Testing for Isoniazid*

An Evaluation of the Arkansas Method

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To better treat and eliminate tuberculosis, patient compliance must be improved. Compliance can be evaluated by measuring a drug or its metabolite in the urine. In Arkansas, a simple colorimetric method of checking the urine for isoniazid (the Potts-Cozart test) has been used for many years, but it is relatively unknown outside that state and its reliability has not been confirmed. To evaluate this test, urine was blindly tested from patients from a tuberculosis clinic. Controls included urine from patients from a substance abuse clinic and Veterans Medical Center. In more than 200 urine samples tested, no false-positives were found. Urinalysis showed normal values for three patients who were supposed to be receiving antituberculosis medication, but it is likely that these patients were noncompliant. A peculiarity of the test was that the color change with positive tests varied. To investigate this variation, absorption spectroscopy of many substances was performed. Nicotine accounted for the different shade of blue associated with the positive test, but the color produced and the absorption spectroscopy were different from isoniazid, so it did not confuse the interpretation of the results. This test for isoniazid in the urine is simple, quick, inexpensive, easy to interpret, and reliable. It also can be used to detect nicotine and its metabolites. (Chest 1990; 98:314-18)

To eradicate tuberculosis, we must improve patient compliance. Patients must take their medicine and we cannot rely on their word alone. Although supervised therapy is an effective treatment method, it is costly and cannot be done in many places. Assuring compliance in unsupervised therapy is, therefore, important. One method of testing compliance is measuring a metabolite of a therapeutic drug. Yet, most centers do not test for compliance even though several authors have emphasized it. The lack of testing may be because paper strips that check urine for isoniazid are expensive, time-consuming, and not always easy to interpret. The Arkansas Department of Public Health has used a simple method to check urine for isoniazid for many years (William Stead, oral communication, 1985). It was developed by Potts, Cozart, and Reagan, but their work was never published. Henderson, who worked in the same laboratory, reported high reliability of the test in an unblinded study of 190 patients who were taking isoniazid and 65 patients who were not. We blindly evaluated this test, and, by studying it spectrophotometrically, we discovered another use for it.

METHODS

Patient Selection

Urine was tested from three groups of patients. To check for sensitivity and specificity, the urine of 94 patients from the tuberculosis clinic was studied. The laboratory personnel doing the test were unaware of the drugs being given to these patients, and the clinical personnel sending the urine to the laboratory were unaware of the laboratory results until the end of the study. Patients in this group included those taking isoniazid alone and rifampin (which gives urine an orange color) without isoniazid, so urine color as a source of bias was lessened. To detect false-positives resulting from other medicines, the urine of 84 hospitalized patients that was sent for routine urinalysis was tested for isoniazid. To estimate how many medicines patients in the hospital were taking, we randomly selected ten patients each from the medical, surgical, and psychiatric services and tabulated the medications they were taking. To detect false-positives from other substances, urine from 52 patients from the substance abuse clinic was tested. These urine samples often contained metabolites of commonly abused drugs and other unknown materials.

Laboratory Materials and Procedures

All chemicals were obtained from Sigma Chemical Co, St Louis, Mo. Acetylsalicyclic acid hydrazide was prepared by reacting 2.0 g of isonicotinic acid hydrazide with 15 ml of acetic anhydride. After one hour, the mixture was centrifuged and the excess acetic anhydride was decanted. The precipitate was washed with small volumes of cold methanol, centrifuged, and decanted. The acetylsalicyclic acid hydrazide was dried in a vacuum desiccator. For spectrophotometric studies, stock standards of acetylsalicyclic acid, isoniazid, nicotine, and cotine were prepared by dissolving these agents in spectro-grade methanol to obtain concentrations of 1.0 mg/ml. Working standards were diluted with water from the stock standards to contain 0.04 mg/ml.

Test tubes (13 × 100 mm) were prepared in advance by adding 30 mg of barbituric acid to each tube and stopping it until use. Chloramine-T, 14 g/dl, was stored in a plastic bottle that could be dispensed by the drop. Potassium cyanide, 5 g/dl, was prepared and stored in a second plastic bottle that could be dispensed by the drop. The optimal urine assay was as follows: add 4 drops of the patient's urine to a test tube containing the barbituric acid. Then
add 2 drops of the chloramine-T solution and 2 drops of the potassium cyanide solution. Agitate the test tube for 10 s. A positive test will have a color change to dark blue or turquoise. A negative test will have a color change to yellow.

Differences in Color Change

The differences in color of the positive urine samples were further investigated by a spectrophotometric study. After performance of the test reaction, 10 ml of methanol diluent was added to the test tube. The sample was then thoroughly mixed to dissolve reaction products and a spectral scan was done in a recording spectrophotometer (Beckman model 35, Berea, CA). To test the hypothesis that this color change may be associated with smoking, the histories of the patients whose urine samples were tested were reviewed for cigarette smoking. Spectrophotometry was performed on the urine of smokers and nonsmokers and on urine with nicotine and cotinine added. Cotinine is the principal metabolite of nicotine. Isoniazid and its metabolites were also added to check for interactions.

RESULTS

Of the 94 patients from the tuberculosis clinic whose urine samples were evaluated, 69 were taking isoniazid and 25 were not. All patients claimed to be taking their medication as directed. The urine samples of all patients who were not taking the medication were negative except one. On further questioning of the nursing home where this patient was staying, it was learned that the patient was still receiving isoniazid after therapy was supposed to have been discontinued. Three patients who claimed to have taken their medication had negative urine tests. One of these patient's sputum later became positive for mycobacteria. One person admitted noncompliance when questioned, and the third died before he could be interviewed.

