Short Communication

Evaluation of a commercially available rapid urinary porphobilinogen test

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Abstract

Background: Demonstration of substantially increased urinary excretion of porphobilinogen is the cornerstone of diagnosing acute porphyria crisis. Because porphobilinogen testing is not implemented on clinical chemistry analysers, respective analyses are available in rather few clinical laboratories. The aim of this study was to critically describe and to evaluate a semi-quantitative rapid test for urinary porphobilinogen determination which is commercially available and recommended by the American Porphyria Foundation.

Methods: Urinary samples from patients with acute intermittent porphyria and control samples were analysed and the semi-quantitative results were compared with the results obtained by a manual quantitative spectrophotometric method.

Results: In all 32 samples studied, acceptable agreement between the results of the rapid test and the quantitative test was observed. Handling of the test was found to be convenient.

Conclusions: The assay was found to be reliable and has the potential to increase the availability of porphobilinogen testing in the field.

Keywords: acute intermittent porphyria; porphobilinogen; rapid test.

Extremely high concentrations of porphobilinogen (PBG; Figure 1A) are pathognomonic for crises of acute porphyrias (i.e., of acute intermittent porphyria, variegate porphyria, hereditary coproporphyria) (1). The clinical symptoms of this rare disease condition are polymorphic, but very intensive abdominal pain (typically requiring opiates) is found in most cases. Profound hyponatremia, and autonomous, peripheral and central neuropathy are also typical features. Whereas central neuropathy can be associated with psychiatric symp-

toms, peripheral neuropathy can progress to tetraplegia. Once diagnosed, porphyria crisis can be terminated in most cases by administration of haem arginate, withdrawal of porphyrinogenic drugs and control of triggers, such as hypoglycaemia, alcohol or infections. Although indeed very rare, acute porphyria crisis has to be excluded not infrequently in patients with etiological obscure abdominal pain or neuropathy of unknown origin. Thus, it is evident that early and reliable biochemical diagnosis or exclusion of porphyria by urinary PBG quantification is of crucial importance to avoid unnecessary interventions (such as laparotomy), but also to initiate efficient therapy using haem arginate in porphyria crisis. Thus, urinary PBG measurement should be available for all emergency departments.

Automated tests for urinary PBG implemented on routine clinical chemistry analysers are not available. The analyte can be quantified using two commercially available photometric tests which are handled manually. These tests require a spectrophotometer and substantial practical expertise. The tests are time-consuming, in particular as reagents have to be freshly prepared for analysis. Consequently, the implementation of these tests (available from Bio-Rad Laboratories and from Recipe, Munich, Germany) is a substantial challenge in the setting of a “24/7” short turnaround time (STAT) clinical laboratory.

Previously, a semi-quantitative colour test for urinary PBG has become available (Thermo Scientific, Waltham, MA, USA; www.thermofisher.com). This test does not require special laboratory equipment because results are classified by visual inspection of a colour reaction; reagents have a long shelf life and the manual workload of testing is limited. Because during acute porphyria attacks no borderline results of urinary PBG with respect to the upper limit of the reference range (<2.3 mg/L; www.porphyria-europe.com) are found but concentrations are typically more than 10-fold increased, such a semi-quantitative test can be useful for making the diagnosis of acute porphyria. Indeed, this test is recommended by the American Porphyria Foundation (www.porphyrifoundation.com). However, so far no data have been reported with regard to the analytical performance of this test. We therefore decided to perform a bicentric investigation of this test regarding practicability and analytical reliability.

The Thermo PBG test kit is based on the Watson-Schwartz test or Hoesch test, respectively. In this test, PBG condenses with 4-dimethylamino-benzaldehyde (DMAB) in acid solution to form a magenta coloured product (Figure 1B). To
obtain adequate specificity of this procedure, interfering compounds (such as urobilinogen) have to be removed from the urine sample before the reaction is initiated. The Thermo PBG test utilises an anion exchange resin which binds the analyte; interfering compounds are removed during a washing step while PBG is finally eluted into the acidified DMAB solution. Colour development is compared with a three-category colour chart, referring to concentrations of 6 mg/L, 12 mg/L, and 23 mg/L.

The DMAB solution has to be activated by mixing two components of the kit (DMAB powder and 6.1 M HCl); once prepared, this solution is claimed to be stable for at least 12 months at room temperature. The pH of the urine sample has to be checked before analyses, and has to be corrected to pH 6–8 if necessary with dilute ammonia solution (approximately 8%; not included in the test kit). Essentially the analytical procedure includes: drawing up 1 mL of urine into the resin-containing syringe; expelling the urine after some seconds of mixing (which is facilitated by an air bubble drawn into the syringe); drawing up of distilled water and expelling the water after mixing; drawing up 1 mL of a ready-to-use elution reagent (1 M acetic acid); expelling the solution into a transparent reaction tube containing 1 mL of DMAB reagent. After 3 min, the colour of the solution observed against a white background is compared to a colour chart showing the typical colours for three distinct PBG concentrations (6 mg/L, 12 mg/L, 23 mg/L). According to the test instruction the results are given as ‘‘ND’’ (PBG < 6 mg/L), ‘‘+’’ (PBG 6–12 mg/L), ‘‘++’’ (PBG 12–23 mg/L), or ‘‘+++’’ (PBG > 23 mg/L). The kit contains extraction syringes with 5 µm pre-filter devices, DMAB powder, 6.1 M HCl for dissolving, elution reagent (1 M acetic acid), reaction vials and a colour chart for 20 determinations.

