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**False-Negative Results for Urinary Phenothiazines and Imipramine in Forrest’s Qualitative Assays**

Gordon P. James, Mary Helen DJang, and Horace H. Hamilton

When a series of patients’ urine samples supplemented in vitro with chlorpromazine or imipramine was assayed with the Forrest qualitative assays, we observed an occasional false-negative result, which we found was attributable to interference by ascorbic acid. It interferes with the reagent, not with the analytes, in both assays. We easily eliminated this interference with the phenothiazine test by using an anion-exchange resin. Eliminating the interference with the assay for imipramine, however, is more difficult; false-negative results can be obtained even after ion-exchange chromatography if the imipramine concentration is <50 mg/L.

Forrest and Forrest (1) were the first to describe a simple, rapid, qualitative test for phenothiazines in urine involving use of ferric chloride, perchloric acid, and nitric acid (FPN). In 1961 Forrest et al. (2) reported on their use of the FPN screen for urinary phenothiazines and claimed that “virtually no false negatives have been encountered.” Campbell (3), however, in 1965 claimed more than 20% false-negative results with the FPN test for urinary phenothiazines. In 1966 Brownstein and Roberge (4) reported a false-negative rate of approximately 25% with the FPN test. Forrest et al. responded in 1966 (5), claiming that “…Campbell’s 20% false negatives were essentially authentic negatives, caused by drug-defaulters. . . .”

The Forrest FPN test has appeared unchanged in several recent texts on analytical toxicology (6) and clinical chemistry (7), and it is still generally accepted that a negative FPN test “effectively eliminates this group of drugs as a factor in diagnosing the condition of the patient” (7).

Forrest et al. (8) also developed a simple color test for imipramine in urine and attempted to use it to estimate daily drug dosage. This test also appears in a recent text (6) and is frequently included as part of a battery of tests for drugs of abuse.

We studied the Forrest tests on a series of patients’ urines supplemented in vitro with chlorpromazine or imipramine to a concentration of 50 mg/L. We found occasional urine samples that gave false-negative results. Subsequent investigation revealed ascorbic acid to be the interfering substance. This is a serious interference because of the many dietary sources of the vitamin and its frequent use in high doses as attempted therapy for infection of the upper respiratory tract.

Ascorbic acid also interferes with urinary glucose assays. A simple anion-exchange procedure has been reported that eliminates ascorbic acid interference with urine glucose procedures (9). We have modified the phenothiazine screening test (1) and imipramine screening test (8) to include an anion-exchange step before addition of color reagent. This procedure eliminates interference by vitamin C and the associated false-negative test results for phenothiazines; it also prevents interference with the Forrest test for imipramine if the urinary concentration of imipramine is at least 50 mg/L.

**Materials and Methods**

*Phenothiazines.* Prepare anion-exchange columns as follows. Weight 10 g of AG 1 × 4 anion-exchange resin, chloride form, 200–400 mesh (Bio-Rad Laboratories, Richmond, CA 94804) into a 125-mL Erlenmeyer flask. Add 35 mL of deionized water and stir. Add 0.5 mL of the anion exchange slurry into a Pasteur pipette containing a small cotton plug. This will produce an ion-exchange column that is about 1 cm × 4 mm i.d. Add 1.0 mL of the urine to be tested for phenothiazines to this ion-exchange column. Discard the eluate; it will contain no detectable phenothiazine but may contain small amounts of ascorbic acid, depending on the original ascorbic acid concentration in the urine. Add 1.0 mL of deionized water to the column and collect the eluate. This eluate will contain phenothiazine if present in the original urine sample. Add 1.0 mL of Forrest’s FPN reagent (1) to the collected eluate. A phenothiazine will be indicated by a pink or purple color.

*Imipramine.* The Forrest test for imipramine is much more sensitive to ascorbic acid interference than is the FPN test for phenothiazines. Use a 2 cm × 4 mm i.d. ion-exchange column to remove ascorbic acid from the urine sample. The procedure is the same as for phenothiazine except that 1.0 mL of the appropriate Forrest’s reagent (8) is added to 0.5 mL of the second eluate. A positive result for imipramine is indicated by a green color.

