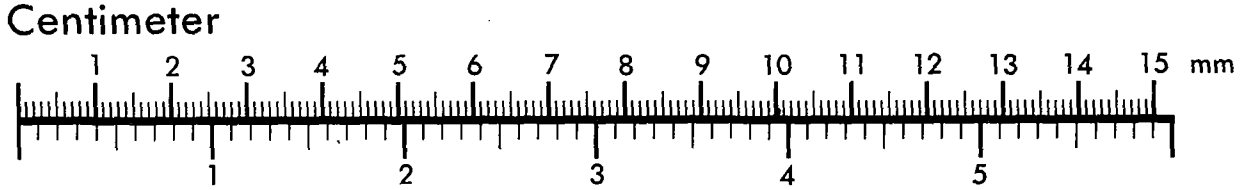
MS303-1980



K864224-A

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
8757 Georgia Avenue
Silver Spring MD 20910

JAN 5 1987

Abbott Laboratories
Attn: Keith Minter
1921 Hurd Street
P.O. Box 152020
Irving, TX 75015

Re: K864224/A
Abbott Spectrum Glucose Reagent
Dated: December 1, 1986
Received: December 3, 1986

Dear Mr. Minter:

We have reviewed your Section 510(k) notification of intent to market the above device and we have determined the device to be substantially equivalent to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments. You may, therefore, market your device subject to the general controls provisions of the Federal Food, Drug, and Cosmetic Act (Act) until such time as your device has been classified under Section 513. At that time, if your device is classified into either class II (Performance Standards) or class III (Premarket Approval), it would be subject to additional controls. Please note: This action does not affect any obligation you might have under the Radiation Control for Health and Safety Act of 1968, or other Federal Laws or regulations.

General controls presently include regulations on annual registration, listing of devices, good manufacturing practice, labeling, and the misbranding and adulteration provisions of the Act. In the future, the scope of general controls may be broadened to include additional regulations.

All regulations and information on meetings of the device advisory committees, their recommendations, and the final decisions of the Food and Drug Administration (FDA) will be published in the Federal Register. We suggest you subscribe to this publication so you can convey your views to FDA if you desire and be notified of any additional requirements imposed on your device. Subscriptions may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Such information also may be reviewed in the Dockets Management Branch (HFA-305), Food and Drug Administration, Room 4-62, 5600 Fishers Lane, Rockville, Maryland 20857.

This letter does not in any way denote official FDA approval of your device or its labeling. Any representation that creates an impression of official approval of this device because of compliance with the premarket notification regulations is misleading and constitutes misbranding. If you desire advice on the labeling for your device or other information on your responsibilities under the Act, please contact the Office of Compliance, Division of Compliance Operations (HFZ-320), 8757 Georgia Avenue, Silver Spring, Maryland 20910.

Sincerely yours,

Jerome A. Donlon M.D. Ph.D.

Jerome A. Donlon, M.D., Ph.D.
Director, Division of Clinical Laboratory Devices
Center for Devices and Radiological Health

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Memorandum

Date 12/17/86

From REVIEWER(S) - NAME(S) Broden Staples Jr.

Subject 510(k) NOTIFICATION K864224/A

To THE RECORD

It is my recommendation that the subject 510(k) Notification:

✓ (A) Is substantially equivalent to marketed devices.

_____ (B) Requires premarket approval. NOT substantially equivalent to marketed devices.

_____ (C) Requires more data.

_____ (D) Is an incomplete submission. (See Submission Sheet).

(b)(4) Confidential and Proprietary Information

[redacted]

The submitter requests: Class Code w/Panel:

✓ No Confidentiality 75CGA

_____ Confidentiality for 90 days

_____ Continued Confidentiality exceeding 90 days

REVIEW: K. Ann (BRANCH CHIEF) 12-30-86 (DATE)

FINAL REVIEW: TMT 1/5/87 S. K. Wedlake for JGAD (DIVISION DIRECTOR) 1/2/87 (DATE)

2

DEPARTMENT OF HEALTH AND HUMAN SERVICES — Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
8757 Georgia Avenue
Silver Spring, MD 20910

DECEMBER 3, 1986

ABBOTT LABORATORIES
ATTN: KEITH MINTER
1921 HURD STREET
P.O. BOX 152020
IRVING, TX 75015

D.C. Number : K864224
Received : 12-03-86
Product : ABBOTT SPECTRUM GLUCOSE REAGENT

The additional information you have submitted has been received.

-- We will notify you when the processing of your submission has been completed or if any additional information is required. You are required to wait ninety (90) days after the received date shown above or until receipt of a "substantially equivalent" letter before placing the product into commercial distribution. I suggest that you contact us if you have not been notified in writing at the end of this ninety (90) day period before you begin marketing you device. Written questions concerning the status of your submission should be sent to:

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
8757 Georgia Avenue
Silver Spring, Maryland 20910

If you have procedural or policy questions, please contact the Division of Small Manufacturers Assistance at their toll-free number (800) 638-2041 or me at (301) 427-8162

Sincerely yours,

Robert I. Chissler
Premarket Notification Coordinator
Office of Device Evaluation
Center for Devices and
Radiological Health

3

ABBOTT LABORATORIES

Diagnostics Division

1921 Hurd St.
P.O. Box 152020
Irving, Texas 75015

K864224/A

December 1, 1986

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
8757 Georgia Avenue
Silver Spring, Maryland 20910

RE: Abbott Spectrum Glucose Reagent Kit (Urine Application) 510(k), D.C. Number K864224

Gentlemen:

We are providing the additional information requested for the Abbott Spectrum Glucose Regent Kit (Urine Application) 510(k):

(b)(4) Confidential and Proprietary Information

[redacted]

4338o-1

4

(b)(4) Confidential and Proprietary Information

[redacted]

Please contact me at (214) 257-6154 if you have any questions or need additional information.

Sincerely,

ABBOTT LABORATORIES

Keith Minter
Regulatory Affairs Manager

KM:rsh

attachments:

4338o-2

5

ATTACHMENT A

with a high lipid content. In this event add 2.0 ml. of isopropyl alcohol to the 8 ml. of final reaction mixture, mix, measure absorbance, and multiply by 10/8. If the mixture is still cloudy, repeat the test on a protein-free filtrate.

The color follows Beer's law and sufficient reagent is present to permit simple dilution of the final mixture with acetic acid for values up to 2000 mg./100 ml. Moderate hemolysis does not interfere. Bilirubin remains unchanged under these conditions and interference is negligible. In some modifications of this method undiluted serum is added directly to the reagent. More intense color is obtained with glucose under these relatively anhydrous conditions, but the interference from bilirubin becomes significant because of its conversion to the green pigment biliverdin.

REFERENCES

Dubowski, K. M.: Clin. Chem., *8*:215, 1962.
Feteris, W. A.: Am. J. Med. Tech., *31*:17, 1965.

GLUCOSE TOLERANCE TESTS

Patients with mild or diet-controlled diabetes may have fasting blood glucose levels within the normal range, but be unable to produce sufficient insulin for prompt metabolism of ingested carbohydrate. As a result, blood glucose rises to abnormally high levels and the return to normal is delayed. In other words, the patient has decreased tolerance for glucose. Therefore, glucose tolerance tests are most helpful in establishing a diagnosis of a mild case of diabetes.

When a standard dose of 100 gm. of glucose is given orally, absorption occurs rapidly and the blood glucose concentration increases. This stimulates the pancreas to produce more insulin with the result that after 30 to 60 minutes the blood glucose level begins to decrease. Since there now exists more insulin than necessary, the blood glucose tends to drop below the fasting level after 1.5 to 2 hours, and then returns to normal levels by approximately 3 hours. Response to glucose in various conditions is shown in Figure 4-11. Values refer to serum or plasma glucose concentrations. As noted earlier, these values are approximately 12 per cent greater than true glucose levels in whole blood.

In a normal response the fasting level of serum glucose is within normal limits; the peak concentration is reached by 30 or 60 minutes and does not exceed 170 mg./100 ml.; and the 2 hour level drops below 120 mg./100 ml. Corresponding values for true glucose in whole blood are 150 and 110 mg./100 ml.

The patient should be placed on a diet containing 1.75 gm. of carbohydrate per kilogram of body weight for 3 days before a glucose tolerance test. If carbohydrate intake has been too low preceding the test, a false diabetic type curve may be obtained.

Oral Glucose Tolerance Test

The test is usually performed in the early morning. The patient should not eat after the evening meal on the day before the test, although water may be taken. The patient should remain at rest during the test and also refrain from smoking or eating. A fasting blood sample and urine specimen are obtained. A solution containing 100 gm. of glucose is given to adults; for children, 0.5 gm. of glucose per pound of

REF. TIETZ, NORBERT W. "FUNDAMENTALS OF CLIN. CHEM."

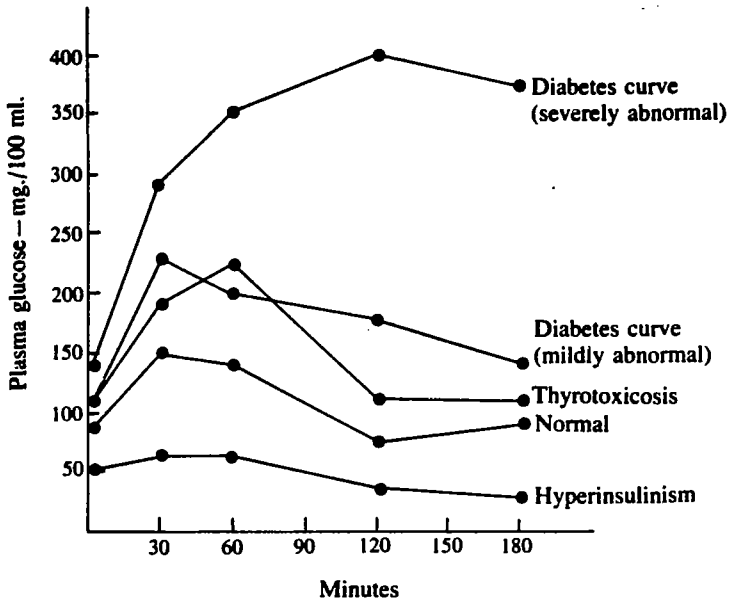


Figure 4-11. Response to oral glucose tolerance test.

body weight is satisfactory. Various commerical preparations are available; otherwise, 100 gm. of glucose is dissolved in about 200 ml. of water and flavored with lemon juice.

Blood specimens are collected at ½, 1, 2, and 3 hours after glucose ingestion. Urine specimens are collected at the same time and are analyzed semiquantitatively for glucose. Normally these should all show a negative reaction. The level of plasma glucose at which glucose appears in the urine is called the renal threshold and is approximately 180 mg./100 ml. Some individuals exhibit lower renal thresholds and excrete glucose in the urine even when the glucose tolerance curve is normal. Although this may be unrelated to any pathologic condition, about one third of these individuals eventually develop diabetes.

A 3 hour test is usually adequate for routine evaluation of diabetes. If hypoglycemia is suspected, additional specimens are taken at 4 and 5 hours. Patients with adrenal insufficiency or with islet cell tumors of the pancreas tend to have low fasting levels of blood glucose. Response to a glucose tolerance test may appear normal over the first 3 hours, but values continue to fall during the fourth and fifth hours. Some patients with latent diabetes tend to show hypoglycemia during this period also, probably associated with a delayed secretion of insulin.

Intravenous Glucose Tolerance Test

Poor absorption of orally administered glucose may result in a "flat" tolerance curve. In this event, glucose may be given intravenously, and preparation of the patient is exactly the same as for the oral test. A fasting blood sample and urine specimen are obtained. A physician then injects 50 per cent (w/v) glucose intravenously. The recommended dose is 0.333 gm. of glucose per kg. of body weight. A simple method to determine the proper amount of 50 per cent glucose to be injected

is to divide the pati
No more than 50 n
Blood and urin
test. The interpret
½ hour specimen an

Two Hour Postpra

Since the 2 hou
in evaluating diabe
determination. The
taining 100 gm. of
glucose determinati
amount of carbohyd
meal. Many physi
routinely in lieu of
usual hospital conc
interval very accura
test meal. To insu
recommended that
postprandial blood
Interpretation.
depends on whether
or serum glucose a
the true whole bloo

Insulin Tolerance T

This test is use
crine disorders. Th
hydrate daily for 2
blood is taken for
intravenously by a
weight. Blood spec
90, and 120 minute
per cent glucose sho
observed closely an
terminate the test sh
Normally the b
30 minutes, and the
two types of abno
delayed decrease in
(Cushing's syndrom
type of response the
or does not occur
pituitary (Simmond
hyperinsulinism. I
half the usual dose
signs of hypoglycem
patients at the end

is to divide the patients's weight in pounds by 3.3. For example, 165 lb./3.3 = 50 ml. No more than 50 ml. should be injected.

Blood and urine specimens are collected at ½, 1, 2, and 3 hours as for the oral test. The interpretation is similar except that the peak blood value occurs with the ½ hour specimen and has little numerical significance.

Two Hour Postprandial Glucose

Since the 2 hour specimen in a glucose tolerance test has the greatest significance in evaluating diabetes, the test may be shortened for screening purposes to a single determination. The patient consumes a breakfast, lunch, or glucose solution, containing 100 gm. of carbohydrate. Two hours after the meal, blood is drawn for a glucose determination. The patient should be instructed to consume the required amount of carbohydrate and to remain at rest during the 2 hour period following the meal. Many physicians now request 2 hour postprandial glucose determinations routinely in lieu of fasting glucose levels as guides to insulin requirements. Under usual hospital conditions it is often difficult, however, to control the 2 hour time interval very accurately since timing may start at the beginning, midway, or end of the test meal. To insure uniformity of carbohydrate intake and accurate timing, it is recommended that 100 gm. of glucose in solution be used routinely as a test meal for postprandial blood glucose determinations.

Interpretation, as with other glucose determinations, varies with the method and depends on whether whole blood or plasma is used for the analysis. In general, plasma or serum glucose at 2 hours postprandially should be less than 120 mg./100 ml; the true whole blood glucose should be less than 110 mg./100 ml.

Insulin Tolerance Test

This test is useful in evaluating patients with insulin resistance or certain endocrine disorders. The patient is placed on a diet containing at least 300 gm. of carbohydrate daily for 2 or 3 days before the test. With the patient in the fasting state, blood is taken for a baseline glucose level, after which regular insulin is injected intravenously by a physician in an amount corresponding to 0.1 unit per kg. of body weight. Blood specimens are then taken for glucose determinations at 20, 30, 45, 60, 90, and 120 minutes after the insulin was given. A syringe containing 50 ml. of 50 per cent glucose should be available for intravenous injection. The patient should be observed closely and a physician should be available to make the injection and terminate the test should a hypoglycemic reaction occur.

