Medical Intelligence

Evaluation of Some Commonly Used Semiquantitative Methods for Urinary Glucose and Ketone Determinations

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SUMMARY

The 2 Drop Clinitest, 5 Drop Clinitest, Tes-Tape, and Keto-diastix semiquantitative urinary glucose methods were evaluated using 300 urine samples from diabetic patients and comparing the results with those obtained by an AutoAnalyzer-glucose oxidase method. At high levels of glycosuria (above 1,500 mg./100 ml.) the 2 Drop Clinitest method gave better quantitation than the other methods. In this range the Keto-diastix method often gave falsely low results. At intermediate levels of glycosuria (376 to 1,500 mg./100 ml.) there appeared to be little or no difference among the methods. The 2 Drop Clinitest method was often insensitive to levels of urinary glucose below 376 mg./100 ml. In this range the other three methods gave comparable results, with the exception that Tes-Tape was sometimes positive with normal levels (1 to 15 mg./100 ml.) of glycosuria. Proteinuria and pregnancy had no effect on any of the methods. Acetest was a more sensitive and accurate indicator of urinary ketone levels than Keto-diastix. DIABETES *23*:474-79, May, 1974.

To be useful, a semiquantitative test for urinary glucose must provide information for evaluation of diabetic control. In selecting such a method, at least four questions arise: (1) What information is needed? (2) Is the test designed to obtain the needed information? (3) Does the test provide the information it is designed to obtain? and (4) Is the test designed so that it can be performed correctly by the patient? The purpose of this investigation was to answer the third question as it applies to four commonly used semiquantitative tests of urinary glucose concentration (Keto-diastix,* Tes-Tape,† 2 Drop Clinitest* and 5 Drop Clinitest*). In a test of 300 urine samples, values obtained with each of these methods and a quantitative glucose oxidase method were compared.

In addition the Keto-diastix* and Acetest* methods for measuring urinary ketone levels were compared.

MATERIALS AND METHODS

Materials. The testing materials were obtained from commercial sources. The reagents used for glucose determinations by the AutoAnalyzer were obtained as a commercial preparation (God-Perid-aa reagent) from Boehringer Mannheim Corporation, New York, N.Y. Lithium salt of acetoacetic acid was purchased from the Sigma Chemical Company, St. Louis, Missouri.

Laboratory methods. Of the 300 urine samples, 275 were obtained at the University of Colorado Medical Center and the Denver Veterans Administration Hospital and twenty-five were collected at the University of Missouri Medical Center. The specimens were obtained at random from diabetic patients, with the exception that the number of aglycosuric specimens was restricted (twenty-eight of 300). All samples were tested within two hours of collection by each of the four semiquantitative methods and were considered to contain glucose when at least one method gave a positive result. By these criteria 272 contained glucose. The samples were then immediately frozen without acidification for subsequent quantitative glucose de-

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terminations. They remained frozen from six to twelve months. After the 275 samples obtained in Colorado had been transported to Columbia, Missouri in the frozen state, they were thawed and Keto-diastix determinations were repeated on all samples. In both states all procedures were performed in air-conditioned buildings.

Ketone levels were determined in fresh urines with the Acetest and Keto-diastix methods. The presence or absence of proteinuria was determined with Uristix*. The Uristix, Tes-Tape, Keto-diastix, and Acetest procedures were performed according to the manufacturers' directions outlined on each container. The 2 Drop and 5 Drop Clinitest methods were performed according to directions with a Clinitest Kit and appropriate color charts. All tests were done by one of us (R.C.J.).

Quantitative glucose determinations were performed by a modification of the AutoAnalyzer-glucose oxidase method described by Furedi et al.[1] The absorbancy of the chromogen was read at 420 nm. instead of 600 nm. In order to remove ascorbic acid and uric acid (substances which may interfere with glucose oxidase methods), urine samples and standards were treated with a mixture of Lloyd's reagent and Norite (1.5 gm: 50 mg.) in a manner similar to that described by Kingsley and Getchell.[2]

Data analysis. The changes in glucose values obtained with the 2 Drop Clinitest, 5 Drop Clinitest, and Keto-diastix methods were evaluated after the urine samples were brought from Colorado to Missouri. At glucose concentrations above 1/4 per cent, the glucose portion of the Keto-diastix often becomes speckled with shades of brown and green. Changes in this phenomenon which occurred upon transportation of the urine samples were studied.