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Received Sept. 5, 1979; accepted Nov. 19, 1979.
Results and Discussion

Phenothiazines. We established the limiting sensitivity of the FPN test for phenothiazines in urine as follows. Aliquots of a fresh urine (negative for protein, glucose, ketones, bilirubin, blood, nitrite, ascorbic acid, and urobilinogen), pH 7.0, were supplemented in vitro with chlorpromazine to the following concentrations: 0, 2.5, 12.5, 25.0, 37.5, and 50.0 mg/L. Each chlorpromazine concentration was split into two aliquots, placed in coded containers, and arranged in an unordered, blind series on the laboratory bench. The resulting 12 samples were tested for phenothiazine by three technologists, with the FPN reagent. All samples with chlorpromazine concentrations of 12.5 mg/L or greater were called phenothiazine positive by all three technologists; all samples with less were called phenothiazine negative. An identical test protocol established the same limiting sensitivity for mesoridazine.

An abbreviated protocol, involving only one technician, indicated the following sensitivities for other phenothiazines: promethazine, 12.5 mg/L; prochlorperazine, 2.0 mg/L; thioridazine, 10 mg/L; and thioridazine, 100 mg/L. Thiethylperazine and thioridazine produce a blue color with the FPN reagent; the other phenothiazines tested produce a pink color.

Ascorbic acid decreases the sensitivity of the FPN assay. An ascorbic acid concentration of 2.0 g/L caused a false-negative FPN test when the chlorpromazine concentration was near the limits of sensitivity of the assay, i.e., 12.5 mg of chlorpromazine per liter, and gave a result that the technologist could not unambiguously interpret at 25 mg of chlorpromazine per liter. Similar ambiguous results were obtained with 0.5 and 1.0 g/L ascorbic acid for the lower concentration of chlorpromazine; 0.25 g of ascorbic acid per liter did not affect the FPN results.

A patient’s urine that was strongly positive for a phenothiazine by the FPN test and by thin-layer chromatography (10) was supplemented in vitro to various ascorbic acid concentrations and retested by the FPN assay. Ascorbic acid concentrations between 2.5 and 4.5 g/L produced questionable results that could easily have been false-negative; a 5 g/L concentration caused a false-negative result. One milliliter of this patient’s urine sample, supplemented with 5.0 g of ascorbic acid per liter, was applied to an ion-exchange column and tested by the FPN assay as described above. A strong positive test for phenothiazine resulted. Aliquots of this patient’s urine sample (one supplemented with 5.0 g/L ascorbic acid and one with no ascorbic acid) were assayed for phenothiazines with a thin-layer chromatographic procedure (10). The two samples were identical, indicating that in the FPN procedure the ascorbic acid was interfering with the FPN reagent, not with the phenothiazine or its metabolites.

A patient’s urine that was strongly positive for ascorbic acid—i.e., >300 mg/L by “C-Stix” (Ames Co., Division of Miles Laboratories, Inc., Elkhart, IN 46515)—was supplemented in vitro with chlorpromazine to 12.5 mg/L. An FPN test on this sample gave a false-negative phenothiazine result. Applying the ion-exchange cleanup described above led to a positive FPN test for phenothiazine.

FPN tests on urines supplemented in vitro with prochlorperazine or thioridazine were falsely negative when the ascorbic acid concentration reached 5.0 g/L. True-positive FPN results were obtained for both of these phenothiazines after ion-exchange cleanup.

We also studied a modified version of the Forrest FPN test (11) for sensitivity to ascorbic acid, by using a urine supplemented in vitro with 12.5 to 100 mg of chlorpromazine per liter. False-negative results for phenothiazines were obtained with ascorbic acid concentrations as low as 250 mg/L. A patient’s urine that was strongly positive for a phenothiazine by the modified FPN procedure gave false-negative results after as little as 0.5 g of ascorbic acid was added per liter.