Normally the blood glucose decreases to about 50 per cent of the fasting level by 30 minutes, and then returns to normal fasting limits by 90 to 120 minutes. There are two types of abnormal response. The insulin resistant type shows only slight or delayed decrease in blood glucose and occurs with adrenal cortical hyperfunction (Cushing's syndrome), in acromegaly, and in some cases of diabetes. In the second type of response the blood glucose falls normally, but the subsequent rise is delayed or does not occur at all. This situation occurs with hypofunction of the anterior pituitary (Simmond's disease) or the adrenal cortex (Addison's disease), and in hyperinsulinism. In cases of suspected Simmond's disease it is recommended that half the usual dose of insulin be given and that the patient be watched carefully for signs of hypoglycemia. Glucose solutions or fruit juice should normally be given to

9

Other Tolerance Tests

Various other tests have been proposed that require serial determinations of blood glucose. Some of these will now be described briefly.

Tolbutamide (1-butyl-3-*p*-tolylsulfonylurea, Orinase) is a compound that stimulates the pancreas to produce insulin. Following intravenous injection, the normal response is similar to that observed with the insulin tolerance test: the blood glucose decreases to about 50 per cent of the fasting level by 30 minutes, then returns to normal. If the blood glucose level at 20 minutes is between 80 and 84 per cent of the fasting value the patient is said to have a 50 per cent probability of having diabetes.[5] In more severe cases the response will be even less inasmuch as the pancreas is unable to secrete adequate insulin. The test has also proved to be valuable in evaluating hypoglycemic states caused by insulomas.[2] In this condition injection of tolbutamide results in marked decrease in blood glucose to values in the range of 20 to 30 mg./100 ml. and persistent hypoglycemia up to 3 hours. As with the insulin tolerance test, patients must be watched carefully for hypoglycemic reactions and the test terminated, if necessary, by intravenous administration of glucose.

The *epinephrine tolerance* test is used to evaluate one form of glycogen storage disease (Type I, von Gierke's), a condition in which there is a deficiency or absence of the enzyme glucose-6-phosphatase in the liver. This enzyme is the catalyst for the final step in the formation of blood glucose from hepatic glycogen. Individuals with von Gierke's disease have low glucose level in the blood, increased liver glycogen, but decreased *availability* of liver glycogen as shown by less than normal or no increase in blood glucose following administration of epinephrine. In a normal person, after intramuscular injection of 1 ml. of a 1:1000 solution of epinephrine hydrochloride, the blood glucose increases 35 to 45 mg./100 ml. in 40 to 60 minutes and returns to the fasting level by 2 hours. Blood specimens are taken at 30, 45, 60, 90, and 120 minutes after injection.

Deficiency of small bowel mucosal lactase has been found to be a rather common condition in healthy adults. Such deficiency may be associated with intolerance to lactose manifested by diarrhea and other symptoms following ingestion of milk. The diarrhea will usually disappear if lactose is eliminated from the patient's diet. A *lactose tolerance test* can be done to evaluate this condition.[1] A standard oral glucose tolerance test is performed first to provide a basis for comparison. On the following day the test is repeated except that 100 gm. of lactose is substituted for glucose. If lactase activity is present the lactose will be split to glucose and galactose and the resultant tolerance curve will be similar to that observed with glucose. With lactase deficiency the lactose tolerance curve will be flat with a rise not exceeding 20 mg./100 ml. over the fasting level. Either a copper reduction method or the *o*-toluidine method may be used to determine blood "sugar," since both galactose and glucose react in either method.

URINARY SUGARS

Occurrence of Sugars in Urine

Urine is examined routinely to detect or determine the presence or amount of glucose; this is done either as a screening procedure or as a guide to insulin therapy. Other sugars may also appear in the urine in certain conditions and interfere with the

detection and determ..
sugars; that is, the
for the very rare case
of pathological signi

Galactose appea
acterized by inability
since half of the milk
found in urine of wo
nancy. The laborat
Fructose may appear
significance. Fructos
confused with diabete

plums, or prunes, or
distinguished from gl
the urine of some pa

Many reducing s
list of the more impo

Qualitative Methods

PRINCIPLE

Benedict's qualit
alkaline solution. G
cuprous ion with res
oxide.

REAGENT

Dissolve 17.3 gm.
with heating, 173 gm.
in 800 ml. of water.
mixing, to the coppe
stable.

PROCEDURE

Add 8 drops (0.4
in a boiling water ba

detection and determination of glucose. The sugars of clinical interest are all reducing sugars; that is, they readily reduce cupric ion in hot alkaline solution. Except for the very rare cases of galactosuria, glucose is the only sugar found in urine that is of pathological significance.

Galactose appears in the urine of infants with galactosemia, a condition characterized by inability to metabolize galactose. Such infants fail to thrive on milk since half of the milk sugar, lactose, is converted to galactose. Lactose is sometimes found in urine of women during lactation and occasionally toward the end of pregnancy. The laboratory may be required to differentiate this sugar from glucose. Fructose may appear in the urine after eating fruits, honey, and syrups, but has no significance. Fructosuria is a rare and harmless congenital defect that should not be confused with diabetes. Pentoses may occur in urine after eating such fruits as cherries,

TABLE 4-1. *Reducing Substances in Urine*

Fructose	Ketone bodies
Lactose	Sulfanilamide
Galactose	Oxalic acid
Maltose	Hippuric acid
Arabinose	Homogentisic acid
Xylose	Glucuronic acid
Ribose	Formaldehyde
Uric acid	Isoniazid
Ascorbic acid	Salicylates
Creatinine	Cinchophen
Cysteine	Salicyluric acid

plums, or prunes, or as a harmless congenital anomaly and, as with fructose, must be distinguished from glucose. Maltose has been reported to occur along with glucose in the urine of some patients with diabetes.

Many reducing substances other than sugars may also occur in urine. A partial list of the more important reducing substances is shown in Table 4-1.

Qualitative Methods for Total Reducing Substances

PRINCIPLE

Benedict's qualitative reagent contains cupric ion complexed with citrate in alkaline solution. Glucose, or other reducing substances, reduces cupric ion to cuprous ion with resultant formation of yellow cuprous hydroxide or red cuprous oxide.

REAGENT

Dissolve 17.3 gm. of $CuSO_4 \cdot 5H_2O$ in 100 ml. of hot water. Dissolve separately, with heating, 173 gm. of sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 100 gm. of Na_2CO_3 in 800 ml. of water. Allow to cool, then add the citrate-carbonate solution, with mixing, to the copper sulfate solution. Dilute to 1 L. with water. This reagent is stable.

PROCEDURE

Add 8 drops (0.4 ml.) of urine to 5 ml. of reagent in a test tube. Mix and place in a boiling water bath for 3 minutes. Remove and examine immediately. Report

as 0 to 4+ according to the following criteria:

Appearance	*Report*	*Approximate Glucose Concentration* (gm./100 ml.)
Blue to green, no precipitate	0	0–0.1
Green with yellow precipitate	1+	0.3
Olive green	2+	1.0
Brownish orange	3+	1.5
Brick red	4+	2.0 or more

A convenient adaptation of the preceding procedure is marketed in tablet form (Clinitest, Ames Co. Div., Miles Laboratories, Elkhart, Ind.). The tablets contain anhydrous cupric sulfate, sodium hydroxide, citric acid, and sodium bicarbonate. Five drops (0.25 ml.) of urine are mixed with 10 drops of water in a test tube. One tablet is added and the mixture is allowed to stand undisturbed for 15 seconds, remixed, and observed for color. A chart provided by the manufacturer is used to interpret the result. Heat is generated by contact of sodium hydroxide and water. The initial reaction between citric acid and sodium bicarbonate causes the release of carbon dioxide, which blankets the mixture and reduces contact with oxygen from the air.

Quantitative Methods for Total Reducing Substances

Although quantitative measurement of total reducing substances in urine provides information of limited diagnostic value, the test is still performed in a number of hospital laboratories. The Folin-Wu or Somogyi-Nelson methods may be used for this purpose. The urine usually needs diluting to below about 300 mg./100 ml. to bring the concentration of glucose within the range of the method. The dilution necessary can be estimated from the qualitative test. The preparation of protein-free filtrate is omitted; instead, the urine is further diluted with water and analyzed in the same manner as a protein-free filtrate. Results are corrected for the initial dilution. A small amount of reducing substances is found in urine specimens that are negative with the qualitative tests. Expressed as glucose, the concentration is less than 150 mg./100 ml. of urine.

SEPARATION AND IDENTIFICATION OF SUGARS

Techniques for separating and identifying sugars include fermentation, osazone formation with phenylhydrazine, specific chemical tests, and paper or thin-layer chromatography. The availability of glucose oxidase test strips has greatly simplified the differentiation of glucose from the many other reducing substances.

Glucose, fructose, maltose, and mannose are fermentable with yeast, but lactose, galactose, and pentoses do not ferment. Of the fermentable sugars, only glucose and, rarely, fructose are likely to occur in urine. The fermentation test can be used, therefore, to differentiate glucose from lactose or other nonfermenting sugars.

Fermentation Test

Bring a portion of the urine to boiling to destroy *E. coli*, which can ferment lactose. Cool to room temperature and reserve a portion for a qualitative test for reducing

sugar. Add about 0.3
and mix with a stirri
Transfer to a large t
1 hour in a 37°C w
qualitative test for re
similar test performe
If the fermented
glucose. If the fermer
or reducing substance
positive, but lower t
glucose. It is good p
Dissolve 0.1 gm. of g

TABLE
Proce

Ferric
Folin-
Somo
o-Tol
Ferme

reducing substances.
should become negati
0.3 gm. of yeast with 1
reducing substances i
centrifuged, and rewa
If desired, a qua
on the fermented and
is considered to be glu
in the same manner a
described for serum.
highly specific enzym
show an upper limit o
Generally, it is n
glucose excretion in
reasonable specificity.
were all negative for re
by an automated alka
Nelson method, and t
yeast fermentation, th
the decrease in value
Results, shown in Ta
creatinine. Of the fou
specific and the ferricy
urine.

12

sugar. Add about 0.3 gm. of dry active baker's yeast to 10 ml. of the boiled specimen and mix with a stirring rod until the yeast is dispersed into a homogeneous mixture. Transfer to a large test tube and incubate unstoppered with occasional mixing for 1 hour in a 37°C water bath. Centrifuge the incubated specimen and perform a qualitative test for reducing sugar on the supernatant. Compare the results with a similar test performed on the unfermented specimen.

If the fermented specimen is negative, all the sugar in the urine is probably glucose. If the fermented sample is the same as the unfermented sample, some sugar or reducing substance other than glucose is present. When the fermented sample is positive, but lower than the untreated sample, the difference is considered to be glucose. It is good practice to include a control to check the activity of the yeast. Dissolve 0.1 gm. of glucose in 10 ml. of urine previously shown to be negative for

TABLE 4-2. *Results Obtained by Four Glucose Procedures on 20 Urine Specimens All Negative for Reducing Substances*

Method	Apparent gm. glucose/gm. creatinine *Range*	*Mean*
Ferricyanide	1.07–2.77	1.80
Folin-Wu	0.83–2.16	1.24
Somogyi-Nelson	0.26–1.26	0.53
o-Toluidine	0.10–0.37	0.20
Fermentation	0–0.18	0.06

reducing substances. This specimen, when carried through the fermentation test, should become negative for reducing sugar. A blank may also be included by mixing 0.3 gm. of yeast with 10 ml of water to rule out the possible presence of nonfermenting reducing substances in the yeast. If necessary, the yeast may be suspended in saline, centrifuged, and rewashed to remove reducing substances.

If desired, a quantitative determination of glucose in urine may be performed on the fermented and unfermented specimens. The difference between the two values is considered to be glucose. For the Somogyi-Nelson procedure, a filtrate is prepared in the same manner as described for blood. The *o*-toluidine method is performed as described for serum. The amount of glucose excreted normally, as determined by highly specific enzymatic methods, is less than 0.5 gm./24 hr. Random specimens show an upper limit of normal of approximately 30 mg./100 ml.

Generally, it is not necessary to resort to fermentation tests to determine total glucose excretion in a 24 hour urine specimen provided the glucose method has reasonable specificity. In one study, 20 morning urine specimens were selected that were all negative for reducing sugar by the copper reduction test. These were analyzed by an automated alkaline ferricyanide method, the Folin-Wu method, the Somogyi-Nelson method, and the *o*-toluidine method. Creatinine was also determined. After yeast fermentation, the specimens were again analyzed by the *o*-toluidine method and the decrease in value recorded as fermentable sugar. Yeast blanks were included. Results, shown in Table 4-2, are expressed as apparent gm. of glucose per gm. of creatinine. Of the four procedures, the *o*-toluidine method was found to be the most specific and the ferricyanide method the least specific for the estimation of glucose in urine.

13

Qualitative Tests for Individual Sugars

GLUCOSE

A convenient paper test strip is commerically available (Clinistix, Ames Co.). The filter paper is impregnated with glucose oxidase, peroxidase, and *o*-tolidine and provides a simple color test according to principles discussed in an earlier section. The test end is moistened with urine and examined after 10 seconds. A blue color develops if glucose is present. The sensitivity of the strip has been adjusted to take into account the presence of enzyme inhibitors normally occurring in urine. Thus, a positive test will be obtained with lower concentrations of glucose in water as compared to urine. For the same reason, a false positive test may be obtained with very dilute specimens.

In one study of 2000 urine specimens, 11 false negative enzyme paper tests were encountered. Among the inhibitors identified were ascorbic acid, dipyrone, and meralluride sodium (mercuhydrin). Several antibiotics contain ascorbic acid as a preservative. The acid is largely excreted unchanged and can cause false negative results. Contamination of urine with hydrogen peroxide or a strong oxidizing agent, such as hypochlorite, produces false positive results. For routine examinations, however, a negative stick test is considered negative for glucose. A positive stick test is further evaluated by one of the copper-reduction methods, such as a Clinitest tablet.

REFERENCE

Free, A. H., Adams, E. C., Kercher, M. L., Free, H. M., and Cook, M. H.: Clin. Chem., *3*:163, 1957.

SELIWANOFF'S TEST FOR FRUCTOSE

Hot hydrochloric acid converts fructose to hydroxymethyl furfural, which links with resorcinol to produce a red-colored compound. To make the reagent, dissolve 50 mg. of resorcinol in 33 ml. of concentrated hydrochloric acid and dilute to 100 ml. with water. Add 0.5 ml. of urine to 5 ml. of reagent in a test tube and bring to a boil. Fructose produces a red color within ½ minute. The test is sensitive to 0.1 per cent fructose provided excess glucose is absent. A 2 per cent solution of glucose will produce about the same color as 0.1 per cent fructose after ½ minute of boiling. A 0.5 per cent solution of fructose should be used as a control. With high concentrations of fructose, a red precipitate forms, which may be filtered and dissolved in ethanol to produce a bright red colored solution.

