A Comparative Study of Qualitative Tests for Ketones in Urine and Serum

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The detection of ketone bodies has become an important clinical measurement during the past 50 years. The two types of reactions most commonly employed for ketone detection are nitroprusside tests, which react with acetone and acetoacetic acid, and ferric chloride tests, which react with acetoacetic acid but not with acetone. Ferric chloride tests are very insensitive, give false-positive reactions with such common drugs as salicylate, and develop, with biologic compounds, other colors which mask the true acetoacetic acid reaction. Each modification of the nitroprusside test has its own sensitivity resulting from variations in concentration and variations in actual chemical compounds used. The nitroprusside test was standardized some time ago by the development of a simple tablet test called Acetest and has recently become available as a dip and read test called Ketostix. The present report describes these tests and presents results obtained with each on ketotic urine and serum samples and on normal samples with and without added acetoacetate.

METHODS

Tablet Test

In this modification of the nitroprusside reaction, the reagents are compounded into a single tablet containing sodium nitroprusside, disodium phosphate, and aminoacetic acid. The test procedure is to

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1Acetest is a registered trademark of the Ames Company Inc., Elkhart, Indiana.
2Ketostix is a registered trademark of the Ames Company Inc., Elkhart, Indiana.
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place a tablet on a clean white surface such as typing paper. A drop of urine is placed on the tablet, and 30 seconds later the color of the top of the tablet is compared with the color chart provided, which has 3 color blocks with various shades of purple representing trace, moderate, and strongly positive reactions. The tablet remains white or turns cream-colored if the urine is negative. The test will easily detect 5 mg. of acetoacetate per 100 ml. or 20 mg. of acetone per 100 ml. of urine, but these concentrations give colors that are lighter than the trace color on the chart.

To use the tablet test with serum or plasma the simplest method is to place a drop of the sample on the tablet. Readings made at 30 seconds will detect 10 mg. of acetoacetate per 100 ml. of serum or plasma but this concentration will give a much brighter purple if the test is read at 2 minutes. The high protein content of serum or plasma prevents a drop from being easily absorbed by the tablet and thus makes color development harder to estimate. An alternative procedure is to place a thin smear of plasma or serum on a white nonabsorbent paper and to place the tablet on the smear. Thirty seconds later, the tablet is turned over and the color compared with the chart as for urine. Here again 10 mg. of acetoacetate per 100 ml. of serum or plasma can be detected with the 30-second reading, but extension of the reading time to 2 minutes gives a brighter positive reaction.

A simple technic for use of the tablet with whole blood is to place a drop of blood directly from the fingertip or a syringe on a tablet. The blood on the tablet is allowed to clot (about 10 minutes) and the clot is then lifted off with a piece of paper or an applicator stick. The surface of the tablet from which the clot was removed is then compared with the color chart. This method will detect 25 mg. of acetoacetate per 100 ml. of blood and with closer scrutiny by an experienced operator somewhat smaller concentrations can be recognized.

**Strip Test**

This test consists of a heavy strip of special paper which is coated on one end with a mixture of sodium nitroprusside, sodium phosphate, and aminoacetic acid. The procedure for use is to dip the coated end of the strip in urine and remove and 1 minute later to compare the color of the dipped portion with the color chart on the bottle label. Three color blocks from pink to purple represent small, moderate, or large amounts of ketone bodies. The test will detect 5 mg. of acetoacetate per 100 ml. of urine. The test is practically nonreactive
to acetone, since concentrations of acetone of 1 or 2 per cent in urine give no color at 1 minute.

The procedure with serum or plasma is the same as that for urine in which the strip is dipped into the sample and compared with a color chart. Readings made at 1 minute will detect 10 mg. acetoacetate per 100 ml. of serum or plasma, but a brighter pink color will be obtained at this concentration with a 3-minute reading time.

