Ferritin Assay using Novel Dried Instant Plasma Spot Collection Technology

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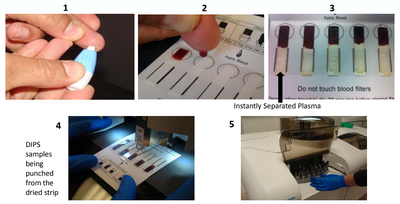
Abstract

Background: The new dried instant plasma spot (DIPS) technology developed by our laboratory uses whole blood spotted on one end of a filter strip, so that the plasma and red cells rapidly separate before the blood has time to clot, leaving dried plasma at one end of the card and residual dried red cells at the other.

Methods: Blood samples were collected from 52 volunteers. Venous blood was drawn into serum separator tubes and non-additive tubes. DIPS were prepared immediately by spotting the blood from non-additive tubes on the collection cards and dried. Serum was separated by conventional methods and stored frozen until analyzed. Two 6.0 mm punches from the dried plasma part of each card were rehydrated and the supernatant removed from the filter paper by centrifugation and transferred into tubes for testing. Both serum and extracted plasma were analyzed for ferritin by the Siemens Immulite auto-analyzer. Ferritin results obtained from the DIPS were corrected for total protein to take into account differences in saturation of the filter paper.

Results: Results showed an excellent correlation between DIPS and serum for ferritin (R^2=0.9). Intra-assay and inter-assay precision for three samples spanning the reference range were <7%. The mean recovery of ferritin from DIPS was excellent at 97.5%.

Conclusion: The convenience of sample collection, storage and transport of DIPS will save considerable costs in blood collection, handling and shipping especially in remote areas without phlebotomists or the equipment necessary to prepare blood serum or plasma, This new self-collection method is ideal for large scale clinical/ research studies due to convenience of point of care combined with the accuracy of testing by auto-analyzers.



Methods

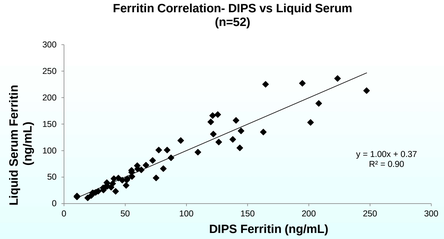
We tested the feasibility of the using the DIPS collection method for testing ferritin, which is not possible using DBS methodology. Blood samples from capillary (finger stick) and venous blood (venipuncture) were collected from 52 volunteers who are employees at ZRT Laboratory. Serum was prepared by conventional methods and stored frozen until analyzed for ferritin by the Siemens Immulite auto-analyzer. For the DIPS dried sample, two 6.0 mm punches from each card were dropped into a Nunc fritted deep 96 well plate (Figure 4). Phosphate Buffered Saline (100 µL) was added to each well containing the punched samples and the Nunc 96-well plate placed on a shaker at 700 rpm for 1 hour. The plate was centrifuged at 3000 rpm for 10 minutes to separate the filter paper from the supernatant, which was transferred to tubes for testing ferritin by the Siemens Immulite auto-analyzer. Following ferritin testing the sample was transferred to a Siemens Dimension Xpand Plus where it was analyzed for total protein content. Ferritin results obtained from the DIPS were corrected for total protein to take into account possible differences in saturation of the filter paper.

Dried Instant Plasma Collection

ZRT Laboratory, in collaboration with MDI Membrane Technologies, has developed a new technology that separates whole blood spotted on one end of a filter strip, so that the plasma and red cells rapidly separate before the blood has time to clot, leaving dried plasma at one end of the card and the residual dried red cells at the other. We have called this method of collection DIPS (Dried Instant Plasma Spots). The pictures on the left show the simplicity and convenience of sample collection (pictures 1, 2), blood separation (3) and testing (4, 5) offered by DIPS technology. It offers the convenience of point of care sample collection combined with the accuracy of testing by auto-analyzers found in most clinical laboratories. This development provides a clear advantage over dried serum spot (DSS) and dried plasma spot (DPS) collection methods, which require blood collection by venipuncture, separation by centrifuge, and then spotting on filter paper.

Results

Data generated by this method showed an excellent correlation between DIPS and serum for ferritin (R^2=0.9), both analyzed by the Siemens Immulite Ferritin Assay



Validation of the micronized ferritin assay using DIPS

Intra-assay and inter-assay precision of the Ferritin method were determined by choosing 3 samples spanning the reference range, and analyzing them multiple times each within the same run (for intra-assay) or in separate assays over the course of a week (inter-assay). The mean recovery of ferritin from DIPS was excellent at 97.5%.

DIPS Ferritin Intra-assay precision

N=10	*Concentration (ng/mL)*	*C.V. (%)*
Sample # 1	*55.3*	*6.1%*
Sample # 2	*100.4*	*6.6%*
Sample # 3	*188.9*	*4.8%*

DIPS Ferritin Inter-assay precision

N=10	*Concentration (ng/mL)*	*C.V. (%)*
Sample # 1	*62.5*	*6.8%*
Sample # 2	*105.7*	*4.8%*
Sample # 3	*205.4*	*3.6%*

Discussion

Methodology for testing analytes in serum or plasma (anti-coagulant treated), spotted and dried on filter paper, has been developed successfully by other laboratories. Dried serum spots (DSS) and dried plasma spots (DPS) have been used for analyses of HIV-1 viral load[1,2], as well as for detecting antibodies against a range of other blood-borne viral infections[3]. DSS/DPS assays have been developed for Vitamin B12[4], and ferritin for iron status[5], and are emerging as a new choice for pharmacology and toxicology laboratories[6,7].

There is currently no accurate, efficient, and convenient blood collection/separation technology available that can be directly substituted for routine, laboratory serum or plasma separation techniques. A more convenient sample collection, storage and transport technique, as presented in this study of Ferritin assay development using DIPS, will help thousands of health care professionals and laboratories around the world to save considerable costs in blood collection, handling, and shipping. This simple blood collection method will also make it easier to collect blood in remote areas without phlebotomists or the equipment necessary to prepare blood serum or plasma. DIPS collection, combined with conventional testing with auto-analyzers, will also make it possible to carry out large scale clinical studies that until now were either not possible or prohibitively expensive. The ultimate outcome of this research could be the widespread availability of DIPS technology to virtually any clinical laboratory using routine testing equipment.

References

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