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Letter to the Editor

# Results of a pilot external quality assessment study on free protoporphyrin in erythrocytes

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Quantification of erythrocyte protoporphyrins (ePP) is essential to establish the diagnosis of erythropoietic protoporphyria, a form of chronic porphyria that is not very rare (prevalence roughly 1:50,000), and clinically characterized by painful phototoxic skin reactions that usually start in early childhood (1, 2), and X-linked dominant protoporphyria, a recently (2008) described metabolic disorder with similar clinical features (3). While PP with zinc chelated as the central ion (zinc PP) is increased in iron deficiency anemia and inefficient erythropoesis, substantial increases (>50-fold) in metal-free ePP is pathognomonic for protoporphyria. Erythrocyte protoporphyrins are measured using various methods, and standardisation of analytic procedures has not been addressed at present.

In order to assess the comparability of free ePP results obtained in clinical samples using different analytical methods, we performed an inter-laboratory comparison study (4). In this trial, lyophilized aliquots of three samples (A–C) were shipped to the participating laboratories. The samples were based on washed red blood cells which were lysed by repeated freeze-thaw-cycles prior to dispensing and lyophilization. Sample B was obtained from an adult male patient suffering from protoporphyria; sample C was from a healthy volunteer; and sample A was a 1+1 mix of samples B and C. Six laboratories from Europe, North-America and Asia-performing ePP quantification routinely, contributed results, expressed as metal-free ePP concentrations of the reconstituted sample in μmol/L. In three laboratories, HPLC methods using fluorescence detection were used, whereas the other three laboratories used fluorometry after extraction without chromatographic separation. Results were evaluated according to absolute concentrations, and as the x-fold of the respective method-specific upper limit of normal (ULN).

All participating laboratories found samples A and B to contain free PP concentrations above the method-specific reference ranges, and sample C was classified as normal by all participants. However, as expressed as the x-fold of the upper limit of the method-related reference range, very substantial between-method differences were observed: In sample A the range was from 1.2- to 123-fold of the upper limit of the reference range, and in sample B from 2.0- to 201-fold (Table 1). All three non-chromatographic fluorometric methods gave substantially lower reference-range related free ePP results when compared to HPLC methods.

Overall, our results disclose poor inter-laboratory concordance of ePP measurements. In the present situation, follow-up of patients should therefore be based on the results of a single laboratory. HPLC methods seem to be superior to non-chromatographic methods for the quantification of ePP with respect to the disease-related dynamics of the results.

To improve standardization of ePP measurement, the introduction of commercially available, human matrix-based, certified materials for calibration and quality control is highly desirable. An external quality assessment scheme for ePP measurements has very recently been initiated by the European porphyria network (EPNET; www.porphyria-europe.org). Standardisation of ePP measurements aiming to stabilize results over time is not only important for the follow-up of patients involving results from different laboratories, but also as a prerequisite of international clinical research on protoporphyria (5).

For rare diseases, laboratory investigations are often of essential diagnostic importance. Since respective analyses are typically performed in rather few laboratories, they are hardly addressed by in-vitro diagnostic companies with respect to standards and quality control materials. Consequently, quality assurance of analyses related to rare disease requires particular attention from the community of clinical chemistry and laboratory medicine. In particular, in-vitro diagnostic companies should be encouraged by the scientific societies to contribute to analytical solutions related to orphan diseases.

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**Table 1** Intermethod comparison of metal-free erythrocyte protoporphyrin measurements; raw data.

| Lab # | Method | Sample | A | B | C | Reference range |
|---|---|---|---|---|---|---|
| 1 | HPLC | Metal-free ePP, µmol/L | 5.9 | 13.0 | 0.06 | <0.12 |
| | | *x-fold of ref. range* | *49.2* | *108* | *0.5* | |
| 2 | HPLC | Metal-free ePP, µmol/L | 11.1 | 18.1 | 0.07 | <0.09 |
| | | *x-fold of ref. range* | *123* | *201* | *0.8* | |
| 3 | HPLC | Metal-free ePP, µmol/L | 8.0 | 15.6 | 0.04 | <0.2 |
| | | *x-fold of ref. range* | *40.0* | *78.0* | *0.2* | |
| 4 | Fluorometry | Metal-free ePP, µmol/L | 5.2 | 8.9 | 0.7 | <2.0 |
| | | *x-fold of ref. range* | *2.6* | *4.5* | *0.4* | |
| 5 | Fluorometry | Metal-free ePP, µmol/L | 1.2 | 2.0 | 0.1 | <1 |
| | | *x-fold of ref. range* | *1.21* | *2.0* | *0.1* | |
| 6 | Fluorometry | Metal-free ePP, µmol/L | 12.8 | 21.0 | 0.71 | <1.5 |
| | | *x-fold of ref. range* | *8.5* | *14.0* | *0.5* | |

## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article. 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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