Of the 84 hospitalized patients who had routine urinalysis, none was taking isoniazid and none of their urine samples was positive. The hospital survey showed that patients were taking, on average, four drugs. This was not different among the medical, surgical, and psychiatric services. The urine samples of 48 patients in the substance abuse clinic were positive for methadone, morphine, cocaine, barbiturates, benzodiazepines, pentazocine, tripelelenamine (Pyrribenzamine), and other drugs. None was taking isoniazid and all urine samples tested negative (Table 1).

The urine of nonsmokers taking isoniazid turned deep blue. The urine of smokers taking isoniazid turned blue-green. When isoniazid or acetylsalicylic acid was added to the urine of nonsmokers, the test caused it to turn dark blue. When these agents were added to smokers' urine, the test produced a blue-green color, with isoniazid producing the most green. The test distinguished isoniazid in the presence of nicotine and its metabolites without error.

The peak absorption spectra of the reaction products were 625 nm for isoniazid, 600 nm for acetylisoniazid, 505 nm for nicotine, and 510 nm for cotinine (Fig 1 and Table 2). The blank urine of nonsmokers had no absorption at these wavelengths (Fig 2). The blank urine of smokers had strong absorption at 505 nm, that nicotine and its metabolite alone produced, and an additional spectral band at 435 nm. In the samples containing the isoniazid and acetylisoniazid, this band shifted to 410 nm (Fig 3).

DISCUSSION

Improving compliance is essential for effective tuberculosis control, and a urine test for isoniazid can help detect noncompliance to stimulate prompt action. For this purpose, the urine test used in Arkansas is excellent. If the three persons with negative tests were not taking their medicine, the sensitivity and specificity of this test would be 100 percent. If both of the patients (the one who developed tuberculosis and the one who died) were compliant, the sensitivity would

Table 1—Results of Urine Testing

<table>
<thead>
<tr>
<th>Test Results</th>
<th>Patients Taking Isoniazid</th>
<th>Patients Not Taking Isoniazid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>3*</td>
<td>154</td>
</tr>
<tr>
<td>Positive</td>
<td>69</td>
<td>0</td>
</tr>
</tbody>
</table>

*There is evidence that these patients were noncompliant. Eighty-four patients were in the hospital; 48 patients were from the drug abuse clinic; and 94 were from the tuberculosis clinic.

Table 2—Spectroscopy

<table>
<thead>
<tr>
<th>Material</th>
<th>Color Produced</th>
<th>Absorption Maximum, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>Dark yellow</td>
<td>505</td>
</tr>
<tr>
<td>Cotinine</td>
<td>Dark yellow</td>
<td>510</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>Dark blue</td>
<td>600</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Powder blue</td>
<td>625</td>
</tr>
</tbody>
</table>
The reagents are readily available and have at least a six-month shelf life at room temperature. The most unstable reagent is chloramine-T. To monitor the reaction, isoniazid, 0.04 mg/ml, can be made as a standard. If the chloramine-T deteriorates, the test with the isoniazid standard will take longer than two minutes. At this point, new chloramine-T reagent should be prepared. If there is doubt in reading a result, a confirmation can be obtained by adding 10 ml of the methanol diluent and reading the spectrophotometer at 610 nm, which will detect both the isoniazid and its metabolite acetylisoniazid. When the reagents become old, the reaction slows, but the color change always occurred within two minutes if the reagents were kept no more than six months.

Shortly after the testing started, we noticed a difference in colors produced by the test. The positive samples usually produced a dark blue color but some samples made a blue-green color. Negative samples produced a light lemon color, but some samples made a yellow-orange color. Many drugs were tested individually to try to understand this difference. Finally, since most of the orange colors were seen in samples that came from the drug abuse clinic and most of these patients were smokers, the possibility of nicotine or its metabolites causing the changes was entertained. Figure 1 shows both nicotine and cotinine react with the reagents to produce a yellow color. Isoniazid and acetylisoniazid produce a blue color. In samples in which isoniazid and nicotine are present, the blue-green develops. The Arkansas test may involve reactions similar to those reported for another test for nicotine that uses barbituric acid.13

REFERENCES
3 Eidus L, Ling GM. Tests for the detection of antituberculous drugs or their metabolites in the urine. WHO Tech Bull 1969; 76:1-9
4 Kilburn JO, Beam RE, David HL, Sanchez E, Corpe RF, Dunn W. Reagent-impregnated paper strip for detection of metabolic products of isoniazid in the urine. Am Respir Rev Dis 1972; 106:923-24

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21616/)  
**Figure 2.** Samples of nonsmokers’ urine containing isoniazid and acetylisoniazid. The urine blank is without added metabolites. It is quicker, more sensitive, and easier to interpret than the paper strips that may take 10 to 20 minutes to do. The Arkansas test usually takes less than a minute. Unlike other therapeutic drug testing, it does not add a large financial or labor burden to the laboratory. The cost of the materials is a few cents per test. In our clinic,11,12 the open policy of urine testing did not have any negative effects on the patients. To the contrary, we believe that it may increase compliance.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21616/)  
**Figure 3.** Samples of smokers’ urine blank and containing isoniazid and acetylisoniazid.

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