Since some of the methods may give better results at certain glucose levels than at others, the urine specimens and data were arbitrarily divided into three groups according to the level of glucose obtained by the AutoAnalyzer-glucose oxidase method: Low range 0 to 375 mg./100 ml.; Medium range, 376 to 1500 mg./100 ml.; High range, > 1,500 mg./100 ml. Within each of these ranges the methods were compared with each other with respect to how well they gave correct results as determined by the AutoAnalyzer-glucose oxidase method.

Of the 300 urine samples tested in this study, twenty-one were from pregnant women and forty-seven contained protein. For each of the semiquantitative methods, values obtained with the urine samples from the twenty-one pregnant and the 279 nonpregnant individuals were compared with each other for proportions correct. Similar comparisons were made between the forty-seven proteinuric and the 253 nonproteinuric specimens.

Finally, differences in the level of ketonuria obtained employing the Keto-diastix and Acetest methods were investigated. Further studies were done using ketone-free urine samples to which the lithium salt of acetoacetic acid had been added in known concentrations.

The data were analyzed for statistical significance by the McNemar, Sign, or Chi-square tests.[3]

RESULTS

With the Keto-diastix method, 187 samples produced the same result in Colorado and Missouri; seventy-eight samples gave a higher and ten a lower value in Missouri than in Colorado. Using the Sign test,[3] this is statistically significant ($P < 0.001$). No such differences were noted using either the 2 Drop or 5 Drop Clinitest methods.

Changes in the speckling phenomenon of the Keto-diastix method upon transport of the urine samples to Missouri were similarly investigated. One hundred and eighty samples showed no change; seventy-nine showed speckling in Colorado but not in Missouri, while the converse was true for sixteen samples. This change is statistically significant ($P < 0.001$). No relationship between salicylate or vitamin ingestion and the changes in glucose levels or speckling with the Keto-diastix method could be found.

To determine whether test results are consistent with those expected, limits of urinary glucose concentrations anticipated to give each possible result with the semiquantitative methods were defined. They are shown on the horizontal scales of figures 1 to 5 and are, with one exception, midway between the values indicated on the corresponding color charts. They differ from one figure to another because the color charts for the four methods are different. The hatched line of figure 3 represents the division between the medium and high ranges and does not correspond to limits of glucose concentrations expected to give certain results.

The figures show the number of urinary glucose determinations giving certain results at each level within the three glucose ranges. P values for comparison of the different methods in each range were ob-

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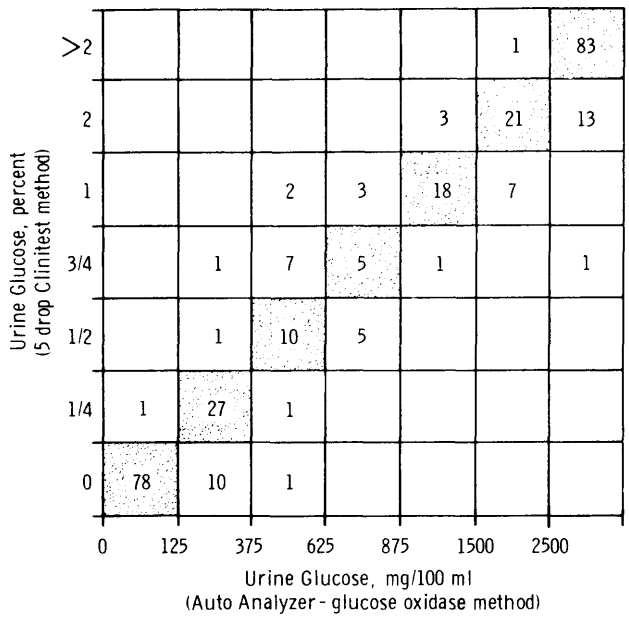