BIAL'S TEST FOR PENTOSE

By heating with hydrochloric acid, pentoses are converted to furfural, which reacts with orcinol to form green-colored compounds.

Dissolve 300 mg. of orcinol in 100 ml. of concentrated hydrochloric acid and add 0.25 ml. of 10 per cent ferric chloride solution. Glucose, if present in the urine, should be removed by fermentation. Add 0.5 ml. of urine to 5 ml. of reagent in a test tube and bring to a boil. Pentoses produce a green color. The test is sensitive to 0.1 per cent pentose. A 0.5 per cent solution of xylose should be used as a control. Glucuronates will produce a similar color if the boiling is prolonged. Fructose, as with Seliwanoff's reagent, produces a red color.

A combination of t... reducing sugar as summ... ferentiated from each ot... paper chromatography.

TABLE

Sugar
Glucose
Fructose
Galactose
Lactose
Maltose
Pentoses

Identification of Urinary

PRINCIPLE

Sugars can be separa... and located after color de... migration depend upon t... Presumptive identification ... with those of authentic s... veniently in a 6 × 18 inch

REAGENTS

1. Solvent. Perform ... *n*-butanol, 40 ml. of pyri... miscible. Pour into the bo... before use.

2. Spray reagent. D... 4 per cent (w/v) sodium h...

3. Reference sugar so... fructose, galactose, maltos... These solutions are stable f...

PROCEDURE

1. Determine the con... qualitative copper reducti... concentration of approxim... use twice as much sample i...

2. Draw a pencil line ... inch section of Whatman N... on the line to indicate start... specimen.

3. Apply approximatel... microhematocrit tube or a ... permitted to dry before ad... spots as small as possible.

14

ATTACHMENT B

CLIN. CHEM. 20/5, 595-602 (1974)

Evaluation of the Adaptation of the Glucose Oxidase/Peroxidase–3-Methyl-2-benzothiazolinone hydrazone–*N,N*-Dimethylaniline Procedure to the Technicon "SMA 12/60," and Comparison with Other Automated Methods for Glucose

R. Neill Carey, Donald Feldbruegge, and James O. Westgard

We have evaluated the Technicon SMA 12/60 modification of a glucose oxidase/peroxidase–3-methyl-2-benzothiazolinone hydrazone–*N,N*-dimethylaniline method, and find it acceptable. Added bilirubin, creatinine, dextrans, and uric acid did not interfere. We compared values for patients' sera by this method to those by the glucose oxidase/peroxidase–2,2′-azino-diethylbenzothiazoline-6-sulfonic acid, neocuproine, *o*-toluidine, ferricyanide, and hexokinase methods. For comparisons to the hexokinase method (which we used as a reference method), n = 371, slope = 1.00, *y*-intercept = 1.57 mg/dl, and bias = 2.05 mg/dl. For the method being evaluated, the run-to-run average monthly standard deviation was 2.9 mg/dl for a control product for which the mean was 85 mg of glucose per deciliter, and 4.3 mg/dl for a product for which the mean was 240 mg/dl. All six methods were compared for effects of uremic sera, icteric sera, and sera from patients receiving ascorbic acid therapy or hypoglycemic drugs.

Additional Keyphrases: *comparison with five other methods for serum glucose • sources of variation • AutoAnalyzer • urinary glucose • normal values • age-related increase in serum glucose • acceptable analytical error, random and systematic • interference studies • "kit" method • Du Pont ACA*

The response of "reducing" glucose methods to substances other than glucose has been well documented, especially for glucose determinations on specimens from uremic patients (*1, 2*). Enzymatic methods should solve this problem, but hexokinase methods are generally too expensive for routine use and glucose oxidase/peroxidase methods are susceptible to interferences (*3, 4*). In an oxidase method (*5*) that is relatively free from interferences, peroxide reacts to couple MBTH[1] and DMA in the presence of peroxidase.

University of Wisconsin Center for Health Sciences, Departments of Pathology and Medicine, Madison, Wis. 53706.

Presented at the 25th National Meeting of the AACC, New York, N. Y., July 15–20, 1973.

[1] Nonstandard abbreviations used: MBTH, 3-methyl-2-benzothiazolinone hydrazone; DMA, *N,N*-dimethylaniline; SMA 12/60, Sequential Multiple Analyzer Model 12/60 (Technicon Corp.); and ABTS, 2,2′-azino-diethylbenzothiazoline-6-sulfonic acid.

Received Dec. 18, 1973; accepted Feb. 22, 1974.

We have evaluated this glucose method as adapted to the "SMA 12/60" by Technicon Instruments Corp., Tarrytown, N. Y. 10591. A limited evaluation of this method (*5*) was described by Romano (*6*) while our report was being prepared. Here, we compare results for this method with those for the hexokinase, glucose oxidase/peroxidase–2,2′-azino-diethylbenzothiazoline-6-sulfonic acid, *o*-toluidine, Cu–neocuproine, and alkaline ferricyanide procedures.

Samples included sera from hospital patients (including uremic and icteric patients), urine specimens, and studies of interference for specimens to which creatinine, uric acid, dextran, ascorbic acid, or bilirubin were added.

Materials, Methods, and Instrumentation

Methods

Glucose oxidase/peroxidase MBTH-DMA method. Stock reagents for this method were supplied by the Technicon Corp., and working reagents were prepared according to their instructions. Final concentrations are those given by Gochman and Schmitz (*5*). Working solution of glucose oxidase (EC 1.1.3.4) was prepared by dissolving the contents of each vial in distilled water to a final volume of 200 ml and adding 4 drops of surfactant ("Brij-35"). Refrigerated, this reagent is stable for two days. Peroxidase (EC 1.11.1.7) working reagent was prepared by dissolving the contents of each vial in acetate buffer (0.1 mol/liter, pH 5.0), to give a final volume of 200 ml. If refrigerated, the peroxidase working reagent is stable for one week. Oxidase and peroxidase reagents prepared by us according to the directions of Gochman and Schmitz (*5*) have been substituted for the Technicon reagents without significant effect. Working MBTH-DMA reagent was a mixture of 4.0 ml of stock MBTH (100 mg/dl), 6.0 ml of stock DMA (10 ml/liter), and 5.0 ml of acetic acid (10 mol/liter), diluted with distilled water to a final volume of 100 ml. This reagent should be kept at room temperature, and must be prepared freshly each working day.

The instrument we used for the method being

evaluated was a survey model SMA 12/60 (Technicon). The flow system we used differs from that of Gochman and Schmitz (*5*) only in that the 7.5-cm (3-inch) dialyzer is replaced with a 31-cm (12-inch) dialyzer. The larger dialyzer provides added sensitivity, which is necessary because the SMA-12 glucose sample is prediluted. Reagent cost per test is about $0.10.

Glucose oxidase/peroxidase–2,2′-azino-diethylbenzothiazoline-6-sulfonic acid method. Reagents for this procedure (*7*) were purchased from Boehringer Mannheim Corp., New York, N. Y. 10017. In this method, a single reagent is used that contains glucose oxidase, peroxidase, and the dye. The reagent was prepared according to the manufacturer's instructions. Two milliliters of the surfactant Brij-35 (Technicon) was routinely added per 2 liters of this reagent, which improved the bubble pattern. This method was run on the SMA-12/60 with a flow system like that used for the neocuproine method, except for the following changes: (*a*) the 460-nm filter was replaced with a 410-nm filter, because the reaction product has a major absorption peak near 410 nm, and others (*8*) have recommended use of a 420-nm filter; (*b*) the 90 °C heating bath was unplugged, so that the entire system was at room temperature (19–25 °C); (*c*) the sodium carbonate reagent pump-tube of the neocuproine method (see below) became the pump-tube for this reagent and water was pumped through the pump-tubes for saline and copper-neocuproine.

Copper-neocuproine method. The copper-reducing method used was the automated one originally described by Bittner and Manning (*9*) and generally available for use with SMA 12/60's (*10*). The copper–neocuproine cartridge (Technicon part No. 157-A055) was not modified in any way, and the flow system used was exactly as described by the Technicon method file (*10*). Copper–neocuproine reagent was supplied by Technicon. Sodium carbonate, 0.25 mol/liter, was prepared by dissolving 26.5 g of sodium carbonate in distilled water to a final volume of 1 liter, to which 1 ml of Brij-35 was then added.

o-Toluidine method. The procedure reported by Sudduth et al. (*11*) was modified slightly (for pediatric use) to decrease the sample volume required. An AutoAnalyzer I (Technicon) system was used. Pump-tube changes (in inches, i.d.) were: *o*-toluidine, from 0.090 to 0.073; air, from 0.056 to 0.100; and sample, from 0.025 to 0.010. *o*-Toluidine reagent was prepared according to Sudduth et al. (*11*).

Ferricyanide method. The procedure used is similar to that given in the Technion method file (*12*), with the following pump-tube changes (in inches, i.d.) for increased sensitivity: air, from 0.065 to 0.025; saline, from 0.090 to 0.081; flow cell, from 0.073 to 0.090. Alkaline potassium ferricyanide was purchased from Simmler (Simmler and Sons, Inc., St. Louis, Mo. 63108) and adjusted to the required sensitivity with a stock solution of potassium ferricyanide (50 g/liter).

Table 1. Glucose Oxidase/Peroxidase MBTH–DMA: Run-to-Run Precision[a]

n	Mean, mg/dl	SD, mg/dl	CV, %
25	85	2.9	3.4
300	165	3.2	1.9
25	240	4.3	1.8
80	335	4.9	1.5

[a] Av monthly values for four months.

Hexokinase method. Glucose was determined by the hexokinase (EC 2.7.1.1) method as used with the Du Pont ACA instrument (E. I. du Pont de Nemours and Co., Inc., Wilmington, Del. 19898). Hexokinase catalyzes the phosphorylation of glucose by ATP to glucose-6-phosphate, which is oxidized to 6-phosphogluconolactone by NAD^+ in the presence of glucose-6-phosphate dehydrogenase (EC 1.1.1.49). The reduced NADH is measured at 340 nm, with use of a blank at 383 nm. All reagents are contained in the ACA glucose test-pack.

Standardization[2]

The hexokinase, *o*-toluidine, and alkaline ferricyanide procedures were standardized by use of aqueous glucose standards (Hyland, Div. Travenol Laboratories, Inc., Costa Mesa, Calif. 92626). All three continuous-flow methods were calibrated with Technicon SMA-12 Reference Serum. The calibration value of each bottle of reference serum was determined by duplicate assays by the hexokinase method.

Results

Linearity, Precision, and Interaction

The oxidase MBTH–DMA method responds linearly to glucose up to 500 mg/dl. Response to aqueous standards, however, was 5–10% too high. We have not investigated the cause of this effect, which was not observed by Gochman and Schmitz (*5*).

Average monthly run-to-run precision data for four months are given in Table 1. Standard deviations increase from 2.9 mg/dl to 4.9 mg/dl as the means of the reported glucose values increase from 85 mg/dl to 335 mg/dl.

[2] Standardization is indeed a problem. However, we have made an effort here to arrive at a common point for all methods. Because there is a difference between aqueous and protein "standards" on the SMA 12/60, and since the samples are a protein matrix, aqueous standards are not appropriate for the methods on the SMA 12/60. This problem is well known, if not well understood. Use of aqueous standards would not provide a common base by which to compare differences caused by the samples themselves, which was our main interest. If aqueous standards were used, then we would observe additional differences caused by the standardization. A good example of this is the recent paper by Miskiewicz et al. (*3*), where aqueous standardization was used. The differences between methods were interpreted as differences in method specificity, even though a portion of the actual difference was probably the difference between aqueous and protein matrixes.

17

Table 2. Results of Tests for Interference in Six Methods for Serum Glucose[a]

Added Interference	Method					
	Oxidase MBTH-DMA	Oxidase ABTS	Neocuproine	o-Toluidine	Ferricyanide	Hexokinase
Ascorbic acid, mg/dl						
2.5	−3	−3	−3	0	+1	0
5	−4	−4	−1	0	+1	0
10	−6	−7	−1	+1	+4	0
15	−10	−12	−1	+2	+5	0
20	−13	−15	+2	+2	+8	−1
25	−17	−20	+2	+3	+11	0
Bilirubin, mg/dl						
3	−2	—[b]	—[b]	+2	—[b]	−1
7	−1	—	—	+4	—	−4
10	−1	—	—	+6	—	−5
13	−3	—	—	+6	—	−8
17	−2	—	—	+8	—	−9
Creatinine, mg/dl						
2.5	0	0	+1	+2	+2	−2
5	−2	−1	0	0	+6	0
7.5	+4	+3	+5	0	+8	0
10	0	0	+3	−2	+10	0
12.5	0	0	+5	−2	+16	+1
Dextran, 40, g/dl						
0.5	+2	+3	+1	+46	0	0
1.0	−2	−1	−4	+124	+2	−1
1.5	−2	−2	−4	+207	0	−1
2.0	−2	−2	−5	+298	+1	−2
2.5	+1	+1	−1	+368	−2	0
Dextran, 75, g/dl						
0.3	−1	0	−1	+32	0	−2
0.6	−1	0	−1	+66	0	−2
0.9	−1	0	−3	+118	−2	−2
1.2	+2	+3	+2	+164	+2	−1
1.5	−2	−1	−3	+220	0	−2
Uric acid, mg/dl						
5	−2	−10	+1	−2	+1	0
10	−2	−18	+1	−2	+6	0
15	−4	−25	+3	−2	+4	−1
20	−1	−27	+5	−2	+6	−2
25	−4	−37	+5	−2	+11	−2

[a] Interference in terms of milligrams of glucose per deciliter; av of duplicate determinations.
[b] Bilirubin interference not measured for these methods.

Sample-to-sample interaction averaged 0.8%, and exceeded 2% only five times during the 115 days on which measurements were made once daily. On these occasions, system maintenance decreased interaction to <2%.

Specific Interferences

We studied the effects of interfering substances on glucose measurements by adding various concentrations of suspected interferers (in saline, 9 g of NaCl per liter) to samples of pooled serum. Results are summarized in Table 2. Control pools had glucose concentrations of 80 to 110 mg/dl.

Ascorbic acid. The hexokinase, neocuproine, and o-toluidine methods are apparently unaffected by increased ascorbic acid; results with the ferricyanide method are elevated 5 mg/dl by an ascorbic acid concentration of 15 mg/dl; and in the oxidase methods there are negative interferences of 10 mg/dl and 12 mg/dl, respectively, for the evaluated and alternative methods.

