Dried Blood Spot Screening of Cardiometabolic Risks

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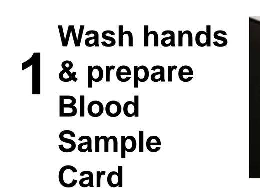
Abstract

Background: The cardiovascular and metabolic risk factors are gaining more attention as potential targets of intervention to combat against increased mortality due to cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). New tools must be made available to clinicians to formulate optimal treatment strategies for effective management of such cardiometabolic risk conditions.
Methods: Dried bloodspot (DBS) samples were evaluated for the presence of insulin and high sensitivity CRP using conventional commercial kits based on a direct sandwich ELISA technique. The triglyceride assay involved enzymatic hydrolysis of triglycerides by lipase to glycerol and free fatty acids. The glycerol produced was then measured by coupled enzyme reactions.
Results: The blood spot CRP values demonstrated good correlation with the serum CRP values, providing the correlation coefficient of 0.9776; slope 0.7317; intercept 0.031. Fasting and non-fasting triglyceride levels, when compared in blood spot and serum samples resulted in a correlation of 0.9. A positive correlation was found in insulin levels from fasting (R2 = 0.99) and non-fasting (R^2 = 0.93) blood spot and serum samples.
Conclusion: The wellness panel in dried bloodspot may be helpful as a first step in comprehensive prevention and treatment approach for patients at cardiometabolic risks (CVD, T2DM or metabolic syndrome). Our preliminary results have also shown the automation feasibility of these assays for high throughput screening studies.

Dried Blood Spot Collection

1 Wash hands & prepare Blood Sample Card

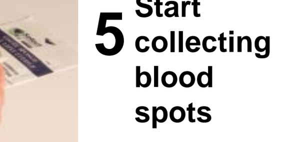


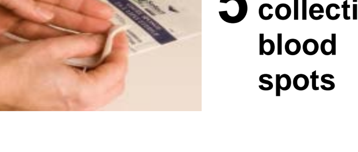
2 Wipe fingertip with alcohol prep pad provided

3 Prick finger with lancet as shown

4 Wipe away first blood drop

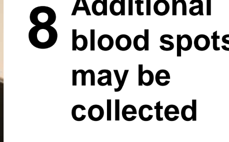
5 Start collecting blood spots





6 Place one drop per circle minimum 3/8" size shown

7 Place one drop in each circle



8 Additional blood spots may be collected

9 Let blood spots air dry

Methods

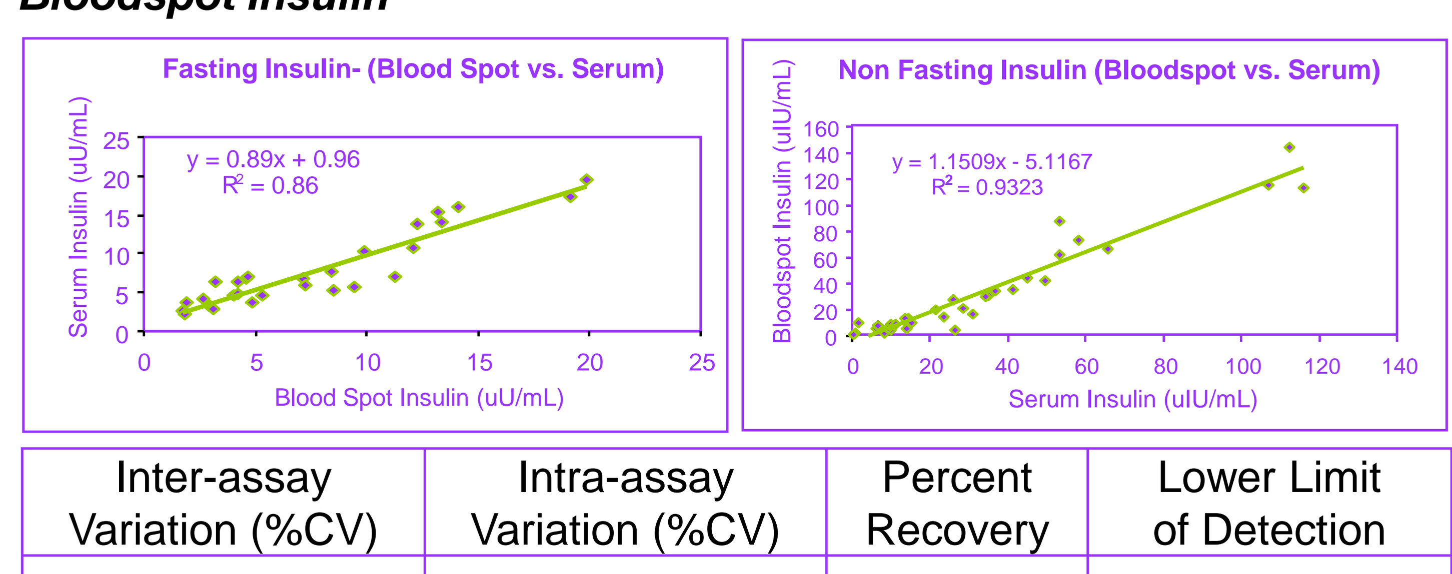
Bloodspot Insulin
Fasting and non-fasting healthy subjects contributed a blood spot sample from a finger-stick and a blood sample through venipuncture for serum isolation. The blood spots obtained from finger sticks were air-dried at room temperature after collection and stored at -20°C within 24 hr of collection. Serum was stored frozen at -20°C. Standards and controls for blood spots were prepared by mixing washed red blood cells 50/50 with insulin kit standards and controls and drying on blood spot filter paper. Final concentrations of standards used in the assay were 75, 37.5, 18.8, 9.3, 4.7, 2.3, 1.2 and 0.6μIU/mL. Serum and rehydrated blood spots were tested in parallel for insulin using the commercial ELISA kits from DRG.

