Stability And Effect Of Storage Of Blood On Hematofluorometer Readings Of Zinc Protoporphyrin

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Citation

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Abstract

Aim: To study the stability and effect of storage, at 4° C temperature in the refrigerator, on blood, on the Hematofluorometer readings of Zinc Protoporphyrin (ZPP) over time.
Study: 48 randomly collected samples were tested for ZPP values using Hematofluorometers. Results were compared using paired t-test.
Results: Data analysis showed that ZPP results were stable up to day 4 from the day of blood collection.
Comments: one should establish their own controls to find the stability of the results according to the locally prevailing conditions for the more reliable results in their practice.

INTRODUCTION

Hematofluorometric determination of Zinc Protoporphyrin (ZPP) is a screening method for the assessment of iron deficiency. Beyond that, ZPP is not only a simple parameter of detecting iron deficiency, but rather a parameter of iron deficient erythropoeisis surveying all the steps of iron metabolism. Therefore, ZPP is used as a kind of end point control to detect real iron deficiency as well as derangements of iron metabolism in chronic inflammatory disorders, neoplastic disorders, sideroblastic disorders or lead poisoning. [1]

Hematofluorometer measures ZPP and a variable proportion of metal-free Protoporphyrin IX (PPN), depending upon the emission and excitation filters of the particular instrument. [2, 3] Only a small drop of blood is required for the test. The measurement takes less than one minute. Its being a simple test, we searched the literature to know the stability of the test and we could only find very few references. Therefore, we decided to study the stability and effect of storage of blood (at 4° C temperature in the refrigerator) on ZPP values over time in our set-up.

MATERIALS AND METHODS

The study was carried out in a tertiary care hospital in the western region of India. 48 samples collected in 3 ml EDTA vacutainer, were selected randomly from the samples sent to our laboratory for various investigations, without any criteria for inclusion or exclusion to represent the whole strata of patients. Samples were tested for ZPP levels using Hematofluorometer (Protofluor-Z, Helena Laboratories). Each sample was subjected to ZPP estimation on the day of collection and then every day till one week from the day of sample collection. Tests were performed at the same time of the day throughout one week. Samples were preserved at the 4° C temperature in the refrigerator. Company provided reagents and calibrators were used for the test performance and calibration, respectively. Tests were performed at the room temperature. Samples and calibrators were allowed to come to room temperature before performing the tests.

RESULTS

Results of the samples of the tests performed daily were analyzed using paired t-test. The ZPP value of day 1 of each sample was compared with that of the respective sample for each subsequent day. The p values of <0.05 were considered significant, meaning thereby that the readings of that particular day were significantly different from those of day 1 results and that implied the instability of the results then onwards. In our study, we observed that ZPP results were stable till day 4 (Table-1). Therefore, it is possible to reproduce and/or get reliable test results till day 4 from the day of collection of the blood under the circumstances prevailing in our laboratory.

Figure 1

Table 1: Comparison of the ZPP results of day 1 with respective day results

Days	Day1/Day2	Day1/Day3	Day1/Day4	Day1/Day5	Day1/Day6	Day1/Day7
P value (Paired t-test)	0.06	0.49	0.27	0.01	0.002	0.0002

DISCUSSION

The principle aim of the study was to find the stability and effect of storage of blood at 4° C temperature, on the ZPP readings using Hematofluorometer. Review of literature of the very few studies that have actually been documented on this subject revealed quite different results.

The studies have reported the stability of the readings up to 10 days at ambient temperature and up to eight weeks if the samples were refrigerated. [4,5] Some studies have shown the stability of the readings for refrigerated samples up to one month. [6] However the blood for analysis was stored in a tube containing 5 % celite in saline in some of the studies, whereas, in our case the samples did not contain any solution apart from EDTA as an anticoagulant. We agree that our results have shown the stability of the results only for four days. But one must compare the results with emphasis on the conditions in the form of preservation techniques, facilities, and climatic conditions as well. We feel that this short term stability of the results are not because of the properties of ZPP as such, but most likely is due to altered physicochemical properties of the stored blood samples. Here, one should take into account the point that freezing causes physicochemical change in blood, which could interfere with the extraction of Protoporphyrin. So blood should not be stored frozen. [5]

In our opinion, even stability of the results for the short time of four days is advantageous because one need not analyze samples immediately or on the day of blood collection. So it makes for the time delay in transporting the samples from the field to the laboratories in routine practice as well as field studies. Looking at the diversity of the result, we also opine that one should establish their own controls to find the stability of the results according to the locally prevailing conditions for obtaining more reliable results and making the best use of this test in their practice.

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