We determined the capacity of our ion-exchange column to remove ascorbic acid from urine as follows. A normal urine was supplemented in vitro with chlorpromazine to 25 mg/L. Aliquots of this urine were supplemented with ascorbic acid to 5.0, 7.0, 9.0, or 12.0 g/L. Aliquots of these samples were tested for a phenothiazine with Forrest’s FPN assay after ion-exchange treatment. The results were strongly positive for urine samples that had contained 5.0, 7.0, or 9.0 g of ascorbic acid per liter, indicating that concentrations up to 9.0 g/L can be tested with confidence when the ion-exchange step is included. The sample containing 12.0 g of ascorbic acid per liter gave a very faint positive result after ion-exchange treatment and could easily have been called false negative.

Imipramine. The limiting sensitivity of the Forrest test for imipramine in urine was about 25 mg/L; below this imipramine concentration, test results cannot be unambiguously interpreted, and very low concentrations of ascorbic acid (125 mg/L) lead to false-negative Forrest test results. The Forrest assay for imipramine was much more sensitive to ascorbic acid interference than was the FPN assay for phenothiazines. Consequently, we used a 2-cm ion-exchange column for the imipramine assay, rather than the 1-cm column for the phenothiazine test. With a 2-cm column one can obtain true-positive results for imipramine on samples containing as much as 4.0 g/L ascorbic acid (Table 1).

A patient’s urine sample, positive for imipramine by the Forrest assay, was supplemented in vitro with ascorbic acid to several different concentrations. After ion-exchange cleanup we assayed various fractions for ascorbic acid (C-Stix) and imipramine (Forrest test). The results are shown in Table 2. This urine sample was only weakly positive for imipramine before ascorbic acid was added. In this situation the ion-exchange cleanup was able to remove as much as 400 mg/L ascorbic acid without affecting the imipramine assay.

Table 1. Results of Forrest Test for Imipramine (50 mg/L) after Ion-Exchange

<table>
<thead>
<tr>
<th>Ascorbic acid, g/L</th>
<th>Forrest assay result with ion-exchange a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>±</td>
</tr>
<tr>
<td>4.0</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>+</td>
</tr>
<tr>
<td>0.5</td>
<td>+</td>
</tr>
<tr>
<td>3.0 b</td>
<td>+</td>
</tr>
<tr>
<td>1.0 b</td>
<td>±</td>
</tr>
</tbody>
</table>

a Without ion-exchange, all Forrest assay results were negative. "±" indicates that the technician was uncertain of the test result.

b Imipramine decreased to 25 mg/L.

Table 2. Effect of Added Ascorbic Acid on Results of Forrest Assay of a Patient’s Urine Sample Positive for Imipramine a

<table>
<thead>
<tr>
<th>Ascorbic acid, g/L</th>
<th>First eluate from ion-exchange column</th>
<th>First water wash from ion-exchange column</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1.0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.25</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a Results of Forrest assay on supplemented urine samples were all negative without ion-exchange step.

b Results of Forrest assay on first eluate were all negative.

c Ascorbic acid assay by C-Stix.
change column was able to handle an ascorbic acid concentration only up to 0.5 g/L. At 1.0 g of ascorbic acid per liter, assay results were falsely negative, even after ion-exchange.

We conclude that urinary ascorbic acid concentrations associated with normal dietary intake of the vitamin do not interfere with the Forrest FPN test for phenothiazines, but as little as 0.1 g/L will give false-negative results for imipramine. To protect against false-negative results, test each urine for ascorbic acid with C-Stix before performing the FPN assay. An ascorbic acid concentration of less than the highest color code (0.4 g of ascorbic acid per liter) will not lead to a false-negative phenothiazine result. If the C-Stix indicates an ascorbic acid concentration of 0.4 g/L or more, use the anion-exchange procedure to remove it before performing the FPN test. Because the C-Stix cannot distinguish between ascorbic acid concentrations of 0.4 and 2.0 g/L, a C-Stix value of 0.4 g/L must be considered suspect. A negative Forrest test for imipramine should not be considered conclusive if any ascorbic acid was detected by C-Stix analysis, even if the ion-exchange step is included.

References