

# Urine Sugar Determination by the Two-drop Clinitest Method

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## SUMMARY

Erroneous urine tests for sugar can result from many causes. The Clinitest method carried out in the usual recommended way (five drops of urine) is accurate in the 0 to 2 per cent sugar concentration range. Over 4 per cent, there is a reversal of color which can be mistaken for 0.75 or 1 per cent and which is referred to as "pass through." When two drops of urine are used instead of five, the range of Clinitest change is extended from 0 to 5 per cent and the "pass through" delayed until a concentration over 10 per cent sugar is reached. In 191 urines from diabetic children, the confusing "pass through" was found to occur seventy times when the urine was tested by the "five-drop method." It did not occur in testing the same urines using the "two-drop method." Quantitative chemical analysis established the range of these two methods. It is recommended that the "two-drop method" should replace the "five-drop method." *DIABETES* 16:557-59, August, 1967.

Reasonable accuracy should be expected from urine tests performed in the home. Insulin-dependent diabetics rely on these tests to make the adjustments frequently necessary in their insulin dosage. This is especially true of juvenile diabetics, who vary so much in their activity and even in their food intake from day to day. Accuracy is crucial during infections where sudden hyperglycemia may occur, and rapid insulin adjustment is necessary to avoid acidosis.

There are many causes for unreliable urine tests. Spoilage of reagent material through exposure to heat and humidity or prior ingestion of drugs can lead to false positive and false negative results. Improper lighting may cause confusion in interpretation of color charts. Often children may attempt to conceal glycosuria. The clinician needs always to be alert to discrepancies when the urine test record is not in accord with clinical and laboratory findings.

At a summer camp for diabetic children, we were

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impressed with the wide difference in results obtained when several test materials were compared. We are in agreement with other authors<sup>1,2</sup> that dip sticks and tapes (Clinistix, etc.,\* and Tes-Tape†) may fail to give useful quantitative information regarding the sugar content of the urine. Having discarded these in favor of Clinitest, we became aware that the results of the latter, done in the recommended fashion with five drops of urine and ten drops of water, were frequently misinterpreted by the children and staff. We therefore decided to evaluate the accuracy of the Clinitest reaction using two drops of urine instead of five.

The Clinitest tablet contains both copper sulphate reagent and a built-in source of heat production (NaOH, NaHCO<sub>3</sub> and citric acid).<sup>3</sup> The instructions sent with the product recommend that the test be made with ten drops of water, five drops of urine and the Clinitest tablet in a test tube. After cessation of boiling, the tester waits fifteen seconds, agitates the tube and compares the color with those on a color chart furnished by the manufacturer which reads between 0 and 2 per cent (figure 1). The instructions caution against the appearance of an orange color during the boiling period. If this color occurs, it is stated, the test should be interpreted as 4<sup>+</sup> or more regardless of the color present in the tube at the end of the reaction. Unfortunately, the instructions are *not* always read, and this important warning is frequently unheeded.

The manufacturers produce a second color chart for the Clinitest reaction (figure 1) available to physicians and laboratories upon request. On this chart, there is a broader scale ranging from 0 to 5 per cent with instructions to use ten drops of water, two drops of urine and a Clinitest tablet. There are no cautionary remarks or other comments.

We became interested in this second method of urine

\*Clinitest, Clinistix and Combistix are registered trademarks of the Ames Company, Inc., Elkhart, Ind.

†Tes-Tape is a registered trademark of Eli Lilly Co. (Canada) Ltd., Toronto.

