A Sensitive Method of Screening for Urinary Porphobilinogen

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In this screening method for urinary porphobilinogen (PBG), urine is added to Dowex 2 resin under alkaline conditions in a test tube and mixed. The supernate is removed and the adsorbed PBG is eluted with acid and reacted with Ehrlich's reagent. We compared results with those by the Watson–Schwartz screening method, using urine samples from normal people with and without added PBG. At a PBG concentration of about five times the upper limit of normal, the resin method gave a sensitivity of 100%; the Watson–Schwartz method gave a sensitivity of 51%. At lower PBG concentrations of just over and twice the upper limit of normal, the sensitivity by the resin method was respectively 97% and 100%. With normal urine samples, the resin method gave negative results for all samples (100% specificity) and the Watson–Schwartz had 95% specificity. Our data indicate that the resin method is sensitive, specific, and reliable and is superior to the Watson–Schwartz method.

A qualitative screening test for urinary porphobilinogen (PBG) is often the first-line laboratory test used to diagnose acute intermittent porphyria. This simple test depends on added Ehrlich's reagent (p-dimethylaminobenzaldehyde) reacting with PBG in urine to produce a pink/red color when the PBG concentration is above normal.

Two methods for screening for PBG, that recommended by the Association of Clinical Pathologists, U.K. (1), and the Watson–Schwartz method as described by McNeely (2), have been assessed in our laboratory (3). Both methods gave about 40% positive results for urine samples having PBG concentrations about fivefold the upper limit of normal, 9 mmol/L (4). At 10-fold normal, detection improved to about 70%. Of the two screening methods the Watson–Schwartz was the preferred method, because it was easier to assess the final color. Despite the poor sensitivity of either screening method, their use has been recommended for use in the initial investigation of acute porphyrin attacks. A negative result does not necessarily exclude the diagnosis, and the urine should be re-assessed later by the Dowex resin column method (5) for a definitive result.

Column-chromatographic methods may not have wide appeal to the busy clinical laboratory, and screening methods will continue to be used. It therefore is important that a screening method be available that is both sensitive and specific. We propose such a method, adapted from the quantitative PBG method of Moore and Labbé (6), that meets these criteria.

Urine is added to Dowex-2 resin under alkaline condition in a test tube. After mixing, the supernate is removed and the adsorbed PBG is eluted with acid and reacted with Ehrlich’s reagent.

Materials and Methods

Freshly voided urine samples were used in this study.

Dowex 2 × 8 (400 mesh) was from Sigma Chemical Co., St. Louis, MO. Porphobilinogen was from Porphyrin Products Inc., Logan, UT 84321. We used disposable transfer-bulb pipettes, graduated 1 × 0.25 mL, bulb size 3.5 mL, style no. 222 (Saint Amand Mfg. Co., San Fernando, CA 91340).

Reagents

**Dowex resin.** Wash the resin with sodium acetate solution (3 mol/L) until free of chloride. Wash with distilled water until free of sodium acetate. Store the resin in a bottle in twice its volume of water, at 4 °C.

**Ehrlich’s reagent.** Dissolve 2 g of p-dimethylaminobenzaldehyde in 100 mL of hydrochloric acid (6 mol/L). This reagent is very stable.

**Ammonia solution (25%).** To 1 volume of concentrated ammonia solution add 3 volumes of water.

**Acetic acid (1 mol/L).** To 1 volume of glacial acetic acid add 17 volumes of water.

Procedure (Resin Screening Method)

For all pipetting and transfers of solution use disposable transfer pipettes.

Transfer 1 mL of Dowex resin slurry (stirred well) into a 13 × 100 mm test tube, centrifuge for 1 min, and remove the supernate. Add 0.5 mL of urine and 0.25 mL of ammonia solution, vortex-mix for 15 s, add water to nearly fill the tube, cover with Parafilm, and invert to mix the resin. Centrifuge for 2 min and carefully remove all the supernatant fluid. (It does not matter if some resin is lost.) Add 1.0 mL of acetic acid, vortex-mix for 15 s, and centrifuge for 1 min. Carefully transfer about 0.5 mL of the supernate into another test tube, and mix with 0.5 mL of Ehrlich’s reagent. Observe for color development within 1 min. A pink/red color is due to PBG and is indicative of an abnormally high PBG concentration in urine.

