Evaluation of the stability of Ferritin, B12 and Folate in serum samples over time

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Introduction

Haematinics is the study of vitamins and minerals involved in the essential function of the body, with the most important and clinically significant of these analytes being Vitamin B12, Folate and Ferritin[1]

If a deficiency were to occur, they would manifest clinically in a multitude of ways, most prevalently; Morphological changes to the Red Blood Cells, Cognitive impairment, developmental issues in the foetus and new-born, and impact the cardiovascular system[2]

Currently within the Cwm Taf Morgannwg University Health Board, Roche guidance on sample storage is followed, which states that from venepuncture, Vitamin B12, Folate and Ferritin are only stable at room temperature for up to 2 hours and 48 hours when stored at 4°C[3-5]

The aim of this study is to determine whether samples for Vitamin B12 Folate and Ferritin are suitable for processing past the recommended Roche guidance on sample stability, as to better fit the heath boards requirements.

This study is important as currently, these samples are only processed in the Prince Charles Hospital, Merthyr. By determining whether the samples are stable for longer periods of time after sample collection it would allow the Biomedical Scientists and Clinicians investigating to have greater confidence in the results produced after storage for 2 hours and 48 hours at room temperature and 4°C, respectively.

Methods and Materials

Samples were taken from 18 Volunteers from within the Pathology department at Prince Charles. Volunteer with any known deficiencies or conditions that may affect the level of B12, Folate and Ferritin were excluded from this study.

Three samples from each volunteer were taken as it was determined that this would provide sufficient volume of serum.

Samples were used to determine how Room Temperature (RT) affected results over time, with 2 samples stored at RT for 0-hours and 1 sample for 7-hours, before being centrifuged at 4000RPM for 10 minutes.

A single Cobas 8000 Analyser using ELICA immunoassay methods were used to quantify the results, and samples ran after morning QCs were completed and authorised, as to ensure minimal variability in the results obtained. QC and reagent lots were recorded daily.

An initial baseline sample was taken from each patient sample after standing at room temperature for 0-hours and 7-hours, and were then stored at -80°C. All samples were moved into -80°C after their allotted time stored at 4°C, as to prevent further sample degradation.

All samples for the volunteer were then thawed on a sample mixer and analysed on the same day, as to further reduce potential variability that may arise, such as changes to QCs and reagent lots used. Samples were also ran in duplicate to ensure the results produced were accurate.

An Acceptable Criteria of ≤10% was outlined in the IFU Assay Kits [3-5], and was used to determine whether the samples were within the expected precision of the analyser.