Bilirubin. Only the oxidase MBTH-DMA, hexokinase, and o-toluidine glucose methods were evaluated for specific bilirubin interferences. Added bilirubin (17 mg/dl) did not affect the oxidase MBTH-DMA method, but depressed glucose values obtained with the Du Pont ACA hexokinase method by 9 mg/dl and increased those obtained by the o-toluidine method by 8 mg/dl.

Creatinine. A positive interference was observed in

results of glucose measurements by the neocuproine and ferricyanide methods when creatinine was added to the pooled serum. Increases of 5 mg/dl and 16 mg/dl, respectively, were observed when 12.5 mg of creatinine was added per deciliter. The other methods were apparently unaffected by such an addition.

Dextran 40 and Dextran 75. Only *o*-toluidine glucose measurements were affected by added dextran. When 15 g of dextran was added per liter of pooled serum, glucose values were increased by 207 mg/dl and 200 mg/dl, respectively, by Dextran 40 and Dextran 75 (Travenol Laboratories Inc., Deerfield, Ill. 60015).

Uric acid. Results for the oxidase MBTH-DMA, *o*-toluidine, and hexokinase methods were not significantly affected by as much as 25 mg of added uric acid per deciliter. Results of the other oxidase procedure, however, were decreased by 37 mg/dl when this amount of uric acid was added. In contrast, results by the neocuproine and ferricyanide methods increased by 5 mg/dl and 11 mg/dl, respectively.

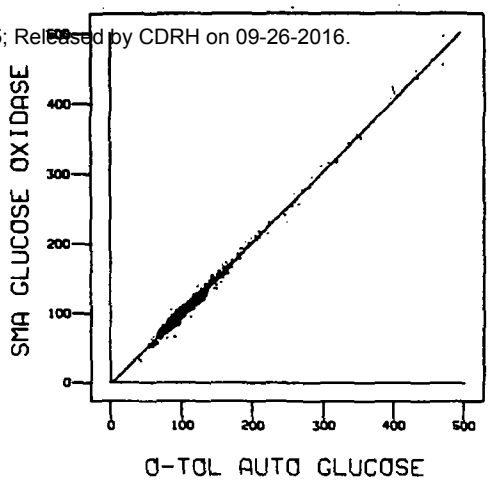


Fig. 1. Comparison of glucose values by the glucose oxidase/peroxidase MBTH-DMA method (*y*-axis) to those by the *o*-toluidine method (*x*-axis) for sera from patients

1020 samples; slope, 1.016; *y*-intercept: −1.73 mg/dl; standard error, 4.53 mg/dl; av *o*-toluidine value, 108 mg/dl; bias, less than 0.1 mg/dl; SD, 4.59 mg/dl; *t*-value, 0.23

Comparison of Oxidase MBTH-DMA to Other Methods

The first comparison studies of the MBTH-DMA glucose oxidase/peroxidase method were made with use of sera from patients, the specimens being chosen without conscious bias from those submitted to the clinical laboratories for routine analysis. Comparison data for 1020 samples analyzed for glucose by both the *o*-toluidine and oxidase MBTH-DMA methods are shown in Figure 1, and paired-sample comparisons (n = 371) between the oxidase MBTH-DMA and hexokinase methods are shown in Figure 2.

Because uremia has been shown to cause errors in some glucose methods (*1, 2, 4*), we compared glucose measurements obtained by use of all six methods from uremic and nonuremic sera. The glucose concentrations of 85 sera from nonuremic patients were used as a control group. These data are shown as the leftmost group of points in Figure 3, where differences between glucose measurements by hexokinase and each of the five other methods are plotted vs. average uric acid concentrations, as determined with the SMA-12 (*10*). For the control group, the average glucose value by the hexokinase method was 97.9 mg/dl, and the average uric acid and blood urea nitrogen concentrations were 5.49 and 14.1 mg/dl, respectively. Comparison results for sera from uremic patients (average blood urea nitrogen, 93 mg/dl) are summarized in groups, according to uric acid concentration, at the right in Figure 3. Grouping by blood urea nitrogen or creatinine concentrations failed to produce trends as regular as those in Figure 3.

The reducing methods show an increasingly positive response as uric acid increases, until at an average of 12.6 mg of uric acid per deciliter, results for the alkaline ferricyanide method differ positively from those for the hexokinase method by an average

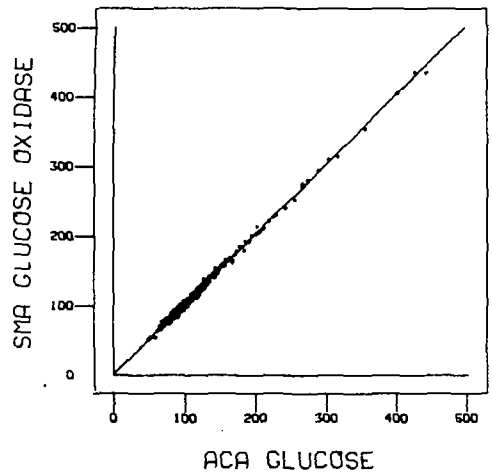


Fig. 2. Comparison of glucose values by the glucose oxidase/peroxidase MBTH-DMA method (*y*-axis) to those by hexokinase (Du Pont ACA) (*x*-axis) for sera from patients

371 samples; slope, 1.004; *y*-intercept, 1.57 mg/dl; standard error, 3.10 mg/dl; av hexokinase value, 113 mg/dl; bias, 2.05 mg/dl; SD, 3.11 mg/dl; *t*-value, 12.7

of 32.3 mg/dl, and average neocuproine results differ by 20.6 mg/dl. Results by the *o*-toluidine method agree well with those of the hexokinase method for sera in which uric acid concentrations are less than 12 mg/dl. However, for the samples with an average uric acid concentration of 12.6 mg/dl, results by the *o*-toluidine method averaged 8.1 mg/dl higher than those for the hexokinase method. Even with this high uric acid concentration, oxidase MBTH-DMA results were only 3.3 mg/dl higher than those by the hexokinase method. Results by the other oxidase method became steadily lower than results by the hexokinase method as uric acid concentration increased, until at the highest uric acid concentrations, they averaged 15.7 mg/dl low. In general, the trends

19

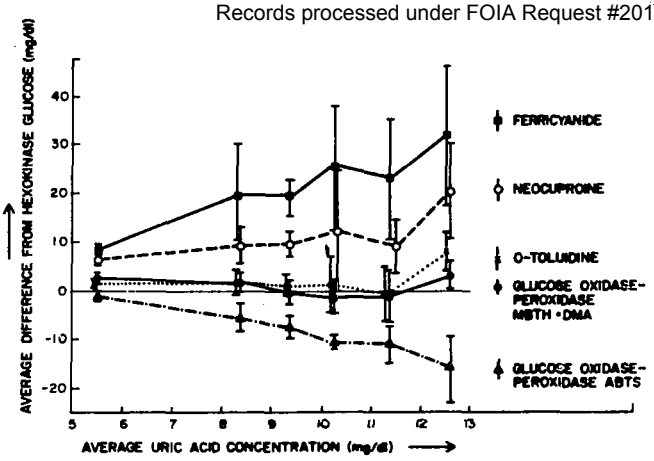


Fig. 3. Comparison of values for serum glucose (patients' sera) by six automated methods

Values are plotted as average differences from results by the hexokinase method for specimens grouped according to uric acid values. Vertical bars surrounding each point indicate 95% confidence limits of the differences (± two standard errors of the means). Number of specimens in each uremic group varies from 6 to 17

suggested by the uric acid addition experiment are observed in uremic sera, but other, unspecified sources of interference may also be present in these sera.

Comparison data for 13 icteric samples (average bilirubin, 20 mg/dl) are summarized in Table 3. Hexokinase results averaged 15 mg/dl lower than oxidase MBTH-DMA and glucose oxidase/peroxidase ABTS results. *o*-Toluidine results, as in the specific-interference study, were higher than the oxidase results.

Table 3 also contains comparison data for 17 patients receiving therapeutic dosages of ascorbic acid. The average differences between results by the two oxidase procedures and by the hexokinase method increased from the average differences observed for the 85 unselected patients. From the data on addition studies, ascorbic acid would have been predicted to cause lower results for both oxidase procedures, but apparently this effect was compensated by other sample variables.

Urinary Glucose

We extended the comparison study to measurements of urinary glucose, to observe the effects of the higher concentrations of interferences present in these samples. Analytical results for each method were averaged, and those values are listed for comparison in Table 4. The data for Group I are from assays for glucose in selected urine samples that had measurable glucose present. Group II samples, from patients, were urines to which glucose standard (100 mg/dl) was added. In both groups of samples, results for the alternative oxidase method were lowest, results for the oxidase MBTH-DMA, hexokinase, and *o*-toluidine methods were closely grouped, and those for the neocuproine and ferricyanide methods were much higher. These results appear to extend the trends established in uremic sera.

Table 3. Comparisons of Serum Glucose Values for Icteric Patients and Patients Receiving Therapeutic Doses of Ascorbic Acid

Method	Mean glucose, mg/dl: Icteric[a]	Ascorbic acid[b]
Glucose oxidase/ peroxidase MBTH-DMA	88.2	113.1
Glucose oxidase/ peroxidase ABTS	87.0	111.4
Neocuproine	95.0	114.7
o-Toluidine	104.2	113.4
Alkaline ferricyanide	99.7	117.6
Hexokinase	73.4	109.7

[a] 13 patients; total bilirubin range: 10–35 mg/dl; mean, 20 mg/dl.
[b] 17 patients; ascorbic acid dosage, 1 g/day or less.

Table 4. Mean Glucose Values for Urine Samples

Method	Mean glucose, mg/dl: Group 1 (n = 29)	Group 2 (n = 16)
Glucose oxidase MBTH-DMA	7	127
Glucose oxidase ABTS	0	68
Hexokinase	27	128
o-Toluidine	20	139
Cu-neocuproine	107	175
Ferricyanide	201	242

Oral Hypoglycemic Drugs

Results for serum glucose for patients receiving oral hypoglycemic drugs are compared in Table 5. Although these data are very limited, no striking differences were apparent when therapeutic concentrations of these drugs were present. Tolazamide interference with the alternative oxidase method has been reported (*13*).

Normal Values for the Glucose Oxidase/ Peroxidase MBTH-DMA Method

Glucose was measured by the oxidase MBTH-DMA method for 336 normal volunteers, including participants in a "Heart Sunday" screening project. For 151 subjects, age 20–49 years, the average glucose concentration was 94 mg/dl (SD, 10 mg/dl). The distribution is not quite gaussian (Figure 4), and use of 2.5 and 97.5 percentiles would give a somewhat higher range (72–116 mg/dl). For older subjects, Figure 5 shows a definite skewing of the distribution toward higher values. The mean is 101 mg/dl, the standard deviation is 11 mg/dl, and the 2.5–97.5 percentile range is 84–128 mg/dl.

Discussion

An almost ideal correlation was found between glucose measurements by the hexokinase method and those by the glucose oxidase/peroxidase MBTH-DMA method, in the randomly selected

Table 5. Comparison of Results of Six Methods for Serum Glucose in Diabetic Patients on Oral Hypoglycemic Drugs

Measured glucose concentration, mg/dl					
Oxidase MBTH-DMA	Oxidase ABTS	Hexokinase	o-Toluidine	Neocuproine	Ferricyanide
		Tolazamide, 100 mg/day			
121	126	123	125	122	119
288	294	294	287	282	275
—	355	353	347	334	339
—	464	472	—	443	435
—	351	342	336	333	339
		Chlorpropamide, 250 mg/day			
155	157	152	156	163	157
125	119	118	128	122	126
258	264	253	253	257	260
334	339	324	335	331	—
191	186	190	195	188	182
		Tolbutamide, 500 mg/day			
104[a]	100[a]	100[a]	105[a]	115[a]	106[a]
244	247	234	240	239	243
207	206	201	207	208	213
93	95	99	99	97	106
204	204	212	209	201	201

[a] Tolbutamide, 250 mg/day.

sample study. The slope is 1.00 and the standard error, 3.10 mg/dl, is only slightly larger than the day-to-day standard deviation of the oxidase MBTH-DMA method, which is 2.9 mg/dl at a glucose concentration of 85 mg/dl. The *y*-intercept (1.57 mg/dl) indicates a small but real (*t*-value, 4.04) constant difference between the methods. Gochman and Schmitz (*5*) observed a slope of 1.01 and a *y*-intercept of −1 mg/dl when they compared their glucose oxidase/peroxidase MBTH-DMA (AutoAnalyzer II) method to a manual hexokinase procedure. Two chemical causes for disagreement between glucose measurements by the oxidase MBTH-DMA method and the hexokinase method were observed in our evaluation: ascorbic acid and bilirubin. Ascorbic acid interferes with both oxidase methods by acting as a reducing substance. Katz and DiSilvio (*14*) observed a small but statistically significant elevation of results by the neocuproine method at an ascorbic acid concentration of 16 mg/dl, which they described as the highest concentration attainable in serum. We observe a 10–12 mg/dl depression of glucose as measured by glucose oxidase methods when 15 mg/dl ascorbic acid is added, but no significant effect on the hexokinase method. Bilirubin interferes with the hexokinase method on the ACA, in which sample absorption is measured at 340 nm and blank absorption at 383 nm. Addition of bilirubin causes a high blank absorbance, resulting in analytical results that are too low. Bilirubin did not effect glucose oxidase/peroxidase MBTH-DMA, but it did elevate values from the undialyzed *o*-toluidine procedure. Comparison data for patients were consistent with this trend.

Miskiewicz et al. (*3*) have described the susceptibility of the alternative method to uric acid interference. Our data agree, in spite of slight system differences, and also indicate that the oxidase MBTH-DMA method is free of uric acid interference, as shown in the initial report by Gochman and Schmitz (*5*). We observed no significant interference from added uric acid (up to 25 mg/dl) on measurements with the *o*-toluidine method; thus our data do not confirm the 4 mg/dl decrease for each 10-mg increment in uric acid per deciliter reported by Powell and Djuh (*1*).

The comparison data from uremic and icteric samples indicate that it is not possible in the case of such sera to convert glucose concentrations measured by a given method into those one would expect to obtain from another method by use of factors, as suggested by Niejadlik et al. (*15*). Their suggestion that neocuproine glucose values be converted to hexokinase glucose values by subtracting 5 mg/dl from the neocuproine value is valid only for samples with no interfering substances. We find that hexokinase and neocuproine glucose measurements in uremic patients disagree by an average of 10 mg/dl, and we observed differences of up to 36 mg/dl for some patients' sera.

