

# False Negative Reactions and Sensitivity in the Urine Glucose Oxidase Test

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## SUMMARY

Some 6,217 random urine specimens were tested with both a glucose oxidase test (Combistix) and a copper reduction test (Clinitest). The ratio of positive Combistix to Clinitest was approximately 3 to 2. There were only eleven false negative enzyme paper tests. The qualitative reaction of the enzyme paper test was not adversely affected over a pH range of 5 to 9 or by addition of significant amounts of uric acid, creatinine, or protein. Positive two-minute Combistix reactions were noted at levels as low as 10 to 12 mg. per 100 ml. of urine glucose. Ascorbic acid completely inhibited the glucose oxidase test for at least two minutes when the ascorbic acid level was two to two and one-half times the glucose level. *DIABETES* 14:224-25, April 1965.

The glucose oxidase test is more sensitive than standard copper reduction tests. Although it can detect urinary glucose concentrations of 0.1 per cent or less,<sup>1</sup> the enzyme paper test is limited in range for urine sugar quantitation.<sup>2-4</sup> The importance of recognition of false negative enzyme paper tests for urine glucose has been stressed by Gifford and Bergerman.<sup>5</sup> After screening 2,000 routine urines, they reported eleven of eighty-four specimens positive to Benedict's test were negative to the enzyme dip stick test. The eleven specimens were negative to enzyme paper tests when glucose was added to the urine. It was of interest to study further the incidence and factors involved in false negative enzyme paper tests for urinary glucose. In addition, the enzyme paper test and copper reduction test were compared for sensitivity and efficiency in screening random urines for glucose.

## MATERIALS AND METHODS

Some 6,217 random urine specimens from inpatients

From the Departments of Pathology, Harbor General Hospital, Torrance, California, and Orange County General Hospital, Orange, California, and University of California and University of Southern California Schools of Medicine, Los Angeles, California.

and outpatients in two large county general hospitals were examined. All urine specimens were tested with both a glucose oxidase paper test (Combistix) and a copper reduction test (Clinitest tablets).<sup>\*</sup> Combistix tests were read at two minutes and trace reactions were judged as positive. Combistix strips giving a negative test for sugar in urine were redipped into a 1 per cent glucose solution and reactions recorded as positive, slowly reactive, or negative after two minutes. The Clinitest reactions were read fifteen seconds after boiling had ceased.

Pooled fresh urine specimens, negative for glucose by the enzyme test, were used to study the effect of ascorbic acid, uric acid, and creatinine on the glucose oxidase reaction. Aliquots of pooled urine were diluted with 1 per cent phosphate buffers of varying hydrogen ion concentration and the pH of samples was adjusted with the aid of a pH meter. These samples were used to study the effect of pH on glucose oxidase reaction.

Glucose concentrations of the pooled urine specimens were adjusted by addition of D-glucose. A thirty-minute period was allowed before testing to allow equilibration of the alpha and beta forms of D-glucose, since glucose oxidase reacts with the beta form.<sup>6</sup>

## RESULTS

Results are shown in table 1. The ratio of positive Combistix test to the Clinitest is approximately 3 to 2. There were eighteen specimens with a negative Combistix reaction and a positive Clinitest reaction. The enzyme paper sticks from such specimens were checked by redipping in 1 per cent glucose solution. Seven of the eighteen showed a positive reaction, probably containing nonglucose reducing substances producing a positive Clinitest reaction. Six specimens remained negative after two minutes and were definitely false

<sup>\*</sup>Combistix and Clinitest are registered trademarks of the Ames Co., Elkhart, Indiana. Portions of material used for study were generously supplied by the Ames Co.

TABLE 1

Reactions for glucose on 6,217 random urine specimens

Combistix (glucose oxidase test)	Clinitest (copper reduction test)	Total number	Per cent total
Positive	Positive	413	(6.6)
Positive	Negative	206	(3.3)
Negative	Positive	18	(0.3)
Negative	Negative	5,580	(89.8)

negative reactions. Five urine specimens displayed a delayed positive reaction and contained inhibitors masking the glucose oxidase reaction.

The effects of pH on the glucose oxidase test were studied by adjusting the hydrogen ion concentration of fresh, normal urine with 1 per cent phosphate buffer. Then, 100, 50, 25, and 12 mg. of D-glucose were added to 100 ml. aliquots of urine; a pH range of 5 to 9 did not affect the qualitative reaction of the enzyme test at two minutes. Combistix reactions were negative at two minutes when the level of added glucose was less than 10 mg. per 100 ml. Addition of 200 mg. per cent of uric acid, 100 mg. per cent of creatinine, or 100 mg. per cent of protein to different aliquots of pooled urine at pH 6 with glucose concentrations of 100 mg. per 100 ml. showed no apparent inhibition of the glucose oxidase reaction. When the amounts of ascorbic acid added to pooled urine containing glucose were varied, ascorbic acid was found to inhibit about two to two and one-half times the level of glucose. For example, 40 to 50 mg. per cent of ascorbic acid can cause complete inhibition of the glucose oxidase test for at least two minutes when the glucose level is 100 mg. per 100 ml.

#### DISCUSSION

The qualitative Benedict's test is more sensitive than the Clinitest.<sup>7</sup> Benedict's test will give clear-cut trace reactions at glucose levels of 50 mg. per 100 ml.; at such levels the Clinitest reacts negatively. Cook et al.<sup>7</sup> have found, however, the sensitivity of the Benedict's test to be a disadvantage because many urine samples from healthy subjects have trace reactions from various nonglucose reducing substances in the urine. Moran et al.<sup>4</sup> demonstrated a positive glucose oxidase test and negative copper reduction test (Clinitest) occurring in 9.1 to 12 per cent of diabetic urines. Also, Fox et al.<sup>8</sup> have reported that routine urines showing very low levels of glucose detectable only by the glucose oxidase test are often clinically significant.

Of 6,217 urines in our series, there were eleven false negative enzyme paper tests. The incidence is

1.8 per cent (eleven of 619) and much lower than the 13 per cent incidence (eleven of eighty-four) cited by Gifford and Bergerman in 1961.<sup>5</sup> The difference may be partially explained by recent improvements in the quality of the commercial enzyme test products although efforts have been made to maintain the sensitivity constant.<sup>9</sup>

In our laboratories all negative Combistix tests are redipped in a 1 per cent glucose solution to check for false negative reactions. If the strip shows a negative or delayed positive after glucose dipping, the urine specimen is checked with the copper reduction test. Caution must be exercised, since ascorbic acid is the most common inhibitor of the glucose oxidase test and can also be responsible for a positive copper reduction test. Patients on therapeutic doses of ascorbic acid (300 to 1,000 mg./day) or receiving injectable antibiotic containing ascorbic acid as a preservative may excrete sufficient amounts of ascorbic acid to inhibit the enzyme test color development.<sup>10</sup> Meralluride and dipyrone are also capable of nullifying the glucose oxidase test.<sup>5</sup> Apparently p-aminosalicylate, streptomycin, didromycin, and penicillin do not inhibit the reactivity of the enzyme test strip to glucose in urine.<sup>11</sup>

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