**Rothera Test A**

The procedure (1) is to place approximately 1 Gm. of solid ammonium sulfate in a test tube and add about 5 ml. of urine. The tube is shaken to dissolve most of the ammonium sulfate. Three drops of freshly prepared 5 per cent sodium nitroprusside solution are added and mixed well. One or two milliliters of concentrated ammonium hydroxide are stratified on the mixture, and 2 minutes later the interface of the solutions is observed for a purple ring. Positive reactions are graded trace to 4+ according to the intensity of purple color. The procedure will detect 1-2 mg. of acetoacetate or 10 mg. of acetone per 100 ml. of urine.

**Rothera Test B**

This modification of the Rothera test (2) is slightly less sensitive than the tests described above. The procedure is to place 5 ml. of urine in a test tube, add 5 drops of saturated ammonium sulfate solution, 3 drops of freshly prepared 5% sodium nitroprusside solution and 5 drops of concentrated ammonium hydroxide, and mix well. Two minutes later, the solution is observed for a purple color which is graded trace to 4+ according to its intensity. Ten milligrams of acetoacetate or 40 mg. of acetone per 100 ml. of urine can be detected by this procedure.

**Ferric Chloride Test**

In this procedure (3) 10% ferric chloride is added by drops to a few milliliters of urine until the precipitate that forms dissolves. The resulting solution is observed for a wine-red color which indicates a positive reaction. An additional test must be carried out on all positives to distinguish between acetoacetate and drug reactions. The preferred method is to carry out a repeat test on urine which has been boiled for 3 to 5 minutes. A less satisfactory method is to boil an aliquot of the completed test reaction with ferric chloride. If the red color is lighter or has disappeared after boiling, the reaction is
due to acetoacetate; if the color of the reaction is the same after boiling as before, it is due to an interfering drug. There is no simple method of telling if both drug and acetoacetate are present. This test will not easily detect less than 80 mg. acetoacetate per 100 ml. of urine and it will not react with acetone.

**METHOD OF ADDING ACETOACETATE TO URINE**

A stock solution of sodium acetoacetate equivalent to 2% acetoacetic acid is made by allowing a mixture of 13 Gm. of ethyl acetoacetate and 500 ml. of 0.2N NaOH to remain at room temperature for 48 hours. Aliquots stored in the deepfreeze are stable for several months. Freshly thawed stock solution or dilutions of it are added to urine in small amounts so that at least 90 per cent of the volume of the final solution is urine.

**RESULTS**

*Comparative Study of Qualitative Tests for Ketonuria*

Both experienced and inexperienced operators tested urines containing no ketones, excreted ketones, or added acetoacetate. All tests were carried out as unknowns using differently coded aliquots of the same urines for each of the tests described above. Results of this study are shown in Figs. 1 and 2. For comparison, positive reactions with all nitroprusside tests have been divided into three groups: *Small*—including trace, 1+, and small reactions depending on which system of grading was used; *Moderate*—including moderate and 2+ reactions; and *Large*—including strongly positive, 3+, 4+, and large reactions. Ferric chloride tests were graded only as negative or positive, with a third classification called "false positive before boiling."

*Sensitivity and Accuracy of Qualitative Tests for Ketonuria*

A total of 5553 values is presented in Fig. 1. Each bar represents 100 per cent of the results obtained with a given test at a given level of added acetoacetate concentration. It is obvious that Rothera A is the most sensitive test, since 15 per cent of the urines which were negative with the other nitroprusside tests gave a small reaction with this test. The least sensitive test is Rothera B, which gave a few small reactions with acetoacetate concentrations as high as 160 mg. per 100 ml. Results obtained with the strip test on the entire series of urines agree well with those obtained with the tablet test. It will easily be seen that the most consistent results are obtained with the tablet and strip tests, which have standard color charts for grading...
**Qualitative Tests for Ketones**

**Fig. 1.** Qualitative ketone tests on urines with and without added acetoacetate (5553 values).

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative</th>
<th>Small</th>
<th>Moderate</th>
<th>Large</th>
<th>False Positive Before Boiling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rothera Test A</strong></td>
<td>60%</td>
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<tr>
<td><strong>Rothera Test B</strong></td>
<td>80%</td>
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<tr>
<td><strong>Tablet Test</strong></td>
<td>75%</td>
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<tr>
<td><strong>Strip Test</strong></td>
<td>75%</td>
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<tr>
<td><strong>Ferric Chloride Test</strong></td>
<td>91%*</td>
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*91% negative results with the ferric chloride test include 17% which were false positive before boiling.

