not likely to be attributable to contamination of the DNA because all of the results obtained by the revised RFLP analysis of DNA solutions in which the standard RFLP analysis produced questionable electropherograms completely coincided with the SSCP analysis of DNA extracted again from the same finger nails. The genotyping results obtained for ADH2/ADH2 by SSCP analysis may be mistaken for those obtained for ADH2/ADH2 by standard RFLP analysis. In addition, ADH2/ADH2 results obtained by SSCP analysis may be mistaken for ADH2/ADH2 results obtained by standard RFLP analysis because of incomplete digestion of PCR products by the restriction enzyme, Msl, because of contamination by various substances (e.g., guanidinium thiocyanate), which may inhibit the activity of Msl. Nucleic acids were successfully extracted from some dirty nails or manicured nails with the present guanidinium thiocyanate method, but not with the proteinase K method; therefore, nails may be a suitable material for mass processing in epidemiologic studies (13). SSCP analysis, because it does not use restriction enzymes, may be preferable to RFLP analysis in genomic DNA analysis using nails.

References


Testing for Porphobilinogen in Urine, Abel Gorchein
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Increased porphobilinogen (PBG) in urine is pathognomonic of an attack or “crisis” of acute porphyria (acute intermittent porphyria, variegate porphyria, hereditary coproporphyria); the absence of increased urinary PBG in a suspected attack excludes the diagnosis. Some patients, especially with acute intermittent porphyria, excrete excess PBG even in remission, but in an attack, this increases above their generally characteristic “basal” concentrations (1). Thus, “screening” of urine for excess PBG and its measurement have an essential role in the initial diagnosis and management of such patients and in their continuing care.
Current screening tests for PBG in urine include the Watson-Schwartz test (2) and derivatives. It is performed by mixing 1 mL of urine with 1 mL of p-dimethylaminobenzaldehyde in acid solution (Ehrlich’s reagent). A reddish-mauve colored compound ($\lambda_{\text{max}}$ 553–555 nm) is formed. The test lacks specificity, however, because of interference from urobilinogen or other endogenous compounds or by drugs and their metabolites. The test also lacks sensitivity, so a concentration of PBG that is 10-fold above normal may be required for detection.

Specificity and sensitivity of the method are increased by adsorbing the PBG on an ion-exchange resin in a column, washing off interfering compounds, eluting the PBG, and reacting it with Ehrlich’s reagent (3). This allows quantification of PBG but this “gold standard” method is relatively time-consuming.

Resin can be directly added to the urine (4), but this method requires time-consuming steps, including centrifugation and transfer of the supernatant to another container. More recently a proprietary screening method (5) has become available that uses a syringe prepacked with ion-exchange resin. The urine sample is adsorbed, washed, and eluted through a series of procedures involving the addition and removal of filters and final color formation with Ehrlich’s reagent. A color chart and a dye color calibrator are provided for visual assessment.

The procedure described here is based on the original method of Mauzerall and Granick (3), but the resin is held in a permeable sack, like a small tea bag, instead of in a column. This provides a sensitive, specific, convenient, and rapid method that can be used for screening and for quantification.

A length of nylon bolting cloth (25-µm mesh size; approximately 25 cm × 25 cm; Sefar; Satake UK Ltd.) was folded over end to end and machine-stitched with cotton thread and a fine needle to provide several rows of sacks (Fig. 1). A cut with fine scissors 2 mm below the horizontal stitch line of the top row, left open at the top, yielded the next row of open sacks. Each sack was filled with 0.5 mL of resin suspension, which was washed down the side with ion-free water until the upper part of the sack was free of resin. After gentle blotting, each row of sacks was closed with a stitch line; sacks were stored in ion-free water at 4°C. Individual sacks (12 mm × 24 mm, with 1.5–2 mm margins) were cut as required. After use, the sacks were briefly rinsed with ion-free water and kept in a saturated solution of sodium acetate for batch regeneration of the resin, followed by washing with water; they were reusable for ≥4 cycles.