The good correlation of measurements by the oxidase MBTH-DMA and hexokinase methods for glucose suggests that the normal range for glucose as measured by the two methods should be nearly identical. We previously observed a mean of 90.4 mg/dl and a standard deviation of 9.9 mg/dl for a normal population by use of the ACA hexokinase method for

21

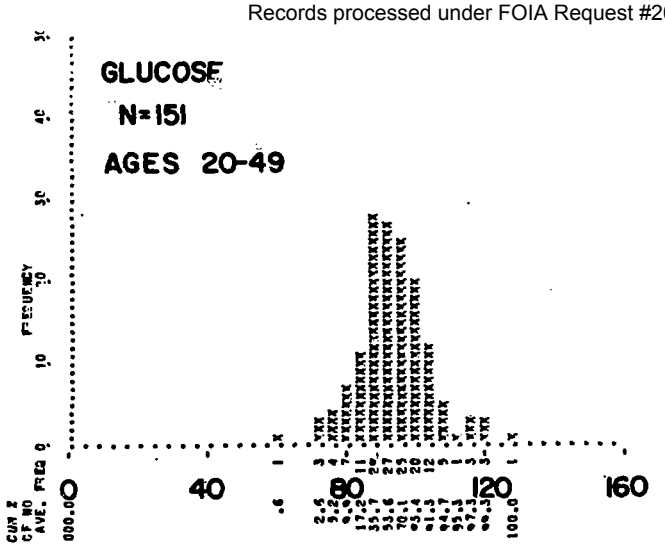


Fig. 4. Histogram of results by the glucose oxidase/peroxidase MBTH–A method for a normal population, age 20–49 years

y-axis, frequency, or number of samples; *x*-axis, figures in top row are the total number of samples in a given class interval, second-row figures (bold type) indicate glucose concentration in mg/dl, and third-row numbers are cumulative percentage figures. Mean, 92 mg/dl; mode, 85 mg/dl; 2.5–97.5 percentile range, 72–116 mg/dl

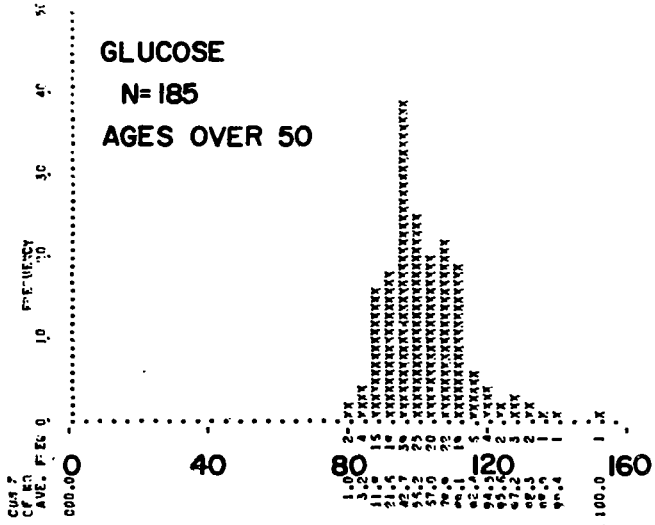


Fig. 5. Histogram of results by the glucose oxidase/peroxidase MBTH–DMA method for a normal population, age >50 years

Axes as in Figure 4. Mean, 111 mg/dl; mode, 95 mg/dl; 2.5–97.5 percentile range, 84–128 g/dl

glucose (*16*). The age distribution for that study was about the same as that of the 20–49 year age group observed in this report. The standard deviations for the previous study and this one are nearly identical (9.9 and 10.0 mg/dl, respectively), and subtracting the bias (2.0 mg/dl) between the oxidase MBTH–DMA and hexokinase methods for glucose from the population mean of 92 mg/dl observed here results in a mean of 90 mg/dl, the same as that of the previous study. The 2.5–97.5 percentile range of the previous study was 72–112 mg/dl, whereas here we observed a range of 72–116 mg/dl. Correction for the bias produces a range of 70–114 mg/dl, which differs by only 2 mg/dl on each end. The higher range for subjects older than 50 years demonstrates the age-related increase in serum glucose.

Acceptability of the Glucose Oxidase/Peroxidase MBTH–DMA Method

Performance of analytical methods must be judged most carefully at the concentrations that may be the basis of critical medical decisions. Barnett has suggested that the critical concentrations for glucose measurements are 50 and 120 mg/dl (*17*), and that an acceptable glucose method should have a standard deviation of 5 mg/dl or less at each of these concentrations. We extend this concept to define an acceptable limit of analytical error that includes random as well as systematic components (*18*). For glucose, the 95% limit of allowable error is 10 mg/dl if we accept Barnett's recommendation (2 SD, 95% limits). For the proposed method to be acceptable, it must produce results that are within 10 mg/dl of the "true" glucose value at 50 mg/dl and 120 mg/dl for all sera, including uremic and icteric specimens.

The systematic component of the error in a proposed method can be estimated by comparing results for specimens from patients, as measured by the proposed and reference methods (*18*). There is no universally accepted "standard" glucose method, so we have chosen the hexokinase method, which Cooper (*19*) has suggested as potentially the best reference glucose method. Therefore, systematic errors at 50 mg/dl and 120 mg/dl are obtained by use of the least-squares slope and intercept from data comparing the oxidase MBTH–DMA and hexokinase methods. The slope is 1.00, so that the systematic error is simply the intercept, 1.6 mg/dl, for both of the "decision concentrations." To systematic error we add the random error (±2 SD, 95% confidence limits) at the decision concentration. The day-to-day standard deviation, measured at a glucose concentration of 85 mg/dl, is 2.9 mg/dl, so the 2 SD value is 5.8 mg/dl. Adding random (5.8 mg/dl) and systematic (1.6 mg/dl) errors results in a total analytic error of 7.4 mg/dl at both 50 mg/dl and 120 mg/dl. No significant interferences were caused by bilirubin, creatinine, or uric acid. Ascorbic acid interfered significantly when added to sera, but comparisons of results for patients receiving ascorbic acid therapy to results produced by methods that are free of ascorbic acid interferences indicate no significant differences attributable to therapeutic administration of ascorbic acid. We, therefore, judge the glucose oxidase/peroxidase MBTH–DMA method to be acceptable for serum glucose measurements, because the analytical error is less than 10 mg/dl at glucose concentrations of 50 and 120 mg/dl. We lack sufficient data to judge with equal assurance the acceptability of this method for measurements of glucose in urine.

The assistance of the Clinical Chemistry Laboratory staff, and especially Lola Jenkins, Julie Hunzicker, Patricia Simmons, and Richard Miller, is gratefully acknowledged. Reagents and supplies were furnished by Technicon Corp.

References

1. Powell, J. B., and Djuh, Y. Y., A comparison of automated methods for glucose analysis in patients with uremia before and after dialysis. *Amer. J. Clin. Pathol.* **56**, 8 (1971).

2. Fingerhut, B., Automated serum glucose levels in uremia. *Amer. J. Clin. Pathol.* **51**, 157 (1969).

3. Miskiewicz, S. J., Arnett, B. B., and Simon, G. E., Evaluation of a glucose oxidase/peroxidase method adapted to the single-channel AutoAnalyzer and SMA 12/60. *Clin. Chem.* **19**, 253 (1973).

4. Peterson, J. I., and Young, D. S., Evaluation of the hexokinase/glucose-6-phosphate dehydrogenase method of determination of glucose in urine. *Anal. Biochem.* **23**, 301 (1967).

5. Gochman, N., and Schmitz, J. M., Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. *Clin. Chem.* **18**, 943 (1972).

6. Romano, A. T., Automated glucose methods: Evaluation of a glucose oxidase/peroxidase procedure. *Clin. Chem.* **19**, 1152 (1973).

7. Werner, W., Rey, H. C., and Wielinger, H., Uber die Eigenschaften eines neuen Chromogens fur die Blutzuckerbestimmung nach der GOD/POD-Methode. *Z. Anal. Chem.* **252**, 224 (1970).

8. Bigat, T. K., and Saifer, A., Some methodological modifications of the Technicon "SMA 12/60 AutoAnalyzer" system. *Clin. Chem.* **18**, 630 (1972).

9. Bittner, D. L., and Manning, J., Automated neocuproine glucose method: Critical factors and normal values. In *Automation in Analytical Chemistry, Technicon Symposia 1966*, **1**, N. B. Scova et al., Eds. Mediad, White Plains, N. Y., 1967, p 33.

10. Technicon Method Files, SMA 12/60, Technicon Instruments Corp., Tarrytown, N. Y. 10591.

11. Sudduth, N. C., Widish, J. R., and Moore, J. L., Automation of glucose measurement using *o*-toluidine reagent. *Amer. J. Clin. Pathol.* **53**, 181 (1970).

12. Technicon AutoAnalyzer Methodology, Method File N-26, Technicon Instruments Corp., Tarrytown, N. Y. 10591.

13. Sharp, P., Interferences in glucose oxidase/peroxidase blood glucose methods. *Clin. Chim. Acta* **40**, 115 (1972).

14. Katz, S. M., and DiSilvio, T. V., Ascorbic acid effects on serum glucose values. *J. Amer. Med. Ass.* **224**, 628 (1973).

15. Niejadlik, D. C., Dube, A. H., and Adamko, S. M., Glucose measurements and clinical correlations. *J. Amer. Med. Ass.* **224**, 1734 (1973).

16. Westgard, J. O., Hunt, M. R., and Lahmeyer, B. L., *Estimates of Normal Range for 15 Determinations on the duPont Automatic Clinical Analyzer*, E. I. du Pont de Nemours and Co., Wilmington, Del., 1972.

17. Barnett, R. N., Medical significance of laboratory results. *Amer. J. Clin. Pathol.* **50**, 671 (1968).

18. Westgard, J. O., Carey, R. N., and Eggert, A. A., Decisions on the acceptability of performance in method comparison studies. *Clin. Chem.* **19**, 650 (1973).

19. Cooper, G. R., Methods for determining the amount of glucose in blood. *Crit. Rev. Clin. Lab. Sci.* **4**, 101 (1973).

23

DEPARTMENT OF HEALTH AND HUMAN SERVICES **Public Health Service**

Food and Drug Administration
Center for Devices and
Radiological Health
8757 Georgia Avenue
Silver Spring, MD 20910

NOVEMBER 20, 1986

ABBOTT LABORATORIES
ATTN: KEITH MINTER
1921 HURD STREET
P.O. BOX 152020
IRVING, TX 75015

Ref : K864224
Product : ABBOTT SPECTRUM
GLUCOSE REAGENT

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by -- the Office of Device Evaluation. This information should be submitted in duplicate to:

Food and Drug Administration
Center for Devices and
Radiological Health
Document Mail Center (HFZ-401)
8757 Georgia Avenue
Silver Spring, Maryland 20910

When your additional information is received by the Office of Device Evaluation the 90-day period will begin again.

If after 30 days the requested information is not received, we will stop reviewing your submission. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and the 90-day time period will begin again.

If you have procedural or policy questions, please contact the Division of Small Manufacturers Assistance at their toll-free number (800) 638-2041 or me at (301) 427-8162

Sincerely yours,

Robert I. Chissler
Premarket Notification Coordinator
Office of Device Evaluation
Center for Devices and
Radiological Health

24

DO NOT REMOVE THIS ROUTE SLIP!!!!

K-86-4224 11/20/86

FROM:	LETTER DATE	LOGIN DATE	DUE DATE
ABBOTT LABORATORIES ATTN: KEITH MINTER 1921 HURD STREET P.O. BOX 152020 IRVING, TX 75015	10/17/86	10/28/86	01/26/87
	TYPE OF DOCUMENT: 510 (k)		CONTROL # K864224

TO:	
ODE/DMC	CONT. CONF.: ? STATUS : H REV PANEL : CH PAN/PROD CODE(S): CH/ / /

SUBJECT:
ABBOTT SPECTRUM GLUCOSE REAGENT

DECISION:	RQST INFO DATE: 11/20/86	INFO DUE DATE: 12/20/86
DECISION DATE: / /	DATE: / /	DATE: / /
	DATE: / /	DATE: / /

25

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Memorandum

Date 11/20/86

From REVIEWER(S) - NAME(S) Braden Staples Jr.

Subject 510(k) NOTIFICATION K864224

To THE RECORD

It is my recommendation that the subject 510(k) Notification:

_____ (A) Is substantially equivalent to marketed devices.

_____ (B) Requires premarket approval. NOT substantially equivalent to marketed devices.

__✓__ (C) Requires more data.

_____ (D) Is an incomplete submission. (See Submission Sheet).

Additional Comments:

The submitter requests:

_____ No Confidentiality

_____ Confidentiality for 90 days

_____ Continued Confidentiality exceeding 90 days

Class Code w/Panel: _____

REVIEW: _____
(BRANCH CHIEF) (DATE)

FINAL REVIEW: _____
(DIVISION DIRECTOR) (DATE)

26

MEMO RECORD | *AVOID ERRORS PUT IT IN WRITING* | DATE 11/20/86

FROM: Broden Staples Jr., Scientific Reviewer | OFFICE ODE

TO: Barbara Booker for Keith Minter, Regulatory Specialist | DIVISION DCLD

SUBJECT: Abbott Spectrum Glucose Reagent

SUMMARY

I called and spoke with Ms. Booker. I indicated the following:

1. The submission should indicate the clinical significance of quantitative urine glucose.

2. The submission should point out an equivalent pre-amendment quantitative urine glucose assay. Although labeling are included for an equivalent assay of Du Pont, it has not been shown to be pre-amendment. Labeling for the Du Pont Assay is dated 7/22/82.

Ms. Booker indicated the information would be assembled and submitted.

27

SIGNATURE: Broden Staples Jr.

DOCUMENT NO. K864224

FORM FDA 2034 (12/80)

DEPARTMENT OF HEALTH AND HUMAN SERVICES **Public Health Service**

Food and Drug Administration
Center for Devices and
Radiological Health
8757 Georgia Avenue
Silver Spring, MD 20910

OCTOBER 29, 1986

ABBOTT LABORATORIES
ATTN: KEITH MINTER
1921 HURD STREET
P.O. BOX 152020
IRVING, TX 75015

D.C. Number : K864224
Received : 10-28-86
Product : ABBOTT SPECTRUM GLUCOSE REAGENT

The Premarket Notification you have submitted as required under Section 510(k) of the Federal Food, Drug and Cosmetic Act for the above referenced device has been received and assigned a unique document control number (D.C. Number above). Please cite this D.C. Number in any future correspondence that relates to this submission.