FIG. 1. Comparison of urinary glucose concentrations measured by the 5 Drop Clinitest method with those obtained by an AutoAnalyzer-glucose oxidase method. Values represent the number of urine samples giving the indicated results with the two methods. Shaded areas indicate agreement between the methods.

tained using McNemar's test[3] and are shown in table 1. In the low range, the 5 Drop Clinitest method gave correct results more often than either the 2 Drop Clinitest method or Tes-Tape. As shown in figures 2 and 3, the 2 Drop Clinitest method showed a considerable number of low values and the Tes-Tape method high values in the low range. Of the sixteen urine samples that contained 15 mg./100 ml. or less glucose, three were positive using Tes-Tape, while half of those with 16 to 50 mg./100 ml. glucose were positive. As indicated in table 1, the Keto-diastix test done in Missouri (but not in Colorado) gave better results in the low range than the 2 Drop Clinitest method. All other comparisons in this range showed no difference.

In the medium range no differences among the methods were evident.

In the high range, the 2 Drop and 5 Drop Clinitest methods produced equally good results. Both of the Clinitest methods gave better results than the Keto-diastix method in Missouri and Colorado. Tes-Tape obtained correct results more often than any other method. Finally, in the high range the Keto-diastix method did better in Missouri than in Colorado. However, in this range it gave many low values in both states (figures 4 and 5).

Neither proteinuria nor pregnancy, as evaluated statistically by the Chi square test for equality of proportions,[3] had a significant effect on any method of testing.

Thirty-three urine samples were positive for ketones by at least one method of testing (Acetest or Keto-diastix). For twenty-eight of these, two methods did not give the same results: Acetest large, Keto-diastix moderate for four; Acetest moderate, Keto-diastix small for six; Acetest small, Keto-diastix negative for seventeen; and Acetest negative, Keto-diastix small for one. According to the Sign test,[3] these differences are statistically significant ($P < 0.001$). Data obtained using known amounts of the lithium salt of acetoacetic acid added to ketone-free urine are shown in table 2. Since the first batch (control no. 1293070) of Keto-diastix used for testing gave a positive result only at levels over 100 mg./100 ml., three additional batches were evaluated. One showed a small level with 40 mg./100 ml. acetoacetic acid while all three showed moderate levels with 100 mg./100 ml. All other results, as well as those obtained with Acetest, were comparable to the manufacturers' claims. Acetest was quite sensitive, detecting as little as 5 mg./100 ml. of urinary acetoacetic acid. The results were not

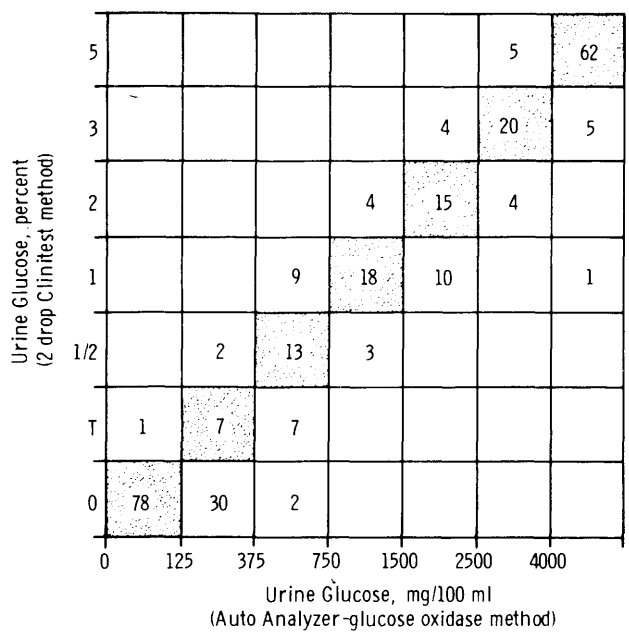

FIG. 2. Comparison of urinary glucose concentrations by the 2 Drop Clinitest method with those obtained by an AutoAnalyzer-glucose oxidase method. Values represent the number of urine samples giving the indicated results with the two methods. Shaded areas indicate agreement between the methods.

