**Rapid Quantitative Method Using Spin Columns to Measure Porphobilinogen in Urine**

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**BACKGROUND:** Large increases of urinary porphobilinogen (PBG) indicate acute porphyria, which may be due to acute intermittent porphyria, variegate porphyria, or hereditary coproporphyria. These conditions are relatively rare but share symptoms with more common conditions, such as acute surgical abdomen, and often must be ruled out rapidly. Reported quantitative methods for PBG measurement are time-consuming and inconvenient. We developed a rapid quantitative method that uses resin-packed spin columns to measure PBG in urine.

**METHOD:** We applied urine to anion exchange resin in a spin column, then performed centrifugal separation and washing. PBG was eluted in 1 mol/L acetic acid and reacted with Ehrlich’s reagent. After 5 min, we measured absorbance at 525, 555, and 585 nm. PBG concentration (mg/L) was calculated as 88 (A555 - 1/2(A525 + A585)).

**RESULTS:** The reportable PBG concentration range was 0.2–15 mg/L. Between-day (total) imprecision (CV) was 8.4% at 1.2 mg/L and 3.5% at 4.4 mg/L. Between-day (total) imprecision (CV) was 8.4% at 1.2 mg/L and 3.5% at 4.4 mg/L. Comparison with our established method (x) yielded a Deming regression equation: y = 1.04x - 0.01 mg/L (R² = 0.98; Sₓᵧ = 0.87 mg/L). No interference was noted from urobilinogen or highly colored urine specimens.

**CONCLUSIONS:** This method for PBG measurement is more rapid and precise than other methods. This test can serve as a quick screening test and facilitates batch analysis for routine quantitative testing.

With the exception of δ-aminolevulinic acid dehydratase deficiency, which is very rare, acute porphyrias that present with neurovisceral complaints (acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria) are characterized by large increases in urine porphobilinogen. Although the incidence of these porphyrias is rather low, an acute porphyria attack can mimic a number of more common acute conditions (e.g., surgical abdomen, acute psychosis). Prompt diagnosis leading to appropriate therapy can reduce the severity and length of these porphyria attacks (1). Accordingly, recent expert guidelines from the American Porphyria Foundation recommend the availability of rapid testing to rule out acute porphyria (1).

Large increases of urinary porphobilinogen (PBG) indicate acute porphyria, and multiple procedures for porphobilinogen testing have been reported, including LC-MS (2). Most methods rely on the reaction of porphobilinogen with Ehrlich’s reagent, coupled with a purification step to remove interfering chromogens. The Watson-Schwartz method uses organic extraction to remove interferences. It is rapid, but has poor sensitivity and specificity (3). The Mauzerall-Granick method and its variants (3–7) incorporate anion exchange purification and can be time-consuming and inconvenient. Two faster ion exchange methods have been described. One is a proprietary qualitative method that is intended for screening only and requires quantitative testing to confirm positive results (4). The other relies on nonstandard laboratory equipment (5). We report a rapid, convenient, and inexpensive method for quantification of PBG based on ion exchange purification in spin columns.

Dowex 2X8 ion exchange resin, acetate form, was prepared by mixing 100 g of Dowex 2X8, 200–400 mesh, (Sigma-Aldrich) with 400 mL of 100 g/L sodium acetate. After settling, the supernatant was decanted and the resin was washed 8 times with 400 mL of Type I water and stored at 2–4 °C as a 500 mL/L suspension in water. Ehrlich reagent was prepared as 20 g/L of p-dimethylaminobenzaldehyde (Sigma-Aldrich) in 250 mL/L glacial acetic acid in concentrated hydrochloric acid (9 mol/L final concentration) and stored for 1 month in a glass bottle. PBG stock solution (250 mg/L) was prepared by dissolving 1 mg of PBG (Frontier Scientific) in 4 mL of 30 mmol/L sodium carbonate solution. Lyophilized control materials (ClinCheck 5-ALA, PBG, and total porphyrins control, levels I and II, Iris Technologies) contained 1.2 and 4.4 mg/L PBG in a urine-like matrix.