We will notify you when the processing of this submission has been completed or if any additional information is required. You are required to wait ninety (90) days after the received date shown above or until receipt of a "substantially equivalent" letter before placing the product into commercial distribution. I suggest that you contact us if you have not been notified in writing at the end of this ninety (90) day period before you begin marketing your device. Written questions concerning the status of your submission should be sent to:

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
8757 Georgia Avenue
Silver Spring, Maryland 20910

If you have procedural or policy questions, please contact the Division of Small Manufacturers Assistance at their toll-free number (800) 638-2041 or me at (301) 427-8162

Sincerely yours,

Robert T. Chissler
Premarket Notification Coordinator
Office of Device Evaluation
Center for Devices and
Radiological Health

28

ABBOTT LABORATORIES

Diagnostics Division

K864224

1921 Hurd St.
P.O. Box 152020
Irving, Texas 75015-2020

October 17, 1986

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
8757 Georgia Avenue
Silver Spring, Maryland 20910

RE: SECTION 510(k) NOTIFICATION, ABBOTT SPECTRUM® GLUCOSE REAGENT KIT

Gentlemen:

Under section 510(k) of the Federal Food, Drug and Cosmetic Act, a manufacturer must notify the Food and Drug Administration at least 90 days prior to introducing a device into interstate commerce. The information following constitutes the 510(k) notification for the addition of a urine application for the ABBOTT SPECTRUM GLUCOSE REAGENT. This product was on the market prior to May 28, 1976, as the Abbott A-GENT® reagent using serum or plasma as a sample source. Under 21CFR part 807.81, paragraph 3, a 510(k) is required if the device is one that the company has in commercial distribution but that is about to be significantly changed. The intended use of the device is being expanded to include urine as a sample source and thus qualifies under this paragraph.

The ABBOTT SPECTRUM GLUCOSE REAGENT KIT (urine application) is substantially equivalent to the DuPont ACA Glucose Assay marketed by DuPont Co., Clinical Systems Division. It is our understanding that this diagnostic kit was on the market prior to May 28, 1976. The Clinical Chemistry panel has recommended that glucose test system be classified as Class II, performance standards. Please contact the undersigned at (214) 257-6154 if questions arise regarding this submission.

Sincerely,

ABBOTT DIAGNOSTICS

Keith Minter

Keith Minter
Regulatory Compliance Specialist

RECEIVED
FDA/BMDDP
1986 OCT 28 AM 8:39
DOCUMENT CONTROL
CENTER

0518Q-1

29

1. Name of Owner Submitting 510 (k): Abbott Laboratories
Abbott Park
North Chicago, IL 60064
Establishment Registration
Number: 1415939

2. Manufacturing Site: Abbott Laboratories
Diagnostics Division
820 Mission Street
South Pasadena, CA 91030
Establishment Registration
Number: 2018433

3. Trade Name: ABBOTT SPECTRUM GLUCOSE REAGENT

4. Common or Usual Name: Reagent system for the determination of Glucose in serum, plasma, or urine.

(b)(4) Confidential and Proprietary Information

[redacted]

6. Substantial Equivalence:

The performance of SPECTRUM GLUCOSE is substantially equivalent to the DuPont ACA Glucose Reagent.

The assays are similar in that:

a. They both utilize the Hexokinase methodology.

b. They both measure an end-point reaction bichromatically in the ultraviolet region (340/380 and 340/383nm for the SPECTRUM GLUCOSE and ACA GLUCOSE, respectively).

The assays are different in that:

(b)(4) Confidential and Proprietary Information

[redacted]

7. Supporting Data Attachments:

A. Comparison of Methods.

B. Performance Characteristics.

C. SPECTRUM GLUCOSE Insert and Procedure Card.

D. SPECTRUM GLUCOSE Label Copy.

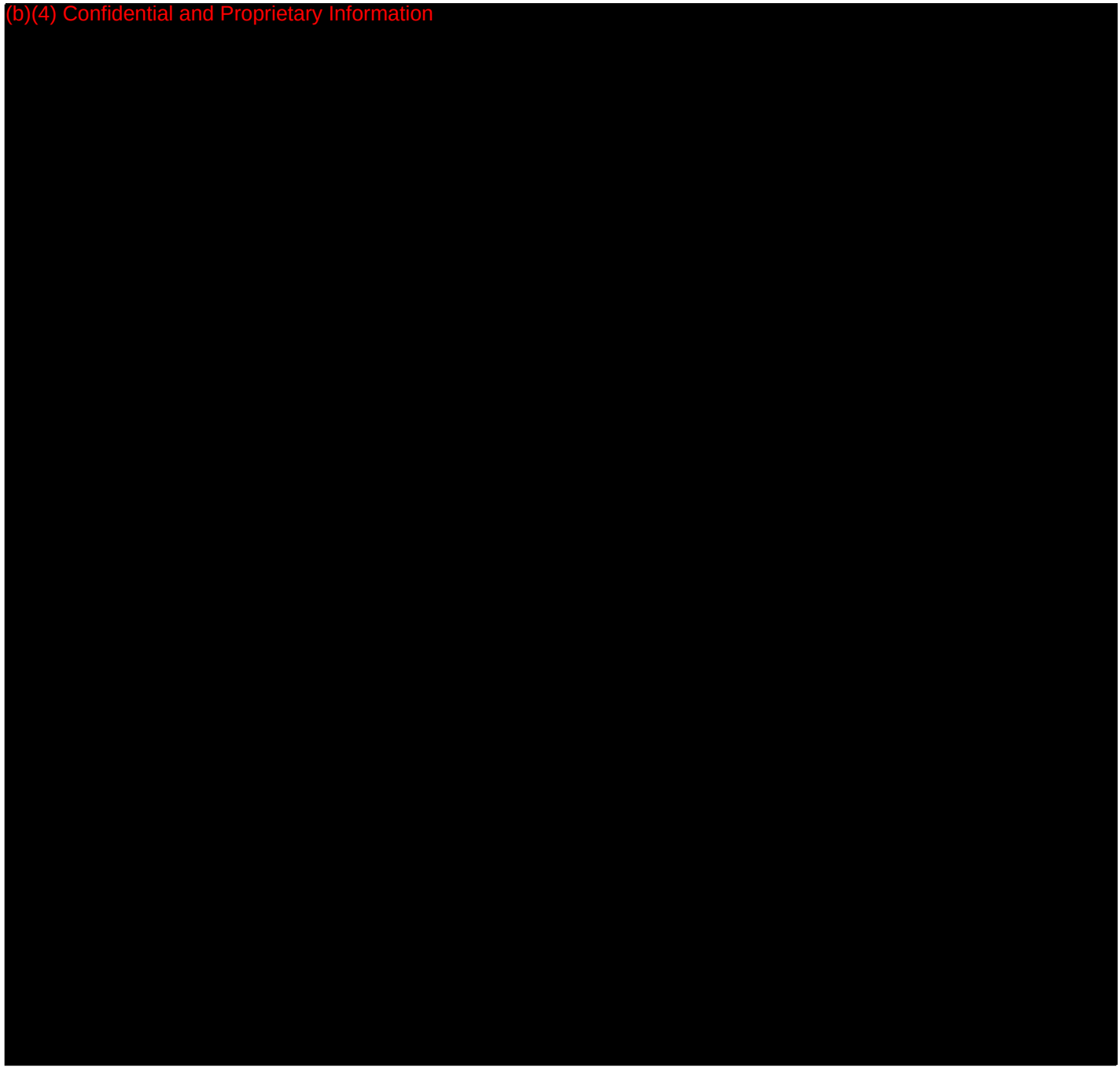
E. Package insert for DuPont ACA Assay Glucose.

0518Q-3

ATTACHMENT A

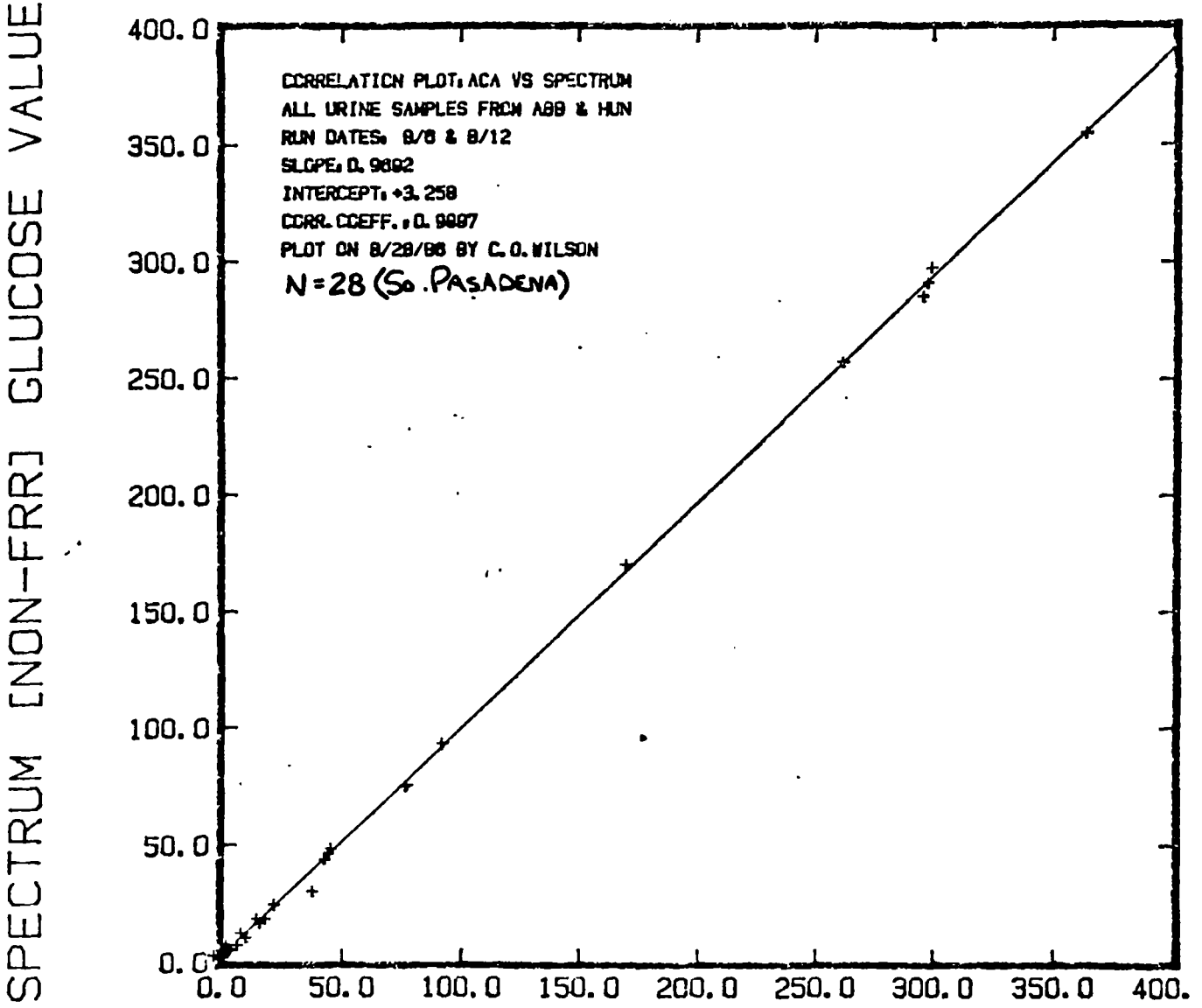
Comparison of Methods

(b)(4) Confidential and Proprietary Information

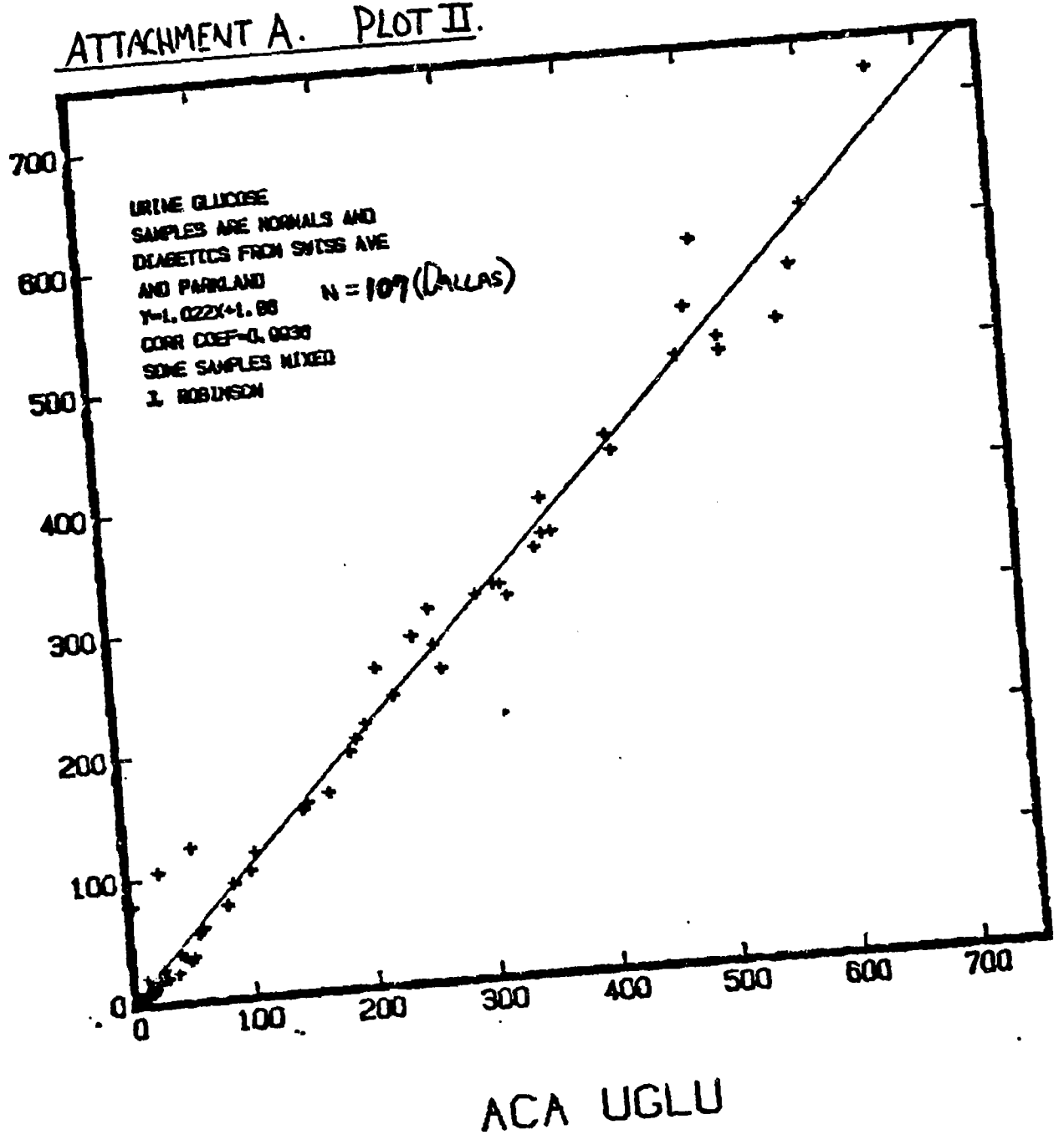


ATTACHMENT A. P LOT 1.