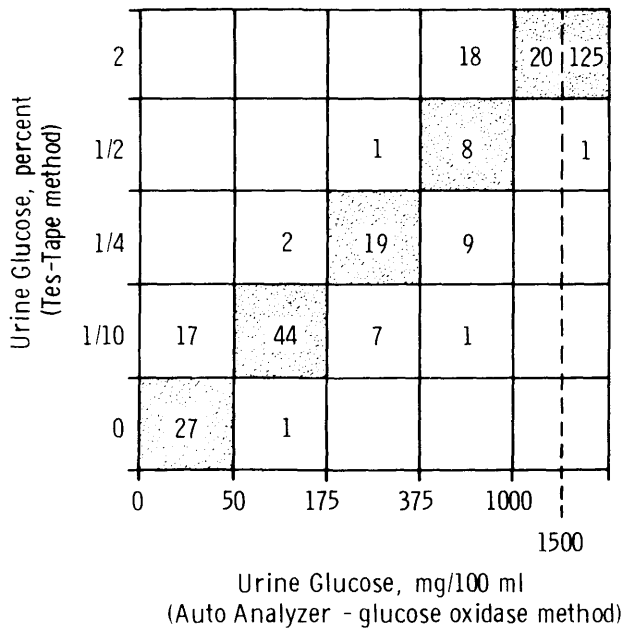

FIG. 3. Comparison of urinary glucose concentrations measured by the Tes-Tape method with those obtained by an AutoAnalyzer-glucose oxidase method. Values represent the number of urine samples giving the indicated results with the two methods. Shaded areas indicate agreement between the methods.

influenced by addition of glucose in concentrations up to 1 per cent.

DISCUSSION

The Keto-diastix, Tes-Tape, 2 Drop Clinitest, and 5 Drop Clinitest methods were selected for study because they are commonly used tests. Clinistix* was not evaluated because it is principally a qualitative test and results are not expressed in terms of per cent glucose present.

It is recognized that interpretation of results with these tests is subjective and that there will be some observer error. The colors developed during the tests fall along a spectrum and often do not match the few colors represented on the charts. Those that fall nearly halfway between two different colors on the charts tend to be particularly difficult to interpret. Errors in urine testing are commonly made by hospital ward personnel.[4] All observations in this study were meticulously carried out according to the manufacturers' directions.

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The Keto-diastix method indicated 2 per cent glycosuria more often in Missouri (eight of twenty-five specimens) than in Colorado (five of 275 specimens). Another apparent geographical discrepancy was made with respect to the occurrence of speckling, which seemed to be decreased in Missouri. For this reason the Keto-diastix method was repeated on the samples which had been collected in Colorado and transported to Missouri. Analysis of data indicated that there is a real difference between the results obtained in the two states. When prescribed medications taken by the subjects were considered, no association between the presence of reducing metabolites (gentisic or ascorbic acids from aspirin or Vitamin C) and changes in the Keto-diastix results upon transport of urine samples to Missouri could be implicated. The cause of these differences is unclear.

Evaluations of substances which sometimes occur in urine and produce false positive or false negative results with glucose oxidase or copper reduction methods have been reported elsewhere[5-9] and, with the above exception, have not been considered in this investigation.

Semiquantitative methods cannot give more accurate results than they are designed to provide. For this

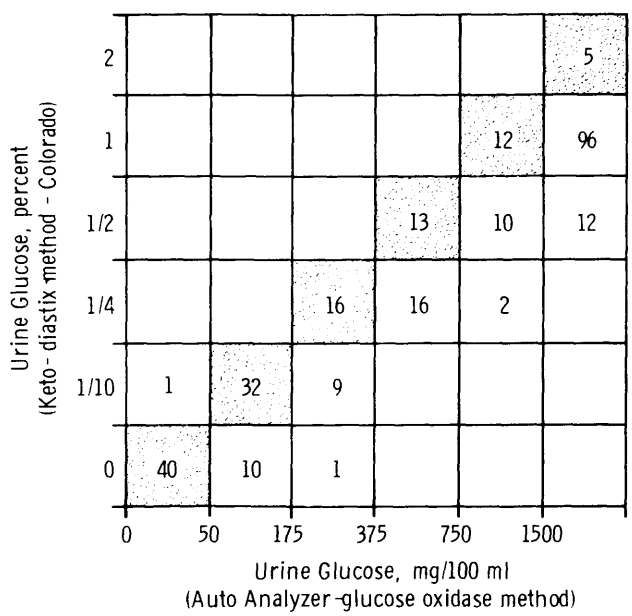

FIG. 4. Comparison of urinary glucose concentrations measured by the Keto-diastix method in Colorado with those obtained by an AutoAnalyzer-glucose oxidase method. Values represent the number of urine samples giving the indicated results with the two methods. Shaded areas indicate agreement between the methods.