The analytical sensitivity and specificity of the present method was compared with the Watson–Schwartz method by 12 laboratory staff. Two analysts carried out the screening procedures to minimize variation in assay technique. Random urine samples (R) were collected from 41 ostensibly healthy laboratory staff who were not taking any medication and represented samples that should be negative for PBG. To a portion of each urine sample was added aqueous PBG solution to increase its concentration by 50 μmol/L (R + 50) — i.e., to about five times normal, a concentration that should be positive by screening tests. Both (R) and (R + 50) represent a urine set (for negative and positive samples, respectively) and were assayed by the two screening methods. The color produced by Ehrlich’s reagent in both methods for each urine set was read by two laboratory staff who independently graded the test as either negative, positive, or equivocal. When there was disagreement between the two observers on any sample, the result was reported as equivocal. A methyl red reference solution (0.5 mg of methyl red sodium salt in 1 L of 0.1 mol/L HCl) was used as a positive reference for the Ehrlich color in color matching.

Drugs reported to interfere with PBG screening methods...
(7, 8) were tested by using urine from patients taking such drugs or urine samples from healthy staff, supplemented with drugs.

Results

Table 1 shows the analytical sensitivity and specificity of the screening methods for the 41 urine sets. The Watson-Schwartz method gave negative results for 39 of the 41 (R) samples (95% specificity), with two equivocal. Results from the (R + 50) samples were positive for 21 (51% sensitivity) and negative for 14, with six equivocal. The same urine samples assayed by the resin screening method gave 100% specificity and sensitivity.

Urine samples from three patients without acute porphyria who were taking chlorpromazine (800–1200 mg daily) tested negative by the resin method. Likewise, urine from four patients without acute porphyria but taking methyldopa and a patient with urobiilinogenuria also tested negative. Urine samples supplemented with pyridium formed an orange color with Ehrlich reagent, distinct from the expected pink due to PBG. Aminosalicylic acid and methyl red added to normal urine gave negative results with the resin method.

Discussion

A previous assessment of the Watson-Schwartz method showed 100% specificity and 42% sensitivity at a PBG concentration about five times normal (3). The present findings of 95% specificity and 51% sensitivity compares well. It is apparent that PBG at five times normal urine concentration is detected with only 40–50% specificity by the Watson-Schwartz method.

The resin screening method detected this PBG concentration with 100% sensitivity. The Ehrlich color was a distinct pink. To investigate the ability of the proposed resin method to detect lower PBG concentrations, we supplemented 36 additional urine samples with smaller amounts of PBG, namely 10 μmol/L (R + 10) and 20 μmol/L (R + 20). At PBG concentrations just over twofold normal (R + 20), the Ehrlich color was an unmistakable pink, and detection sensitivity was 100%. At (R + 10) PBG, sensitivity was 97% (35/36). Concentrated urine samples after resin treatment sometimes develop an orange tinge with Ehrlich reagent, which may mask a pale pink color due to low PBG concentrations. One of the 36 (R + 10) samples tested appeared orange instead of pink and was very concentrated (1070 mOsmol/kg). All 36 random (R) urine samples, without added PBG, were negative by the resin method.

Drugs reported to interfere with previously established PBG screening methods did not affect the resin method. The Dowex resin bound most drugs avidly, and gave a negative Ehrlich reaction with the acid eluate. The drugs we tested gave no false positives by the Watson-Schwartz method except for methyldopa. However, the methyldopa–Ehrlich complex was extractable into butanol, which distinguished it from PBG.

Since completing this project, our attention was drawn to an abstract reporting a screening test for PBG using a polybenzimidazole ion-exchange resin (9). This method appears to have a visual detection limit of 44 μmol/L of PBG. Our method is more sensitive and involves a much simpler screening procedure.

In conclusion, the resin screening method is able to detect PBG concentration at about two times normal with 100% sensitivity. This is far superior to the Watson-Schwartz method. For the laboratory wanting only a screening test for PBG, the resin method is simple, sensitive, and reliable.

References