The PBG assay was performed as follows. We pipetted 0.8 mL of well-mixed resin suspension, 500 mL/L, into an 0.8-mL spin column/2-mL collection tube combination (Product 69702, Pierce Biotechnology) and centrifuged the mixture for 15 s at 14000g, and the eluted water was discarded. A 1-mL urine specimen was alkalinized with 25 μL of 280 g/L ammonium hydroxide, and 200 μL was added to the packed resin. This mixture was allowed to stand for 1 min, then was centrifuged for 15 s and after the eluate was discarded was washed twice with 400 μL of water.
The end of the column was blotted with absorbent paper and placed into a new collection tube. The PBG was eluted by adding 0.4 mL of 1 mol/L acetic acid and centrifuging for 25 s. The resin was eluted with a second 0.4 mL of 1 mol/L acetic acid, and both eluates were combined. Next, we added 0.4 mL Ehrlich reagent to the 0.8 mL combined eluate and also to a reagent blank (0.8 mL of 1 mol/L acetic acid). After 5 min, we measured the absorbances at 525, 555, and 585 nm against the reagent blank. The ratio of A525 to A555 should be approximately 0.8; a different ratio indicates probable interference (3). Color is stable up to 11 min.

PBG concentration was determined from the absorbance at 555 nm, after nonspecific background absorbance was subtracted, estimated as the average of A525 and A585 (a variant of an Allen correction described in (8)). Calibrators at concentrations of 0.3, 0.5, 1, 5, 10, and 15 mg/L were prepared by adding stock PBG to normal urine with an endogenous PBG concentration <0.1 mg/L. A plot of the corrected absorbance vs PBG concentration of the calibrators was linear up to 15 mg/L. Rearranging the equation for the best-fit calibration line yielded PBG (mg/L) = 88 (A555 – ½(A525 + A585)). Samples exceeding the linear range could be read after a 6-fold dilution with reagent blank. Samples with up to 90 mg/L of PBG were linear on dilution. A limit of quantification was established at 0.2 mg/L, the point at which the CV was extrapolated to exceed 20% (based on an SD of 0.04 mg/L at a PBG concentration of 0.3 mg/L and an assumed constant SD at low concentrations).

Recoveries of PBG added to urine were 102.5% and 99% at 1 mg/L and 5 mg/L, respectively (n = 3). Within-day imprecision was 4.0% at 1.2 mg/L and 1.7% at 4.4 mg/L (n = 20). Between-day imprecision values were 8.4% and 3.5%, respectively (n = 20). In contrast, the between-day imprecision values were 14.3% and 10.1%, respectively, when we used our previous bulk ion exchange method, which was based on ref (7) and included the variant Allen correction described above.

We compared the new method with our previous method by using 28 patient samples and 40 samples with PBG added at several concentrations (Fig. 1). This gave a best-fit Deming regression equation of \( y = 1.04x - 0.01 \) (95% CI, 0.95–1.13) \( x = 0.01 \) (−0.14 to +0.13), \( R^2 = 0.98, S_y,x = 0.87 \) mg/L. Bland-Altman difference analysis showed that the new method results were nonsignificantly larger, with a mean difference of 0.1 mg/L (95% limits of agreement, −1.6 to +1.8 mg/L).

Lack of interference by medications and urobilinogen with ion exchange methods has been demonstrated previously (5–7). We verified lack of interference for our method by using 10 abnormally colored urine samples, including 4 with increased urobilinogen and 4 containing hemoglobin. All gave results <1 mg/L, compared with a suggested screening threshold for random specimens of 6 mg/L (1). In 6 months of experience with more than 100 specimens, no false positives were encountered. Only 2 patients had increased PBG concentrations. Both of these patients had previous diagnoses of acute intermittent porphyria and were symptomatic at the time of collection.

The time required to analyze a specimen with the new method was about 20 min, if controls were prepared in advance. The old method required approximately 150 min. Because the major expense for both methods is labor, approximately proportional cost savings should result.

In conclusion, we have developed a rapid, inexpensive, and simple test to measure PBG concentration in urine. This test can serve as a quick screening test, as recommended by recent guidelines (1), and facilitates batch analysis for routine quantitative testing. Implementation in our laboratory resulted in improved pre-
cision and significant reductions in performance time and in the anticipated cost of testing.

**Grant/funding Support:** None declared.

**Financial Disclosures:** None declared.

**References**


DOI: 10.1373/clinchem.2007.096461