URINE SUGAR DETERMINATION BY THE TWO-DROP CLINITEST METHOD

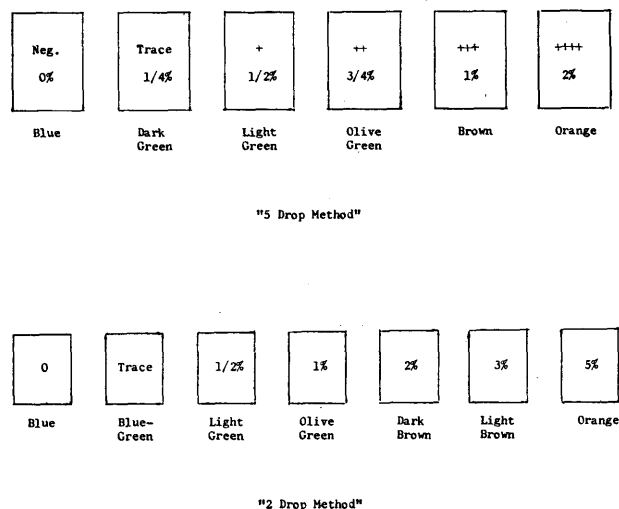


FIG. 1. Comparison of Clinitest methods.

testing after repeatedly noting erroneous interpretations of Clinitest results by children, parents, and hospital personnel. Urines containing large quantities of sugar give a fleeting orange color with Clinitest done by the "five-drop method." This occurs during the period of boiling. The color rapidly fades and a greenish brown solution with precipitate remains. Indeed, the orange color may be only a streak in the midst of the boiling brown liquid with duration so short that it goes quite unnoticed. The final color can then easily be misinterpreted for .75 or 1 per cent. Children can use this reaction to conceal heavy glycosuria.

Previous authors have reported on this phenomenon which has come to be known as the "pass through." In 1958, Cook and Free,<sup>4</sup> working at the Miles-Ames Research Laboratory in Elkhart, Indiana, concluded "that the two-drop modification of the Clinitest provides an improved means of quantitating urine sugar over a wide range of concentration from 0 to 5 per cent." In 1962, Tsifutis, Wohltmann and Hartmann<sup>5</sup> further pointed to the inaccuracies possible with the "five-drop Clinitest method."

METHOD AND RESULTS

The following study was made to establish the frequency of "pass through" in urine collected at our clinic for diabetic children. In addition, an assessment was made of the accuracy of the two Clinitest methods.

Table 1 gives the results obtained when one-hundred diabetic urines were tested by the "five-drop" and "two-drop" Clinitest methods. The urines were collected consecutively from one-hundred children attending the clinic. They were all second voided specimens obtained between 11:00 a.m. and noon. The children had previ-

TABLE 1  
Clinitest results on 100 urines obtained from our Diabetic Clinic

Gm. per cent sugar	0	0.25	0.5	0.75	1	2	3	4	5	"Pass-through"
Clinitest "five-drop" (100 tests)	14	11	5	3	13	13	*	*	*	41
Clinitest "two-drop" (100 tests)	14	11	7	*	15	7	5	7*	34	0

\*Not included on color scale. A 4 per cent reading can be recognized, however, for the "two-drop" test and seven urines are listed as such.

ously received their usual insulin dosage, breakfast and midmorning snack.

The correlation between the two tests is absolute between 0 and 0.25 and close between 0.5 and 1 per cent. At 2 per cent or over, there is a difference in response. "Pass through" was encountered in forty-one of these 100 urines tested by the "five-drop method." No "pass through" occurred using the "two-drop" test.

Table 2 gives the results obtained when ninety-one urines were tested for sugar by chemical analysis (Asator and King method<sup>6</sup>) and the two Clinitest methods. The urines were obtained from diabetic ward patients and chosen so that their sugar contents ranged from zero to the highest concentration occurring naturally. This proved to be 8.2 per cent.

