip readings.

## Conclusion

Results of verification by three levels of standard solutions show that although the Nordson ASYMTEK X-1020 dispenser has fairly accurate jet dispensing volume, the weight deviation of droplets of jetting dispensing cannot easily be controlled to within ±1.5%. The most stable dispensing volume can be achieved by turning the stroke adjustment knob adjust the stroke value and thus control the spring, which varies the closing stroke speed of needle assembly hitting on the seat. This study identifies the significant impact of the stroke value on the test strip. The stroke scale value of 1.5 generates droplets within the reaction zone of test strip in the shape of an arched bead, which can surely cover the reaction zone of the golden electrode of a blood glucose test strip. The measured statistics show that the stroke scale value of 1.5 yields rather low STD and CV, and no overflow or lack of dispensed solution occurs within the reaction zone of test strip. Thus, the proper adjustment of the stroke adjustment knob can indeed improve the accuracy and stability of reading of golden electrode blood glucose test strips.

## Declaration of conflicting interests

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