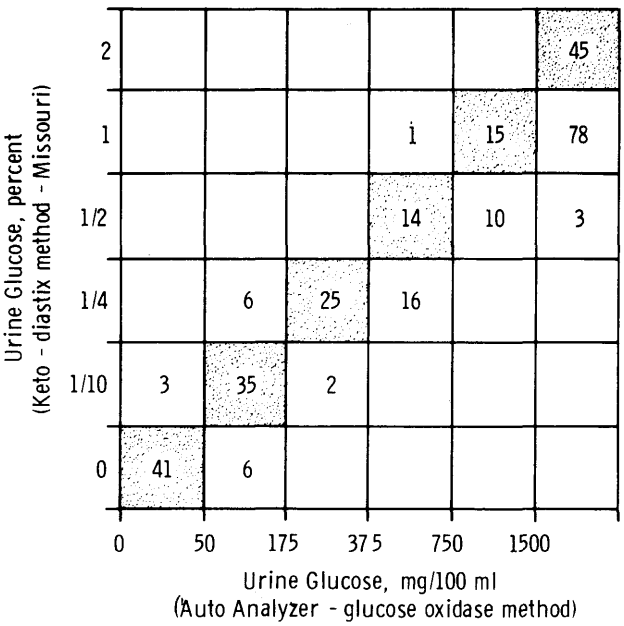

FIG. 5. Comparison of urinary glucose concentrations measured by the Keto-diastix method in Missouri with those obtained by an AutoAnalyzer-glucose oxidase method. Values represent the number of urine samples giving the indicated results with the two methods. Shaded areas indicate agreement between the methods.

reason the various methods were evaluated on the basis of the information they were expected to give. In the low range, thirty of 118 samples read lower than expected by the 2 Drop Clinitest method, suggesting that this method is relatively insensitive in this range. It is a poorer measure of urinary glucose in the low range than the 5 Drop Clinitest or, in Missouri, the Keto-diastix method. The 2 Drop Clinitest method was as good as Tes-Tape in the low range, apparently because the Tes-Tape detected glucose at levels below the set lower limit of 50 mg./100 ml. Tes-Tape gave positive results in half the urine samples containing 16 to 50 mg./100 ml. glucose. The observation that it detected glucose in three of sixteen urine specimens containing 15 mg./100 ml. or less glucose indicates that it may be so sensitive that it will give positive results with glucose levels found in the urine of healthy persons.[10] Thus, the 5 Drop Clinitest and Keto-diastix methods are the tests of choice for glycosuria in the low range.

In the medium range, there appears to be little difference among the various methods. Although the Keto-diastix method tended to produce low results in this range, it did not give less accurate results than the other methods since the latter gave a spread of values both above and below the expected readings.

In the high range, the Keto-diastix method gave a low result in 108 of 113 urine specimens tested in Colorado and in 81 of 126 in Missouri. This method is, therefore, a poor means of measuring high urinary glucose levels. Tes-Tape produced the expected results more often than the other methods, probably because with the Tes-Tape method, all values above 1,000 mg./100 ml. are correct if they give a reading of 2 per cent or more. The 2 Drop and 5 Drop Clinitest procedures were equally effective in obtaining expected results. However, with the 5 Drop Clinitest method all values above 2,500 mg./100 ml. are correct if they give a reading of greater than 2 per cent. Thus, few errors were produced by the Tes-Tape or 5 Drop Clinitest methods in attempting to quantitate glucose levels within the high range. These observations notwithstanding, the authors prefer the 2 Drop Clinitest method in the high range because it distinguishes between three levels of glycosuria (2 per cent, 3 per cent, and 5 per cent). In addition, it is preferable to the 5 Drop Clinitest method because the "pass through" phenomenon occurs less often in the high