33



ATTACHMENT A. PLOT II.



ATTACHMENT B

Performance Characteristics

(b)(4) Confidential and Proprietary Information

[redacted]

35

Attachment B., Continued

(b)(4) Confidential and Proprietary Information

[redacted]

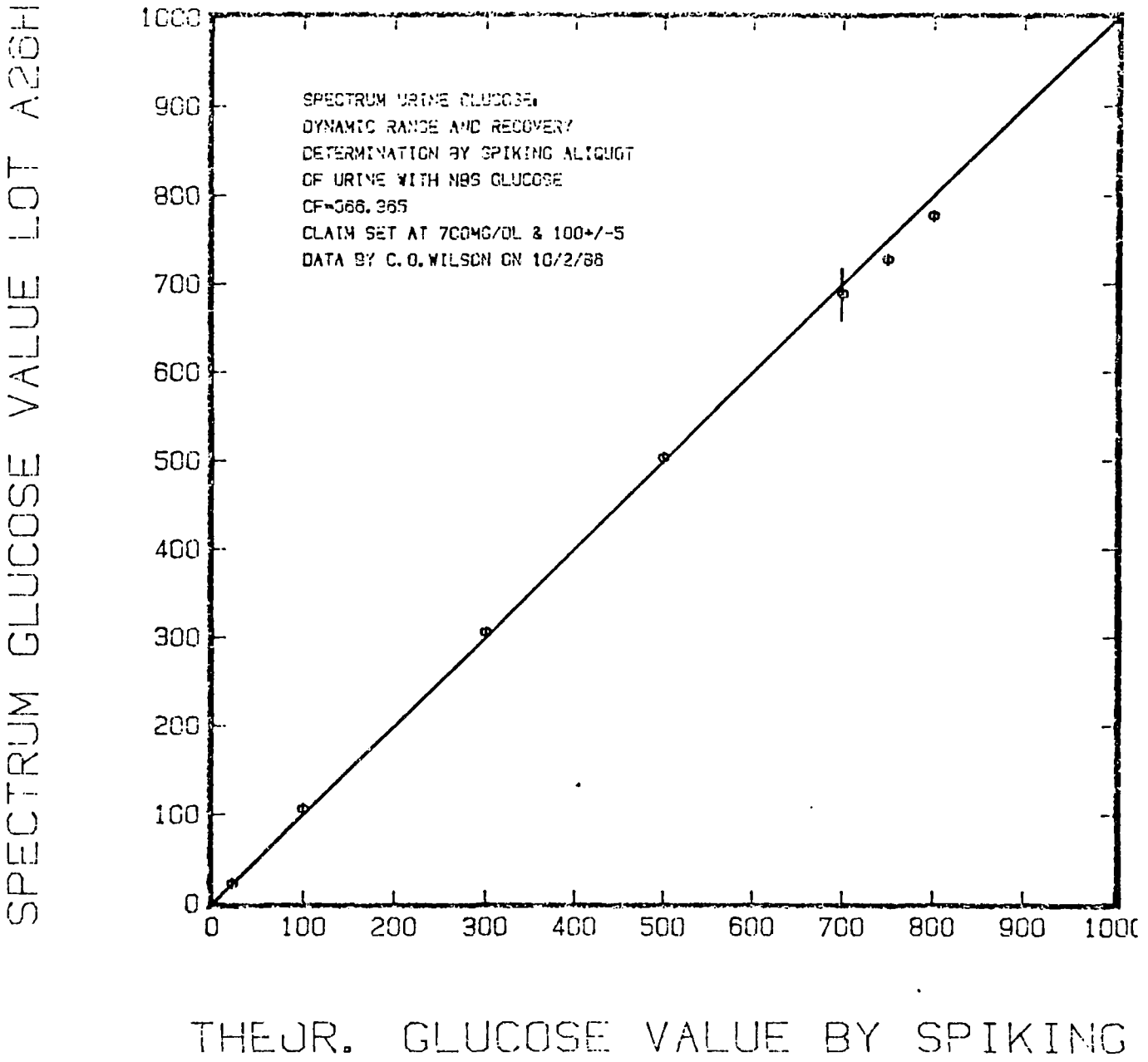
36

Attachment B., Continued

(b)(4) Confidential and Proprietary Information

[redacted]

37



PROPOSED LABELS AND LABELING

39

GLUCOSE

Intended Use

ABBOTT SPECTRUM Glucose Reagent is used with the ABBOTT SPECTRUM High Performance Diagnostic System for the quantitation of Glucose in serum, ~~or~~ plasma, OR URINE. Refer to the ABBOTT SPECTRUM Operator's Guide for details of instrument operation and specifications.

Reconstitution

Step 1. Record the lot number and expiration date found on the polyfoil bag, then open the bag and remove the reagent cartridge.

Step 2. Remove the reagent cap and place on the cap retainer.

Step 3. Tap the cartridge both upside down and right side up on a flat surface to facilitate reconstitution. Remove the seal.

Step 4. Add the volume of distilled or deionized water stated on the reagent cartridge label.

Step 5. Swirl the contents gently and ensure that reagent is adequately mixed. Place the septum on the cartridge opening.

CAUTION: Avoid splashing reagent on the septum as this will cause Motor O aborts or incorrect reagent fluid sensing.

Step 6. Write the date and time of reconstitution in the space provided on the cartridge label.

Step 7. Place the cartridge in the refrigerated reagent section.

Expected Results

Normal Range
FOR SERUM: 76 – 115 mg/dL and Plasma 4.22 – 6.38 mmol/L FOR URINE: **

To convert to SI units (mmol/L), multiply mg/dL by 0.0555.

It is recommended that each laboratory determine its own normal range based upon its particular locale and population characteristics.

**To be determined prior to market entry of urine application.

Storage and Stability

- Store the unopened reagent cartridge in the polyfoil bag at 28°C or less.
- Refer to the chart for reconstituted reagent.

Storage Stability	18°C–28°C	8 hours
	2°C–8°C	7 days
On Board Stability	Refrigerated	4 days

- Instability or deterioration of the reagent should be suspected if turbidity of the reagent is detected by the ABBOTT SPECTRUM at 660nm.

Precautions and Limitations

- For *In Vitro* diagnostic use.
- Do not use the components beyond the expiration date.
- Do not mix material from different lots.
- Do not mix reagents prepared at different times.
- Do not reuse the reagent cartridges or septum caps due to the risk of contamination and potential loss of reagent performance.
- This procedure is specific for glucose except as described under Specificity.

Specimen Collection and Preparation

Suitable Specimens	Serum ~~or~~ Plasma, OR URINE	Specimen Storage	
		RT	Several hours
		2°C–8°C	Several days

- Serum or plasma should be separated rapidly from the cells, since, at room temperature, whole blood glucose levels will decrease due to glycolysis.

Reagent Contents

	mmol/L
ATP, Na_2	1.8
Aspartate, Mg	1.2
NAD	1.5
Triethanolamine HCL	50.0
Na_2CO_3	16.5
Oxalate, K_2	9.0
	U/L
Hexokinase	833
Glucose-6-Phosphate Dehydrogenase	1,667

Inert ingredients: fillers and enzyme stabilizers.

40

Chemical Principles

Glucose in the sample is phosphorylated by Hexokinase in the presence of excess ATP. The product of the phosphorylation reaction, glucose-6-phosphate (G-6-P), is subsequently oxidized by Glucose-6-Phosphate Dehydrogenase (G-6-PDH) *with* the concomitant reduction of NAD^+ to NADH. The extent of NADH formation is measured at 340nm and is proportional to the amount of glucose present in the sample.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\text{Hexokinase}} \text{ADP} + \text{G-6-P}$$

$$\text{G-6-P} + \text{NAD}^+ + H_2O \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{NADH} + H^+$$

Procedure and Results

Materials Provided:

ABBOTT SPECTRUM GLUCOSE Reagent

List Number	Test Size	Reconstituted Volume
1340-03	6 x 50 tests	14mL
1340-05	6 x 150 tests	40mL
1340-06	6 x 250 tests	65mL

6 Septum Caps per List Number

Materials Required but Not Provided:

- ABBOTT SPECTRUM Multi Calibrator Set
- Distilled or Deionized Water
- Pipet for reagent reconstitution

Calibration

It is generally considered good laboratory practice to include control sera with unknown samples. Values obtained using commercial sera should be within range specified by the manufacturer as acceptable for this method.

Test Parameters (FOR SERUM and Plasma)

TEST PARAMETER FILE. TEST DEFINITION

ENTRY NAME GLU	TEST NO 16	SAMPLE VOL (uL)	+2.50	
REPORT NAME	GLUCOSE	TEMPERATURE (C)	37	NEXT TEST
REACTION TYPE	UP LIN CAL	OUT RANGE SAMPLE DIL	0	DELETE TEST
MATH MODEL	LIN REG END PT	SEC UNITS FACTOR	+.055	
UNITS (PRIM) MG/DL	(SEC) MMOL/L	NO OF REAGENTS	1	
NORMAL RANGE +70.0 TO +115.		SERUM BLANK SAME CELL		SAVE TEST
		INST FACTOR MULT	+1.00	
		INST FACTOR INTR	0.00	
CALIBRATOR NAME NO				
LEVEL 1 user* MCC1 1		CAL MODE CAL ON CMD		
LEVEL 2 user* MCC2 1		CAL LEVEL	0	DEFINE REAGENT
LEVEL 3 user* MCC3 1		CAL INTERVAL (HR)	24	
LEVEL 4 0.00 WATER 1		REF CAL FACTOR	+.383	
LEVEL 5 0.00 0		% TOL OF CAL FACTOR	10	
LEVEL 6 0.00 0		% TOL OF CAL	10	
AUTO PRINT (time & date)				RETURN

*user programmed values

TEST PARAMETER FILE REAGENT DEFINITION

REAGENT NUMBER 1 FOR TEST GLUCOSE

REAGENT NAME	GLUCOSE	ASSAY RANGE HIGH	+700	ABORT
REAGENT VOLUME (uL)	+236	ASSAY RANGE LOW	[illegible]	
TIME OF ADDITION (SEC)	0	INITIAL Ad	+.250	
		ABS LIMIT (Ad)	[illegible]	
FIRST READ TIME (SEC)	240	REAGENT BLANK	NO	
LAST READ TIME (SEC)	1500			SAVE
NUMBER OF READS	1	OUT RANGE READ DIL	2	NEXT
READ INTERVAL (SEC)	+60.0			REAGENT
WAVELENGTHS E.F		MIX TIME (SEC)	+1.00	
1 340/ 380 +4.76		MIX DELAY (SEC)	0	
2 340/ 380 0.00		DEGRADATION (Ad)	0.00	
3 340/ 380 0.00		INTEGRITY (COUNTS)	0	
4 340/ 380 0.00		COOLING		
5 340/ 380 0.00				
6 340/ nA 0.00				
AUTO PRINT (time & date)				

+7.0 cm
2.40 cm

41

(b)(4) Confidential and Proprietary Information

[redacted]

42

GLUCOSE

(b)(4) Confidential and Proprietary Information

[redacted]

43

ATTACHMENT C. SPECTRUM
GLUCOSE INSERT

ABBOTT

SPECTRUM®

High Performance Diagnostic System

GLUCOSE Reagent

NAME AND INTENDED USE

ABBOTT SPECTRUM Glucose Reagent is optimized for use with the ABBOTT SPECTRUM High Performance Diagnostic System for the *quantitation of Glucose in serum or plasma*, OR URINE

SUMMARY OF THE TEST

The enzymatic determination of glucose is based on the coupled reaction of hexokinase and glucose-6-phosphate dehydrogenase. Glucose-6-phosphate which is generated by the phosphorylation of glucose, reacts with NAD in the presence of glucose-6-phosphate dehydrogenase. The increase in absorbance in the ultraviolet region as NAD is reduced to NADH is directly proportional to the concentration of glucose in the sample.

REAGENTS

Each reagent cartridge contains:	mmol/L		U/L
ATP, Na_2	1.8	Hexokinase	833
Aspartate, Mg	1.2	Glucose-6-Phosphate	
NAD	1.5	Dehydrogenase	1,667
Triethanolamine HCl	50.0		
Na_2CO_3	16.5		
Oxalate, K_2	9.0		

Inert ingredients: fillers and enzyme stabilizers.

RECONSTITUTION

- Remove the reagent cartridge from the polyfoil bag.
- Remove the plastic cap from the reagent cartridge opening and place onto the cap retainer (located directly behind the opening).
- Tap the cartridge on a flat surface to dislodge any powder adhering to the foil seal.
- Remove the foil seal.
- Reconstitute the reagent by adding the volume of distilled or deionized water stated on the reagent cartridge label.
- Swirl the contents gently.
- Note date and time of reconstitution in the space provided on the reagent cartridge label.
- Position the cartridge in the refrigerated section of the reagent carousel.

STORAGE

- Store the unopened reagent cartridge in the polyfoil bag at 28°C or less.
- Reconstituted reagent is stable for 8 hours at 18° to 28°C or for 7 days if immediately refrigerated at 2° to 8°C.
- Refer to the Glucose Procedure Card/Assay Manual for on-board stability information.

PRECAUTIONS

- For *In Vitro* diagnostic use.
- Ensure that contents are adequately mixed.
- Do not use the components beyond the expiration date.
- Do not reuse the cartridge due to the risk of contamination and potential loss of reagent performance.

SYSTEM INFORMATION

44

LABEL COPY FOR SPECTRUM GLUCOSE

A. POUCH LABEL

Lot No. Expiration Date

ABBOTT
SPECTRUM
GLUCOSE Reagent
List No. 1340-03

14 mL
Contents: 50 test
cartridge

For In Vitro Diagnostic Use
Store at 28°C or less
Active Ingredients:
Hexokinase
Glucose-6-Phosphate Dehydrogenase
ATP
NAD

ABBOTT HEALTH CARE PRODUCTS, INC.
NORTH CHICAGO, IL 60064 USA

B. CARTRIDGE LABEL

Lot No. _____ Exp. Date _____
ABBOTT
SPECTRUM
GLUCOSE
Reagent
14 mL
Date and Time of Reconstitution

ABBOTT HEALTH CARE PRODUCTS, INC.
North Chicago, Il 60064 USA

C. CARTON LABEL

Lot No. _____ Exp. Date _____
For the Determination of/Zur Bestimmung von/Pour la determination du
Para la determinacion del/Per la determinazione del
GLUCOSE
GLUKOSE/GLUCOSA
in Serum, Plasma or Urine
List No. 1340-03
Composition: 6 x 50 test
cartridges
Reagenzbehalter
Cartouches de reactif
Cartuchos de reactivo
Cartucce di reagente
6 Septum Caps/Septum-Verschlusse/Capuchons en caoutchouc
Cabezales de proteccion/Tappi di protezione

Store at 28°C or less
Bei maximal 28°C lagern
Conserver a 28°C ou moins
Conservar a 28°C o inferior
Conservare non superiore a 28°C
For In-Vitro Diagnostic Use
See package insert

ABBOTT HEALTH CARE PRODUCTS, INC.
NORTH CHICAGO, IL 60064 USA

45

ATTACHMENT E:

PACKAGE INSERT FOR DUPONT ACA

GLUCOSE ASSAY

aca™ DU PONT DISCRETE CLINICAL ANALYZER

GLUC
GLUCOSE

INTENDED USE:

The GLUC pack is used in the Du Pont aca™ discrete clinical analyzer to quantitatively measure glucose in serum, plasma,[1] spinal fluid,[2] and urine[3] samples.