Bloodspot hs-CRP
The serum and dried blood spot samples from fifty volunteers at ZRT were collected and stored as explained above. Standards and controls were also prepared in a similar fashion. The high concentration standard (5μg/mL) was serially diluted to prepare the standard curve. One 6.0 mm disk of dried bloodspot was punched and 200 μL of extraction buffer was added and incubated at room temperature on a shaker for two hours. A 20 μL of extracted sample and 100 μL of CRP enzyme conjugate from the assay kit was added into a 96 well plate. The plate was incubated at room temperature for 45 minutes and washed five times thereafter with distilled water. To this, 100 μL of substrate TMB solution was added and incubated in dark for 20 minutes. The reaction was stopped by adding 100 μL of stop solution and the plate was read at 450 nm within 15 minutes.

Bloodspot Triglycerides
Bloodspots were collected from healthy volunteers from ZRT, after overnight fasting for triglyceride determination. Standards and controls were prepared as explained earlier. The high standard was serially diluted to get a standard curve with concentrations 200, 100, 50, 25 and 12.5 mg/dL. The bloodspot extract was prepared by punching out two 6.0 mm disks from bloodspots in a 96 deep well plate and 200 μL of methanol was added to each well. The plate was then incubated for 2 hours on a microplate shaker at 37°C. Triglycerides were measured by modifying the method of Qurashi et al. 50 μL of sample, standards, controls or blanks were added to 250 μL of triglyceride reagent (Randox) and incubated at 37°C for 10 minutes. The absorbance was read using the Wallac Victor2 1420 Multilabel Counter set to 490 nm. Serum triglyceride levels were determined using the same assay kit.