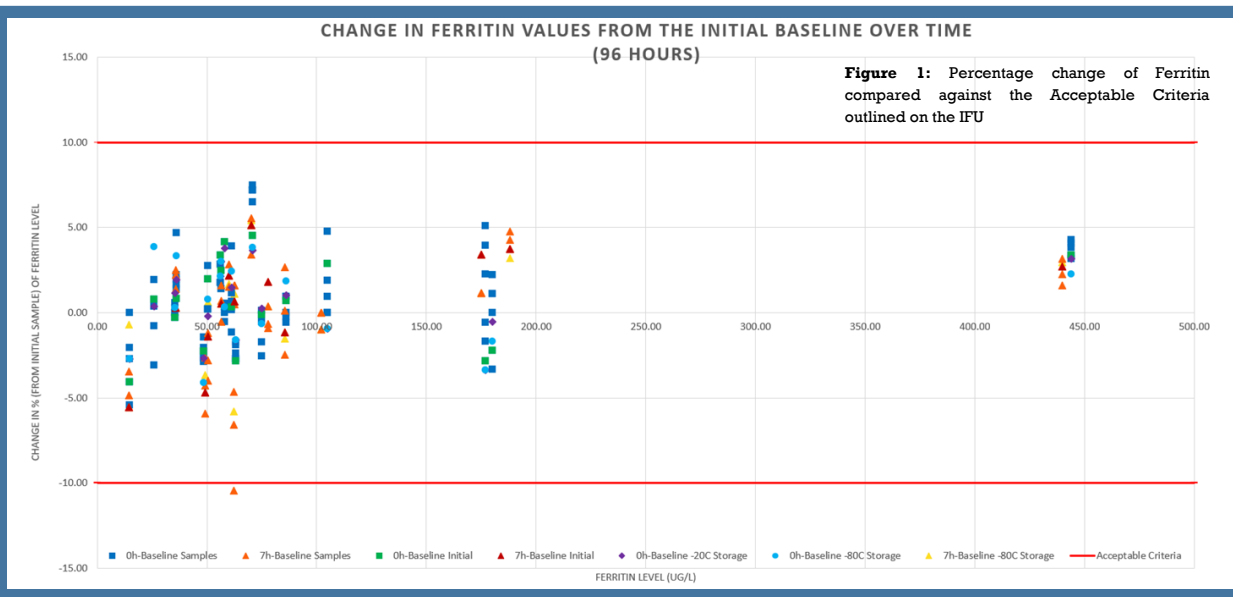

Figure 1: Percentage change of Ferritin compared against the Acceptable Criteria outlined on the IFU

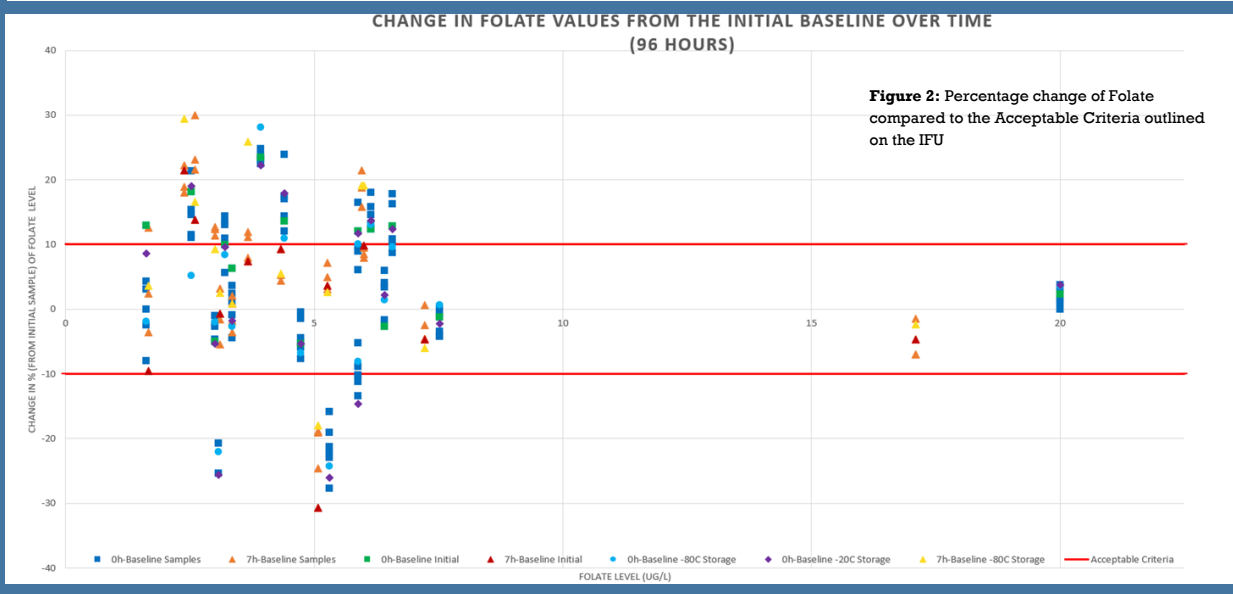

Figure 2: Percentage change of Folate compared to the Acceptable Criteria outlined on the IFU

