Validation of ELISA method for serum erythroferrone evaluation

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OBJECTIVES / PURPOSES
Human erythroferrone is often measured in patients with iron deposition in their organs, including iron deficiency anemia or sideroblastic iron bone marrow accumulation in Parkinson disease or high prevalence. Patients are subjected to phlebotomy in order to remove iron from organs and/or suppression of iron delivery to bone marrow in polycythemia vera, iron deposition from liver cirrhosis or hepato-metabolic diseases, hemochromatosis, hyperferritinaemia, and prion disease trauma. Some patients must lower transfusion doses iron deposition in organ. Investigation on erythroferrone might bring to new therapeutic approaches. Through influence on hepatic iron sequestration and absorption, depression and mobilisation of iron, which may lead to more specific and quick results compared to methods used in our days, such as iron supplementation, sclerotherapy and transplantation.

Validation of ELISA serum erythroferrone quantification follows the next steps: determination of analytical range through calibration curve (four parametric, X−logarithmic, and Y−linear); determination on limit of detection (0.056 ng/ml); verification of accuracy of the curve by low level of quantification (ULOQ), middle point of quantification (MPQ) and upper level of quantification (ULOQ); determination of reliability by recovery method (added/found); and determination of intra-assay and inter-assay precision (average 2.73% and 2.63%, resp.).

RESULTS
Using three of standards (0.04 ng/mL, 0.21 ng/mL and 1.0 ng/mL) and measured such five times (ULOQ) MPQ and ULOQ were established:

- ULOQ: 4.25 ± 0.13 ng/mL
- MPQ: 1.01 ± 0.02 ng/mL
- Low limit of quantification: 0.056 ng/mL

Recovery of added erythroferrone quantification was established by procedure recovery (standard). The area of recovery was 99.3 ± 7.9%.

SUMMARY/CONCLUSION
During the steps of validation of ELISA method for serum erythroferrone quantification we found: wide range of quantification; very low limit of detection; very high diagnostic sensitivity; and low analytical variation. All these analytical characteristics showed that selected ELISA method for serum quantification is reliable for routine laboratory application in diagnosis and monitoring of therapy in different diseases, that involves dysregulation of iron homeostasis.

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