Urine (1 mL; adjusted, if necessary, to pH 6–8 with aqueous ammonia solution) was dispensed into a 7-mL screw-stoppered plastic bottle (e.g., 7-mL Sterilin), and 1 mL of ion-free water was added. A sack containing Dowex-2 (8 × 8 crosslinked, 400 dry-mesh size; acetate form) was added, and the bottle was shaken for 3 min manually (for multiple samples, a mechanical shaker would be more convenient). The liquid was discarded, and the resin-containing sack was washed twice by shaking briefly with aliquots of 2–3 mL of ion-free water, which were poured off to waste. The PBG was then eluted by shaking the sack for 3 min with 2 mL of 0.2 mol/L acetic acid. The sack was then taken out (to be regenerated and reused as described above). Ehrlich’s reagent (2 mL; 1 g of p-dimethylaminobenzaldehyde in 50 mL of 6 mol/L HCl) was added, the bottle was stoppered, and the contents were mixed rapidly by inversion. Maximum color was reached within 5 min and could be determined spectrophotometrically for quantification or visually by comparison with a color chart according to photographs of quantitative yields.

Urine from 20 anonymous patients receiving drug therapy, including antihypertensive medication (bendrofluazide, amiloride, enalapril, ramipril, amlopidine, doxazosin), psychotropic drugs (diazepam, chlorpromazine, prochlorperazine, sertraline), antibiotics (amoxicillin, clarithromycin, metronidazole), as well as omeprazole, alendronate, and gliclazide, were tested. A sample from a patient with obstructive jaundice was also tested. None of the eluates showed any color after reaction with Ehrlich’s reagent, in contrast to two samples from a porphyric patient diluted to give a concentration of 4 µmol/L PBG, run in the same batch. A urine sample from a patient with acute intermittent porphyria in long-term remission, but with continuing excretion of PBG, had a PBG concentration of 25 µmol/L. Serial dilutions were made with nonporphyric urine samples and electronic absorption spectra (450–600 nm) of the Ehrlich reaction products were obtained with a Pye-Unicam SP1800 double-beam recording spectrophotometer. PBG concentrations were calculated from $A_{553}$. A linear response ($y = 23.353x + 0.5017; R^2 = 0.9965$) down to 2 µmol/L was obtained by spectrophotometry. With visual inspection, concentrations of 3–5 µmol/L were unequivocally detectable.

In another assay, PBG was added to give concentrations of 5, 15, and 25 µmol/L to nonporphyric urine samples, which were then subjected to the entire procedure. Recovery of added PBG was 69–72%, uncorrected for loss in the “dead volume” of the sacks, as described below; duplicate samples differed by 3–4%. The response flattened at PBG concentrations >30 µmol/L, presumably because of a capacity limitation of the resin in the sacks,

![Fig. 1. Manufacture of resin-containing sacks.](image)

The pattern of stitch lines is shown for bulk preparation of resin-containing sacks. Horizontal seams are 30–32 mm apart, and pairs of vertical stitch lines (4 mm apart) are placed at 13–14 mm intervals. The finished resin-containing sacks are ∼28 mm × 13 mm. Further details are provided in the text.
which was 25% of that used in this author’s application of the column method of Mauzerall and Granick (3), but this could be readily overcome by testing such urine samples at higher dilution.

The procedure removed ~70% of the PBG compared with the column method. A further elution under the same conditions yielded another 15–20%, consistent with retention in the dead volume (0.50–0.55 mL) of the resin-containing sack. Although the total color yield was less than that of column methods after a single elution step with 2 mL of 0.2 mol/L acetic acid, this was offset by the simplicity and increased speed of the procedure.

Columns are generally eluted with at least 3 column volumes, but only 2 mL of eluate is used in the Ehrlich reaction. The reaction concentration with the present method is therefore likely to be greater, with a corresponding increase in sensitivity.

In summary, a simple modification of the method of Mauzerall and Granick (3) extends its application as a sensitive and specific screening test for urinary PBG.