TABLE 1

Comparison of the relative accuracy of the semiquantitative urine glucose methods in low, medium, and high ranges

Test methods compared*	Low range (0 to 375 mg./100 ml.)	Medium range (376 to 1,500 mg./100 ml.)	High range (> 1,500 mg./100 ml.)
2 Drop* vs. 5 Drop	5 Drop† $P < 0.005$	N.S.‡	N.S.
2 Drop vs. Tes-Tape	N.S.	N.S.	Tes-Tape $P < 0.005$
2 Drop vs. Mo. Keto*	Mo. Keto $P < 0.01$	N.S.	2 Drop $P < 0.005$
2 Drop vs. Colo. Keto*	N.S.	N.S.	2 Drop $P < 0.005$
5 Drop vs. Tes-Tape	5 Drop $P < 0.025$	N.S.	Tes-Tape $P < 0.005$
5 Drop vs. Mo. Keto	N.S.	N.S.	5 Drop $P < 0.005$
5 Drop vs. Colo. Keto	N.S.	N.S.	5 Drop $P < 0.005$
Tes-Tape vs. Mo. Keto	N.S.	N.S.	Tes-Tape $P < 0.005$
Tes-Tape vs. Colo. Keto	N.S.	N.S.	Tes-Tape $P < 0.005$
Mo. Keto vs. Colo. Keto	N.S.	N.S.	Mo. Keto $P < 0.005$

*2 Drop = 2 Drop Clinitest method.
5 Drop = 5 Drop Clinitest method.
Mo. Keto = Keto-diastix method done in Missouri.
Colo. Keto = Keto-diastix method done in Colorado.
†Methods indicated are those giving the best results.
‡N.S. = $P > 0.05$.

TABLE 2

The relative accuracy of the Acetest and Keto-diastix methods in the determination of urine acetoacetic acid concentrations

Acetoacetic acid* concentration	Acetest	Keto-diastix† 1293070§	Keto-diastix† 2229020	Keto-diastix‡ 1226109	Keto-diastix‡ 1256082
0 mg%	Negative	Negative	Negative	Negative	Negative
5 mg%	Small	Negative	Negative	Negative	Negative
15 mg%	Small	Negative	Small	Small	Small
40 mg%	Moderate	Negative	Small	Moderate	Moderate
100 mg%	Large	Negative	Moderate	Moderate	Moderate
200 mg%	Large	Small	Large	Large	Large

*As the lithium salt.
†Containers from which strips had been periodically used over a period of months.
‡Containers not previously opened.
§Manufacturer's control numbers.

range than with the latter method. This helps eliminate errors in interpretation when large amounts of glucose are present.

Other differences among the four methods are also evident. The Tes-Tape and Keto-diastix methods are specific for glucose while the Clinitest methods measure reducing substances. Use of Clinitest requires special equipment and collection of a urine specimen, while the other methods can be performed by dipping the strips directly into the urinary stream. Timing is less critical with the Clinitest methods than with the other two. The Keto-diastix has tests for both ketones and glucose on the same strip. They are mounted on single, stiff, plastic strips, which eliminate the curling and difficulty with tearing often encountered with Tes-Tape. Tes-Tape has a chromatographic effect which separates interfering substances but produces a nonuniform color along the strip, making interpretation difficult. When selecting a test, these features as well as the ability of the person performing the test must be taken into consideration.

The observation that neither pregnancy nor proteinuria affected the results with any test method suggests that these methods may be used to measure glucose in urine from individuals with these conditions.

Not only did the Keto-diastix method indicate lower concentrations of ketones in urine than the Acetest method, but it gave variable results with different lots of the reagent strips when used to test known concentrations of acetoacetic acid lithium salt. The Actetest method gave results which compared well with those claimed by the manufacturer. The variations with the Keto-diastix method may be related to the frequency with which the strips are exposed to room air before use. It is quite possible that, unless strips are used soon after the container is initially opened, deterioration may occur and low readings result. For this reason, Acetest is probably the preferred method.

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