SUMMARY:

The glucose (GLUC) method is an adaptation of the hexokinase — glucose-6-phosphate dehydrogenase method.[4] This method is more specific than general reducing methods and will give results 5-10% lower than those obtained by such reducing methods.[5]

Split-sample comparison between the GLUC method and the National Glucose Reference Method gave a slope of 1.022, an intercept of 3.3 mg/dL and a standard error of regression of 1.7 mg/dL. Typical within-run and day-to-day coefficients of variation were less than 1.5%.[6]

PRINCIPLES OF PROCEDURE:

Hexokinase (HK) catalyzes the phosphorylation of glucose by adenosine-5'-triphosphate (ATP) to glucose-6-phosphate, which is oxidized to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase (G-6-PDH) with simultaneous reduction of nicotinamide-adenine dinucleotide phosphate (NADP). One mole of NADP is reduced to one mole of NADPH for each mole of glucose present. The absorbance due to NADPH (and thus the glucose concentration) is determined using a two-filter (340—383 nm) end point technique.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{HK} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconolactone} + \text{NADPH} + \text{H}^{+}$$

REAGENTS:

Compartment[a]	Form	Ingredient	Quantity[b]	Source
#1	Liquid	HK, G-6-PDH, Stabilizers and Microbial Inhibitors	10 U 1.6 U	Yeast Yeast
#2	Tablet[c]	Buffer and Activator		
#3	Tablet[c]	ATP and Buffer	5.1 μmol	
#4	Tablet[c]	NADP	3.4 μmol	

a. Compartments are numbered 1-7, with compartment #7 located closest to pack fill position #2.

b. Nominal value at manufacture.

c. Tablet contains excipients.

PRECAUTIONS:

USED PACKS CONTAIN HUMAN BODY FLUIDS; HANDLE WITH APPROPRIATE CARE.

FOR *IN VITRO* DIAGNOSTIC USE

MIXING & DILUTION:

The aca™ analyzer automatically aspirates a 40 μL sample of body fluid from the sample cup and injects it into the pack, along with the 4.960 mL of Purified Water. The sample cup must contain a sufficient quantity of body fluid to accommodate the 40 μL sample size plus the 120 μL "dead volume" of the cup. Precise filling of the cup by the operator is not required. The micro sample cup insert, with a total volume of 500 μL and a "dead volume" of 10 μL, may also be used.

STORAGE INSTRUCTIONS:

Store under refrigeration (2—8°C). Do not freeze. Do not expose packs to temperatures above 35°C. Do not expose packs to direct sunlight.

EXPIRATION:

Refer to EXPIRATION DATE on the tray label.

SPECIMEN COLLECTION:

Normal procedures for collecting and storing serum, urine, or cerebrospinal fluid may be used for samples to be analyzed by this method. Unless specimens contain sodium fluoride or sodium iodoacetate, avoid prolonged contact of the serum with the separated red cells.[7] EDTA-plasma must be analyzed within 10-15 min. of collection (see below).

KNOWN INTERFERING SUBSTANCES[8]

Specimens collected with EDTA and analyzed within 10-15 minutes of collection will give glucose values approximately 0-1.5 mg/dL [0.08 mmol/L] higher than values from a corresponding serum specimen. However, if these specimens remain in contact with cells, the glucose concentration will decrease at an approximate rate of 5-10 mg/dL [0.28-0.56 mmol/L] per h.

47

- Sodium fluoride and sodium iodacetate do not interfere.[1]
- Bilirubin at 17 mg/dL [0.29 mmol/L] has been reported to depress GLUC results by 9 mg/dL [0.50 mmol/L].[3]
- Visibly hemolyzed samples may give falsely depressed results.[9]
- It has been reported that ascorbic acid, 2.5-25 mg/dL [142-1420 μmol/L]; creatinine, 2.5-12.5 mg/dL[221-1105 μmol/L], dextran-40, 0.5-2.5 g/dL; dextran -75, 0.3-1.5 g/dL and uric acid; 5-25 mg/dL [0.30-1.48 mmol/L] have negligible effect on GLUC readouts.[3]
- Turbidity associated with lipemic specimens has been shown to cause a positive interference at triglyceride levels ≥500 mg/dL [54.5 mmol/L]. At this level the interference is equivalent to approximately 3 mg/dL [0.17 mmol/L] glucose and increases at an average rate of approximately 2 mg/dL [0.11 mmol/L] glucose per 100 mg/dL [11 mmol/L] increments of triglyceride. Some samples show considerable deviation from this average.[10]
- Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

Preset Glucose Test Conditions

• Sample Size:	40 μL
• Diluent:	Purified Water
• Test Temperature:	37.0 ± 0.1°C
• Reaction Period (Initiation to measurement):	261.5 seconds
• Wavelengths:	340 and 383 nm
• Type of Measurement:	Two filter, end point
• Decimal Point Location:	0000. mg/dL [00.00 mmol/L]

aca™ I/II analyzer:

• Assigned Starting Point:	9983. [99.06]
• Scale Factor:	0.0448 (mg/dL)/count[d] [0.2489 mmol/L/count]

aca™ III analyzer:

• Assigned Offset C_0:	−1.700 E-1 [−9.436 E-1]
• Linear Term C_1:	−4.484 E-1 mg/dL[d] [−2.489 E-2 mmol/L]

d. The preset scale factor (linear term) was calculated from an absorbance to concentration relationship (sensitivity) of 2.23 mA/(mg/dL) [40.17 mA/(mmol/L)]. Due to small differences in filters and electronic components between instruments, the actual scale factor (linear term) may differ from that given above.

PROCEDURE:

TEST MATERIALS

Quantity	Item	Du Pont Cat. #
1	GLUC Test Pack	705104901
1	Sample System Packet or	701989901
1	Micro Sample System Packet and	702694901
	Micro Sample System Holders	702785000
	Dylux® Photosensitive Printer Paper	700036000
	Purified Water	704209901
	Cell Wash Solution	701864901

®Registered trademark, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE.

TEST STEPS

When running analytical test packs, the operator need be concerned only with loading the sample and appropriate test packs into a properly prepared instrument. The instrument automatically advances the packs through the test steps and prints the result. For details of sample preparation and pack processing, refer to Section III of the Instrument Manual.

CALIBRATION

The general calibration procedure is described in the Instrument Manual.

The following information should be considered when calibrating the GLUC channel:

• Range of Linearity:	0—500 mg/dL [0-28 mmol/L]
• Reference Materials:	Primary standards or secondary calibrators.
• Suggested Calibration Levels:	460, 115, and 85 mg/dL [25, 10, 5 mmol/L]
• Starting Point (Offset C_0) Adjustment:	For aca™ I analyzer, relace zero offset (ZO) on the photometer method switching board (no adjustable ZO). For aca™ II analyzer, use adjustable starting point for all four digits. For aca™ III analyzer, enter offset C_0 into Method Memory.
• Scale Factor (Linear Term C_1) Adjustment:	May be required for different pack lots.

48

- Count By (aca™ I/II analyzer): One (1)
- Readout Units: The aca™ analyzer prints out in 1-mg/dL [0.01 mmol/L] increments.

QUALITY CONTROL

Two types of quality control procedures are recommended:

- **General Instrument Check.** Refer to the Filter Balance Procedure and the Absorbance Test Method described in the Instrument Manual. Refer also to the ABS Test Methodology literature.
- **Glucose Method Check.** At least once daily run a GLUC test pack on a solution of known glucose concentration such as an assayed control or calibration standard other than that used to calibrate the GLUC channel. For further details review the Quality Assurance Section of the Chemistry Manual. The result obtained should fall within acceptable limits defined by the day-to-day variability of the system as measured in the user's laboratory. (See SPECIFIC PERFORMANCE CHARACTERISTICS for guidance.) If the result falls outside the laboratory's acceptable limits, follow the procedure outlined in the Chemistry Troubleshooting Section of the Chemistry Manual.

A standard deviation for five consecutive packs greater than 3.6 mg/dL [0.20 mmol/L] for a level of 100 mg/dL [5.55 mmol/L] or greater than 12.3 mg/dL [0.68 mmol/L] for a level of 500 mg/dL [27.75 mmol/L] indicates a possible system malfunction.

RESULTS:

The instrument automatically calculates and prints the concentration of GLUC in mg/dL [mmol/L] using the general scheme #1 illustrated in the Calculation of Results Section of the Chemistry Manual.

Information specific to the GLUC calculation is listed below:

aca™ I/II analyzer:

- Count By: One (1)
- Scale Factor: 0.0448 (mg/dL)/count[d] [0.2489(mmol/L)/count]

aca™ III analyzer:

- Linear Term C_1: −4.484 E-1 mg/dL[d] [−2.489 E-2 mmol/L]

LIMITATION OF PROCEDURE:

GLUC readouts in excess of 500 mg/dL [28 mmol/L] should be repeated after diluting the sample with Purified Water to produce a sample concentration within the range of linearity. The resulting readout must then be multiplied by the dilution factor to give the GLUC concentration of the undiluted sample. A readout in the vicinity of 1000 mg/dL for an undiluted sample may be 10—15% low due to non-linearity.[11] EDTA-plasma will give low results unless it it analyzed within 10-15 minutes of collection.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing a letter code or word immediately following the numerical value should be held for follow-up. Refer to the Instrument Manual.

REFERENCE INTERVAL (Normal Range):

70—110 mg/dL [3.9-6.1 mmol/L] (serum)[12, 13]

Each laboratory should establish its own reference interval for GLUC. This reference interval was calculated non-parametrically and represents the central 95% of a population of 217 apparently healthy, fasting (12 h) subjects, equally distributed between males and females and ages 20 to 65.

SPECIFIC PERFORMANCE CHARACTERISTICS[e]:

REPRODUCIBILITY (Precision)

	Within-Run[f]		Day-to-Day[f]	
MEAN	S.D.	(% C.V.)	S.D.	(% C.V.)
87.9 mg/dL [4.88 mmol/L]	1.2 [0.07]	1.4	0.9 [0.05]	1.0
221.1 mg/dL [12.28 mmol/L]	1.5 [0.08]	0.2	2.1 [0.12]	1.0
429.6 mg/dL [23.87 mmol/L]	2.3 [0.13]	0.5	3.6 [0.20]	0.8

LINEARITY

0—500 mg/dL [0-28 mmol/L]

e. All SPECIFIC PERFORMANCE CHARACTERISTICS tests were run after normal recommended equipment quality control checks were performed (see Instrument Manual).

f. Lyophilized control material was reconstituted and analyzed in triplicate, twice a day for 20 days for a total of 120 observations at each level. The within-run and day-to-day precision were calculated by the analysis of variance method.[4]

BIBLIOGRAPHY:

[1] DuBois, J.A., Roberts, G. and Beekman, J., An extended evaluation of the Du Pont aca glucose (GLUC) method: a comparison of blood collection systems. *Clin. Chem.* 25:1126 (1979).

[2] Greenawald, K.A., Speicher, C.E., Evers, W. and Henry, J.B., Glucose content in cerebrospinal fluid, *Am. J. Clin. Pathol.* 53:518-520 (1973).

[3] Carey, R.N., Feldbruegge, D., Westgard, J.O., Evaluation of the adaptation of the glucose oxidase/peroxidase-3-methyl-2-benzothiazolinone hydrazone-N,N-dimethylaniline procedure to the Technicon SMA 12/60 and comparison with other automated methods for glucose. *Clin. Chem.* 20:595-602 (1974).

49

[4]Slein, M.W., D-Glucose: Determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*, H-U. Bergmeyer, Ed., Academic Press, New York, N.Y. 1965, pp. 117-119.

[5]Westgard, J.O., and Lahmeyer, B.L., Comparison of Results from the Du Pont ACA and Technicon SMA 12/60. *Clin. Chem.* 18:340-348 (1972).

[6]Garber, C.C., Westgard, J.O., Milz, L. and Larson, F.C., Du Pont ACA III performance as tested according to NCCLS guidelines. *Clin. Chem.* 25:1730-1738 (1979).

[7]Tietz, N.W., *Fundamentals of Clinical Chemistry*, W. B. Saunders Co. Philadelphia, Pa. (1976), pp. 47-52 (general specimen collection and handling recommendations) and pp. 242-244 (glucose specific recommendations for specimen collection and handling).

[8]Supplementary information about the effects of drugs and patient condition on in vivo or in vitro levels of glucose can be found in "Effects of Drugs on Clinical Laboratory Tests," *Clin. Chem.* 12(5) (1975), and "Effects of Disease on Clinical Laboratory Tests," *Clin. Chem.* 26(4) (1980), Supplementary Issue.

[9]Gochman, N., Ryan, W.T., Sterling, R.E. and Widdowson, G.M., Interlaboratory comparison of enzymatic methods for serum glucose determination. *Clin. Chem.* 21:356-361 (1975).

[10]Merrill, C. and Statland, B.E., The effect of turbidity on serum glucose results as determined on the Du Pont ACA, *Clin. Chem.* 27:1051 (1981).

[11]Perry, B.W., Hosty, T.A., Coker, J.G., Doumas, B., Straumfjord, J.V., "A Field Evaluation of the Du Pont Automatic Clinical Analyzer," University of Alabama Medical Center, Birmingham, Ala., 1970. (An evaluation of eight of the Du Pont ACA test methodologies.)[g]

[12]Westgard, J.O., Hunt, M.R., Lahmeyer, B.L., "Estimates of Normal Range for 15 Determinations on the Du Pont Automatic Clinical Analyzer," University of Wisconsin Center for Health Science, Madison, Wisc. (Describes 12-hour fasting samples from a group of 217 apparently normal subjects, approximately equally distributed between males and females ages 20 to 65.[g]

[13]Speicher, C.E., Mohammed, M.C., Fetrat, E., Fiske, M.L., and Henry, J.B., "An Automatic Clinical Analyzer: A Critical Evaluation," *Am. J. Clin. Pathol.*, 57, p. 643, 1972.

g. Reprints available from the Du Pont Company, Clinical Systems Division.