References

Preanalytical Factors in the Measurement of Intact Parathyroid Hormone with the DPC IMMULITE Assay, Paul Glendenning,* Alexander A. Musk, Mario Taranto, and Samuel D. Vaskikaran (Department of Core Clinical Pathology & Biochemistry, Royal Perth Hospital, Perth 6000, Western Australia; * author for correspondence: fax 618-9224-2491, e-mail paul.glendenning@health.wa.gov.au)

Intact parathyroid hormone (iPTH) is commonly measured with two-site immunometric assays. The DPC IMMULITE 2000 assay (Diagnostics Products Corporation) measures iPTH by a chemiluminescence reaction with a monoclonal murine capture antibody and a polyclonal caprine signal antibody conjugated to alkaline phosphatase. Accurate and precise iPTH measurements are needed for the correct triage of individuals with hyper- or hypocalcemia and for the evaluation of PTH function in bone and mineral disorders (1, 2). In end-stage renal failure, iPTH measurements are commonly used to determine medical management (administration of vitamin D) and surgical therapeutic options (subtotal parathyroidectomy for extreme hyperparathyroidism) (3, 4). Collection of whole blood into EDTA-containing sample tubes offers increased iPTH stability at room temperature (5) compared to serum samples (6) and is recommended by the manufacturer of the DPC IMMULITE 2000 iPTH assay. However, insufficient sample volume can lead to increased final EDTA concentration in the sample-reagent mixture, causing chelation of metallic cations, and this can affect the activity of the alkaline phosphatase label used in the chemiluminescent reaction. We determined the magnitude of effect of limited sample volume in EDTA-containing tubes on results of the DPC IMMULITE iPTH assay.

We evaluated randomized samples from 20 patients with end-stage renal failure: 12 with increased iPTH, 5 with iPTH within the reference interval, and 3 with suppressed iPTH, along with 5 patients with healthy renal function. We collected ~15 mL of whole blood from each patient, before the commencement of dialysis in end-stage renal failure, into preweighed sample tubes; one EDTA tube was completely filled (reference sample) and five other EDTA tubes were underfilled by approximately 20%, 33%, 50%, 67%, and 80%, as estimated visually and confirmed by weighing after sample collection. PTH was determined by the DPC Intact PTH assay with the IMMULITE 2000 automated immunoassay analyzer. Subsequently, PTH was remeasured after the addition of 120 mmol/L magnesium sulfate to the DPC IMMULITE assay reagent in 10 of the patients (60 individual samples).

When EDTA tubes were <50% filled with whole blood, the reported iPTH value was <75% of the target value (where the iPTH was >10 pmol/L) or, in absolute terms, >2.5 pmol/L (where the iPTH was <10 pmol/L) less than the target value (Fig. 1A). Both of the above criteria are used by the Royal Australasian College of Pathologists Quality Assurance Program to determine allowable limits of performance for this assay. The effect was demonstrable with both the old and the new (reformulated) DPC IMMULITE iPTH assays. Furthermore, the effect was present regardless of renal function or whether the target iPTH value was suppressed, within the reference interval, or increased. Statistical analysis (repeated-measures ANOVA and Dunnett’s post hoc test) of results, expressed as a percentage of the target value, indicated that all sample iPTH values were significantly different from the target value when the EDTA sample tube was ≤50% filled with blood (P < 0.001).

Using five samples from patients with target iPTH values within the reference intervals and five samples from patients with increased target iPTH values, we performed repeat assays with the magnesium-supplemented DPC reagent. All samples, except those <33% full, conformed to the criteria used by the Royal Australasian College of Pathologists Quality Assurance Program for allowable limits of performance with values ≥75% of the target value (Fig. 1B).

We conclude that filling EDTA-sample tubes to ≤50% affects iPTH measurements by the DPC IMMULITE iPTH assay. Incomplete sample filling increases the final EDTA concentration in the sample and consequently affects the alkaline phosphatase secondary enzyme used in the DPC IMMULITE chemiluminescent assay. The addition of supplementary magnesium to the DPC IMMULITE iPTH assay reagent appears to be a simple solution to this.