Again a close relation is noted between the "two-drop" and "five-drop" methods in the range 0 to 2 per cent sugar content. Above this level, the "two-drop" method is quantitative up to 5 per cent. Concentrations between 5 and 8.2 per cent cannot be differentiated, but the phenomenon of "pass through" was not encountered. In the "five-drop" method thirty urines gave a "pass through" reaction. It will be noted that with either method a few discrepancies exist between the chemical analysis and the Clinitest. Search for protein or other reducing substances in these urines was unrevealing.<sup>4,7</sup>

A series of recovery experiments were next carried out. Pooled sugar free urines were obtained from non-hospitalized patients and glucose added in concentrations ranging from 0.25 per cent to 11 per cent. Recoveries were determined by chemical analysis in duplicate and the results compared with the "five-drop" and "two-drop" methods. The results are shown in table 3. Both Clinitest methods gave results with acceptable accuracy in the range between 0 and 2 per cent. Over 2 per cent, the "five-drop" method continued to give

**TABLE 2**  
Comparative results in ninety-one urines tested by chemical analysis and by the "two-drop" and "five-drop" Clinitest methods

Chemical analysis Sugar gm. per cent— Range	Clinitest Method			
	"Five-drop"		"Two-drop"	
	Sugar gm. per cent	Number of specimens	Sugar gm. per cent	Number of specimens
0 (17 tests)	Negative	17	Negative	17
	Trace	2	Trace	2
0.2 to 1.0 (10 tests)	0.5	6	0.5	6
	0.75	1	0.75	*
	1.0	1	1.0	2
1.2 to 2.0 (9 tests)	0.5	0	0.5	3
	1.0	9	1.0	6
2.1 to 3.0 (15 tests)	1	1	1	2
	2	14	2	8
	3	*	3	5
3.1 to 4.0 (10 tests)	2	10	2	1
	3	*	3	8
	4	*	4	1*
	"Pass-through"	30	"Pass-through"	0
4.1 to 8.2 (30 tests)	3	*	3	1
	4	*	4	7*
	5	*	5	22

\*Not included on color scale. A 4 per cent reading was recognized in eight urines tested by the "two-drop" method.

a 2 per cent reading until 4 per cent, beyond which concentration the "pass through" was regularly encountered. With the "two-drop" method the test was accurate from 0 up to 3 per cent, and from 3 to 5 per cent yielded values slightly low but acceptable. From 5 per cent to 10 per cent the reading remained stable orange (5 per cent) without "pass through." Over 10 per cent, "pass through" was encountered with the "two-drop" test.

Thus the "two-drop" method extends the quantitative range of the Clinitest from 0 to 5 per cent, and the "pass through" occurs at levels of sugar concentration rarely encountered clinically. In fact, of 191 urines obtained from well diabetic patients none reached 10 per

cent. Recently, we have encountered "pass through" with the "two-drop" method in urines from patients in diabetic acidosis. Parents and nurses had misread the result as 2 per cent. Accordingly, a warning about "pass through" should accompany even "two-drop" Clinitest color charts. As a semi-quantitative test the "two-drop" method is as sensitive as the "five-drop" in the 0 to 2 per cent range. Therefore, it would seem reasonable to do away with the "five-drop method" and replace it with the "two-drop" color chart to which detailed information about "pass through" should be added. Retaining both methods would serve no purpose and lead to confusion.

**COMMENT**

Errors in urine testing for sugar are described. The "two-drop" Clinitest is preferred to the "five-drop" method in testing urines from diabetic patients, particularly juvenile patients whose sugar spillage may be very large. It is recommended that the "two-drop" method be generally adopted in lieu of the other and that "pass through" be carefully explained. We would like to emphasize that urine tests are inaccurate only if conducted inaccurately. When properly carried out, they provide valuable information with which the patient and doctor can judge insulin dosage.

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**TABLE 3**  
Comparative results in pooled sugar-free urines to which glucose was added

Amount of glucose added	Estimated urine glucose (gm. per cent)										
	0.25	0.5	1.0	2.0	3.0	4.0	5.0	7.0	9.0	10.0	11.0
Recovery by chemical analysis	0.23	0.5	1.03	2.1	3.08	3.96	5.05	7.0	9.1	10.2	10.8
Clinitest "five-drop"	0.25	0.75	1	2	2	2	"Pass-through"				
Clinitest "two-drop"	0.25	0.5	1	2	3	3	4*	5	5	5	"Pass-through"

\*Not included on color scale but recognized here as such.