The test kit was evaluated in two laboratories: Site 1 (Karlsruhe): five leftover samples (stored at –25°C for <2 weeks) of patients with acute porphyria, and 12 samples from healthy volunteers were analysed with the Thermo rapid test and a quantitative, spectrophotometric PBG test based on the procedure of Mauzerall and Granick (2) (Recipe, München, Germany). All samples from patients were identified as pathological and all samples from healthy volunteers gave negative readings with the Thermo test. The patients’ results were as follows (quantitative result, mg/L, vs. concentration specified for the field of the colour standard (Figure 2). The manufacturer recommends confirming positive tests using quantitative analysis in an experienced laboratory.

![Figure 1](image1.png)  
**Figure 1** (A) Molecular structure of porphobilinogen. (B) Reaction of DMAB and porphobilinogen forming a magenta coloured complex.

![Figure 2](image2.png)  
**Figure 2** Components of the Thermo PBG test kit. 1, Elution reagent (1 M acetic acid); 2, 4-dimethylamino-benzaldehyde (DMAB), working solution; 3, reaction tube; 4, resin-filled syringe; 5, filter; 6, colour chart.
that the Thermo PBG test kit is applicable in the setting of
ing solution.
11 months prior to the study by making up the DMAB work-
ducibility of results.
semi-quantitative read-outs, demonstrating unit-to-unit repro-
itative and semi-quantitative results was found (Table 1).
For the 15 aliquots of all five samples agreement of quan-
titative screening test is, in particular, essential for the fol-
low-up of diagnosed patients undergoing therapy.

We conclude from our data and our handling experiences
concentrations. The sequential number of the aliquot and the
determined PBG determination were documented. A total of 15
sample aliquots (3 × quality control sample; 5 × healthy
urine from a healthy volunteer were used. An aliquot of the
ative after reconstitution as well as in dilution 1:2 with the volunteer’s urine. PBG con-
fconcentrations of the original patient’s sample, of the quality
control samples and of the blended samples were quantified
using a commercially available spectrophotometric PBG kit
(Bio-Rad Laboratories). From each of these samples, several
aliquots of 1 mL each were prepared and identified with
serial numerical labels, not disclosing the respective PBG
concentrations.

The Thermo test kit users’ instructions recommend
quality control by analyzing “known positive urine”. Such
material is rarely available for most laboratories. Thus, the
kit could be improved by providing PBG pure compound
aliquoted quantitatively in reaction vials which could be dis-
olved with water before used as a (positive) quality control.
Furthermore, the practicability of the kit would be improved
by inclusion of dipsticks for urinary pH measurement and of
ammonia solution for pH adjustment if necessary in individ-
ual samples.

Rapid testing for urinary PBG is not at all new. The test
described by Schwartz and Watson with visual inspection of
a colour change is still used in a number of laboratories as
well as a modified procedure, the “Hoesch test” (3). How-
ever, these tests are inconvenient because solutions are not
stable and have to be freshly prepared for analysis. The reli-
ability of these screening tests has been reported to be poor
(4). They are known to be very unspecific owing to side
reaction with many interfering substances, such as urobili-
nogen or aminoacetone, leading to false-positives, or high
urea concentrations potentially leading to false-negatives.

<table>
<thead>
<tr>
<th>#</th>
<th>Sample description</th>
<th>Thermo test (semi-quantitative) PBG mg/L</th>
<th>Bio-Rad assay (quantitative) PBG mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quality control sample “RECIPE”</td>
<td>12</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>(lyophilisate reconstituted with water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Target: PBG 14.9 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Healthy volunteer’s urine</td>
<td>Undetectable</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>3</td>
<td>Quality control sample “RECIPE”</td>
<td>6</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>diluted 1 + 1 with volunteer’s urine [#2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expected: PBG 7.5 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Porphyrin patient’s urine</td>
<td>23</td>
<td>22.0</td>
</tr>
<tr>
<td>5</td>
<td>Patient’s urine [#4] diluted</td>
<td>12</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>1 + 1 with volunteer’s urine [#2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expected: PBG 11.0 mg/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Identical results of several determinations. Conversion of units for PBG: [μmol/L] × 0.226 → [mg/L]; [mg/L] × 4.420 → [μmol/L]. Linear regression between “best matching chart field” concentration and quantitative test result: r = 0.99; Bio-Rad = Thermo × 0.88–1.9 mg/L.
screening test approach has been described previously (5, 6).
However, individually configured, not commercially avail-
able tests are a challenge to be implemented in STAT labo-
ratories. A semi-quantitative rapid PBG test kit using
pretreatment with ion-exchange resin and a colour reaction
was described in 1998 (7); however, this test did not find
widespread use and is to our knowledge not available any
more.

When compared to quantitative PBG test kits, it is an
essential advantage of the Thermo rapid test that no labora-
tory instruments are required and that solutions are ready-to-
use with a shelf life of 1 year after activation. The rapid test
for urinary PBG evaluated here indeed offers a very con-
venient handling; this might enable a more widespread avail-
ability of PBG testing for emergency units and could finally
improve patient care.

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Conflict of interest statement

Authors’ conflict of interest disclosure: Research support played
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pretation of data; in the writing of the report; or in the decision to
submit the report for publication.

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