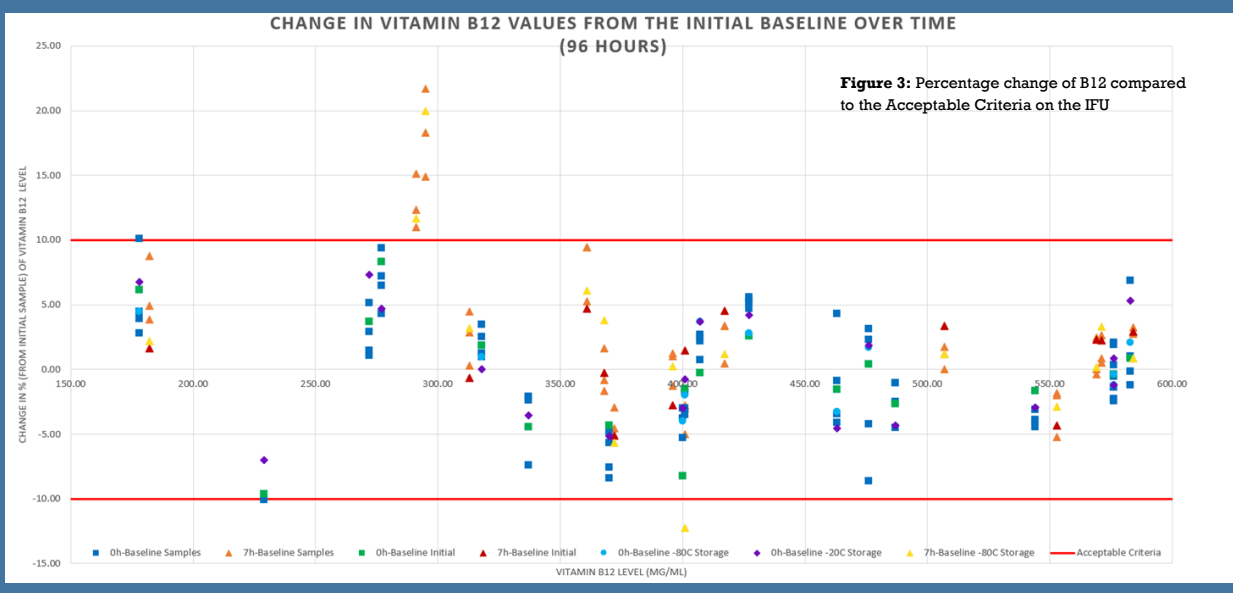

Figure 3: Percentage change of B12 compared to the Acceptable Criteria on the IFU

Discussion

Kruskal-Wallis and ANOVA methods were used as the data produced was not normally distributed, and a high p-value of <0.9 was produced for each analyte. This indicates that no statistically significant difference was seen between the baseline samples stored at RT for 7 hours to samples stored at RT for 0-hours. No significant change was observed in the samples stored for up to 96 hours.

As no significant change was seen, this indicates that samples are stable at RT for up to 7 hours and up to 96 hours when stored at 4°C.

Vitamin B12 and Ferritin were deemed to be most suitable for analysis after 2 hours stored at RT and 48 hours at 4°C, as most datapoints were found to be within of ≤10% of the acceptable criteria, as outlined by the Assay IFU.

However, Folate was found to be unsuitable for analysis after 2 hours, with significant portion of folate found outside of the Acceptable Criteria of ≤10%, however this may be due to external factors, such as the Freeze-Thaw cycle of inserting and removing the samples into the -80°C freezer, and potentially sample degradation caused by UV exposure during its time stored at RT.

Conclusion

To conclude, only Vitamin B12 and Ferritin were found to be suitable for processing after prolonged storage at RT and when stored at 4°C for up to 96 hours, and were found be unaffected as significantly as Folate by external factors. Therefore, Roche guidance on the processing and analysis of vitamin B12 and Ferritin can be ignored.

Folate was found to be unsuitable for analysis after storage at room temperature for 2 hours and 48 hours at 4°C, and therefore processing and analysis of these samples must continue to adhere to guidance outlined by Roche.

The findings of this study may impact practice of the Biomedical Scientists investigating the results as it would allow them to have more confidence in the results produced, as samples that may not have been delivered to the lab in time would still produce reliable results, and would also result in the urgency of sample processing to decrease.

However, further studies will need to be done to investigate and eliminate potential causes of sample instability, particularly when assessing Folates suitability in future.

References

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