**Fig. 2.** Qualitative ketone tests on random urines from hospital patients (4066 values).
the degree of reaction. Inconsistency of results is particularly noticeable with Rothera A at the 20 mg. per 100 ml. and 40 mg. per 100 ml. levels, and with Rothera B at the 10 mg. per 100 ml. level, where reactions from negative to 4+ were obtained with the same acetoacetate concentration. Figure 1 also shows that only half the tests were positive with the ferric chloride test when 80 mg. acetoacetate per 100 ml. were present, a concentration that usually gave a strongly positive reaction with the nitroprusside tests.

Figure 2 shows 4066 results obtained with the five qualitative tests on a series of random urines from hospital patients. Again the variation in sensitivity of the Rothera procedures is apparent. It is interesting to note that all five tests gave about the same number of strong positive reactions. Again the results obtained by both tablet and strip tests agree well.

**Specificity of the Tablet and Strip Tests**

A series of 400 random urines from hospital patients was tested with both the tablet and the strip. There were 77 urines which gave positive reactions with both tests. Each of these urines also gave a positive reaction with the more sensitive Rothera A. The remaining 323 urines gave negative reactions with both the tablet and the strip. These negative urines were tested again with the tablet and the strip after the addition of 10 mg. acetoacetate per 100 ml. In each case, a trace or small reaction was obtained with both tests. These results indicate that in a series of random urines from hospital patients with a variety of disorders and under diverse drug therapy, no urine contained any substance which reacted to give a false-positive with the tablet or the strip or which caused a false-negative reaction with either test with a minimal amount of added acetoacetate.

**Blood and Serum Studies on Two Patients in Diabetic Coma**

From two patients in diabetic coma, 16 ketone-positive blood or serum samples were obtained. In one case, the initial blood sugar was 366 mg. per 100 ml. and in the other case, 1045 mg. per 100 ml. Diffusion studies, using Conway micro-diffusion units in a method developed in our laboratory (4), indicated that in these samples most of the nitroprusside reactive material was acetoacetate and not acetone. Approximate acetoacetate concentrations of the serum samples ranged from 10 to 60 mg. per 100 ml. (as estimated by dilution and comparison with acetoacetate standards using the tablet test). As
shown in Table 1, results with the tablet and with the strip agreed well. The tablet procedure with whole blood gave results similar to those given by serum when either venous or fingertip samples were used.

**DISCUSSION**

It has been established previously (4) that most of the nitroprusside reactive material in ketotic urine is acetoacetate and not acetone. Similar studies (summarized in Table 1) indicate that this is also the case with ketotic serum and blood. Acetone is present, but its concentration is so small and its reactivity to nitroprusside is so much less than that of acetoacetate that its contribution to color formation with nitroprusside is minimal. This concept is further established by the agreement of results with the tablet and strip tests on ketotic urines and serums. Since the strip test is essentially nonreactive with acetone, samples containing only acetone would give a negative reaction with the strip and a positive reaction with the tablet. This type of result did not occur throughout the entire series of tests on ketotic urines and serums by experienced operators.

It is obvious from the results of this study that the tablet and the strip are far superior to the ferric chloride method as a test for acetoacetate. The strip test is many times more sensitive and much more specific than the older time-consuming ferric chloride test. A
limited experience with Lindemann's test for acetoacetic acid (5) shows that it, too, is quite insensitive and nonspecific.

SUMMARY

Results are presented of a comparative study of several methods for detection of ketone bodies in urine involving nearly 10,000 tests on urines containing no ketones, naturally occurring ketones, and added acetoacetate. Acetest (a tablet nitroprusside test) and Ketostix (a strip nitroprusside test) are more specific and accurate, and have the proper sensitivity in comparison with test tube Rothera nitroprusside modifications or the ferric chloride test. Both the tablet and strip are also satisfactory for detection of ketones and serum or plasma. The tablet test may be used with whole blood for detection of ketonemia.

REFERENCES