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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 symptoms, 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.porphyriafoundation.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

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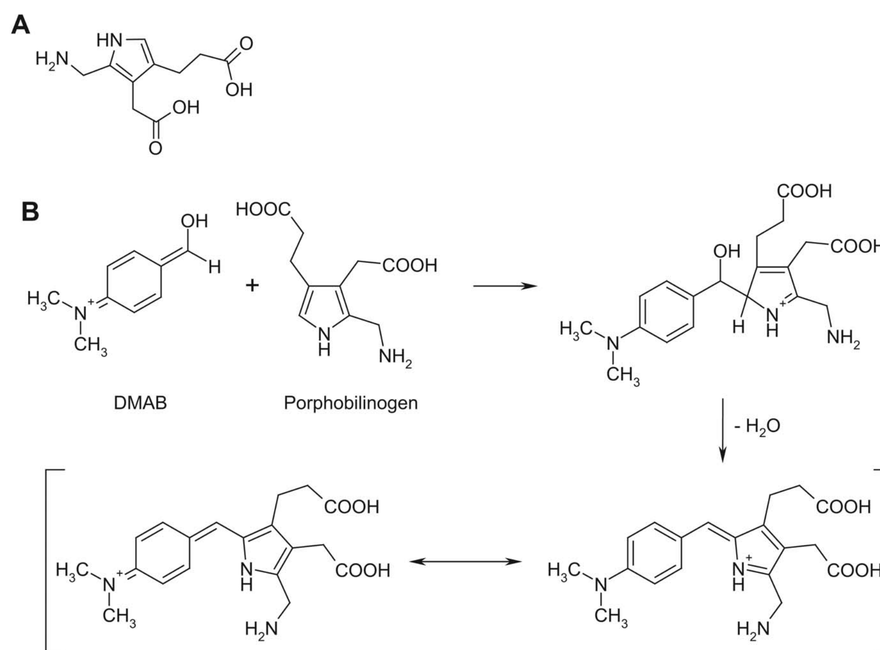


**Figure 1** (A) Molecular structure of porphobilinogen. (B) Reaction of DMAB and porphobilinogen forming a magenta coloured complex.

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 (Figure 2). The manufacturer recommends confirming positive tests using quantitative analysis in an experienced laboratory.

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

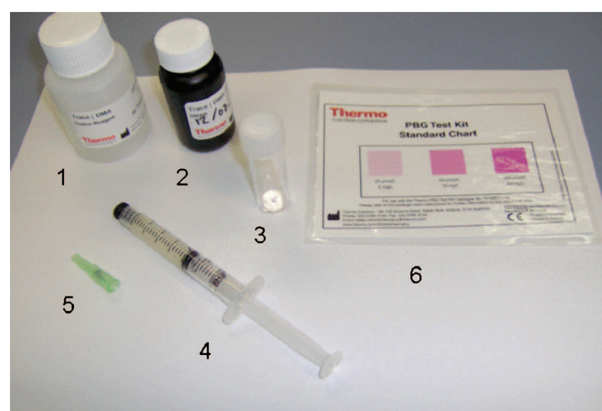


**Figure 2** Components of the Thermo PBG test kit. 1, Elution reagent (1 M acetic acid); 2, 4-dimethylamino-benzaldehyde (DMAB), working solution; 3, reaction tube; 4, resin-filled syringe; 5, filter; 6, colour chart.

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from the Thermo test with the closest agreement to the patient's sample): 45.6 vs. 23; 10.1 vs. 12; 16.8 vs. 12; 4.2 vs. 12; and 43.3 vs. 23.

Site 2 (Munich): a residual native urinary sample from a patient suffering from an acute crisis of porphyria as well as urine from a healthy volunteer were used. An aliquot of the patient's urine was diluted 1:2 with the volunteer's urine. Furthermore, a commercially available quality control sample was investigated (Recipe), native after reconstitution as well as in dilution 1:2 with the volunteer's urine. PBG concentrations 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 sequential number of the aliquot and the determined PBG determination were documented. A total of 15 sample aliquots (3× quality control sample; 5× healthy volunteer's urine; 2× diluted quality control sample; 3× patient's urine; 2× patient's diluted urine) were then analysed using the Thermo semi-quantitative test on the same day in a blinded manner by a regularly trained technician who was not involved in the preparation of the samples. According to visual inspection of the solutions obtained, the results were assigned to the colour reference field with the closest agreement (6 mg/L, 12 mg/L, 23 mg/L). Finally, the results were unblinded by the investigator and the listings of distributed samples' concentrations and the findings were matched. For the 15 aliquots of all five samples agreement of quantitative and semi-quantitative results was found (Table 1). Several fold analysis of individual samples gave identical semi-quantitative read-outs, demonstrating unit-to-unit reproducibility of results.

The Thermo test kit used for validation was activated 11 months prior to the study by making up the DMAB working solution.

We conclude from our data and our handling experiences that the Thermo PBG test kit is applicable in the setting of a STAT clinical laboratory and that reliable screening results can be obtained. Owing to the semi-quantitative nature of the assay, standard clinical chemistry method validation protocols were not applicable; and because acute intermittent porphyria is a very rare disease, the availability of native positive samples was limited. However, using several original patients' samples (native and in dilution) we were able to demonstrate that no misleading results were obtained in our concise evaluation program.

In our laboratories, the Thermo PBG test is applied in case of any diagnostic request outside the routine working hours, whereas a quantitative test is applied whenever possible. The availability of a quantitative test in addition to a semi-quantitative screening test is, in particular, essential for the follow-up of diagnosed patients undergoing therapy.

The Thermo PBG 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 dissolved 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 individual 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). However, these tests are inconvenient because solutions are not stable and have to be freshly prepared for analysis. The reliability 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 urobilinogen or aminoacetone, leading to false-positives, or high urea concentrations potentially leading to false-negatives.

Compared to traditional in-house screening tests, the Thermo PBG assay has the essential advantage of increased specificity owing to a sample extraction step using a resin. This

**Table 1** Raw data of a method comparison study between a semi-quantitative rapid test for urinary porphobilinogen measurement (Thermo Scientific) and a quantitative spectrophotometric test (Bio-Rad).

| # | Sample description | Thermo test (semi-quantitative) PBG mg/L[a] | Bio-Rad assay (quantitative) PBG mg/L |
|---|---|---|---|
| 1 | Quality control sample ''RECIPE'' (lyophilisate reconstituted with water) Target: PBG 14.9 mg/L | 12 | 13.4 |
| 2 | Healthy volunteer's urine | Undetectable | <0.5 |
| 3 | Quality control sample ''RECIPE'' [#1] diluted 1+1 with volunteer's urine [#2] Expected: PBG 7.5 mg/L | 6 | 6.8 |
| 4 | Porphyria patient's urine | 23 | 22.0 |
| 5 | Patient's urine [#4] diluted 1+1 with volunteer's urine [#2] Expected: PBG 11.0 mg/L | 12 | 11.6 |

[a]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.

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screening test approach has been described previously (5, 6). However, individually configured, not commercially available tests are a challenge to be implemented in STAT laboratories. 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 laboratory 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 convenient handling; this might enable a more widespread availability 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 no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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