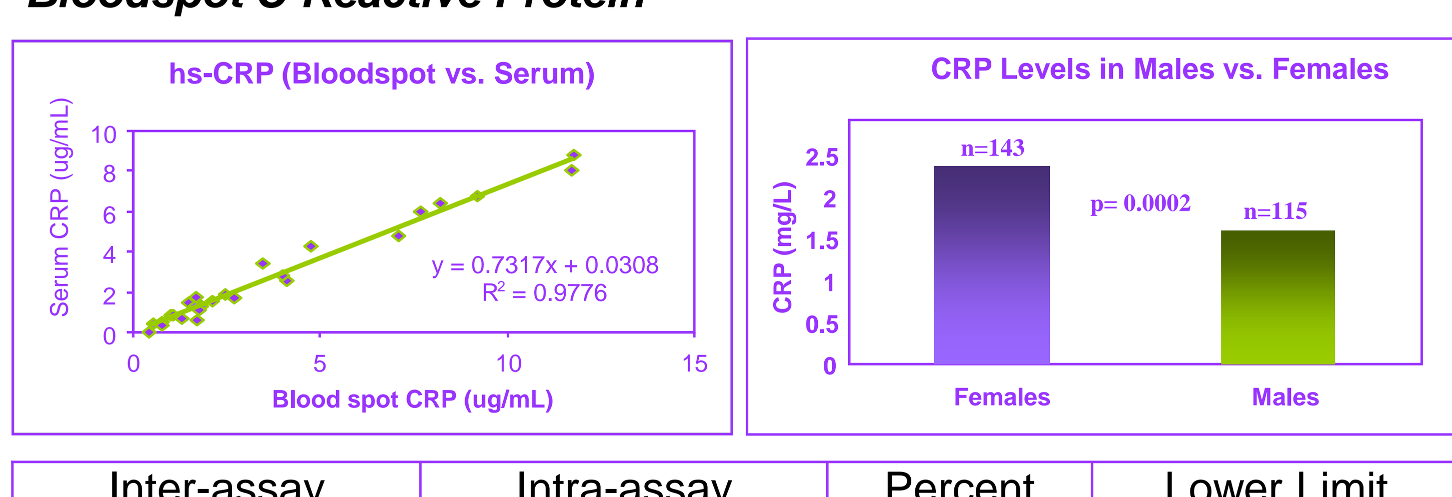
Results

Bloodspot Insulin



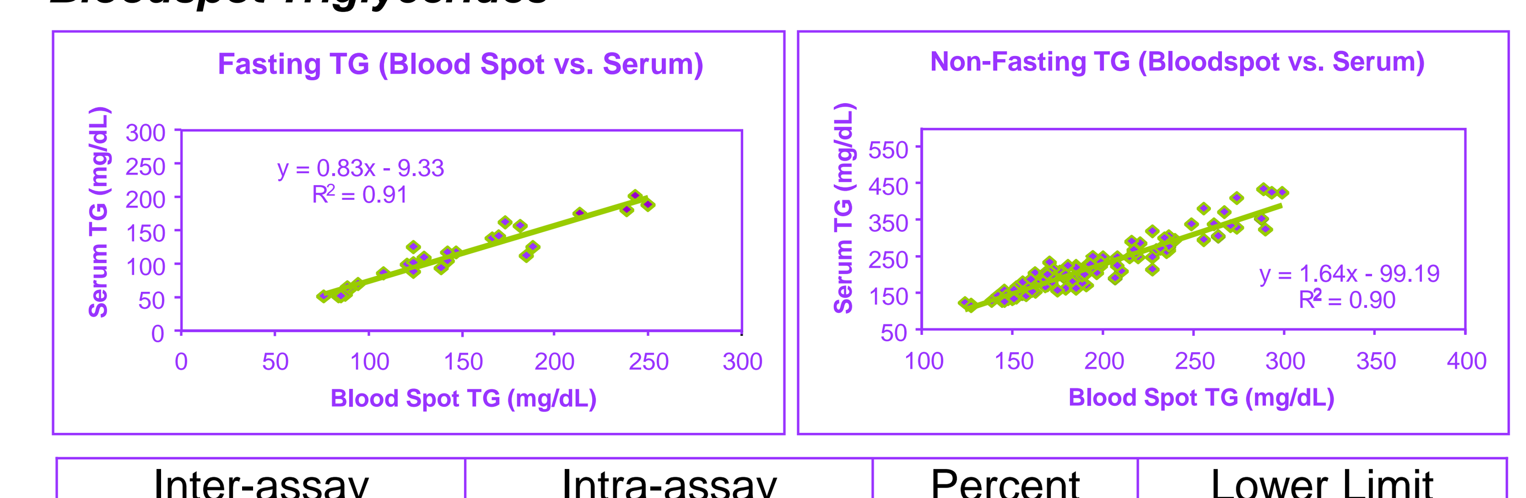
Inter-assay Variation (%CV)	Intra-assay Variation (%CV)	Percent Recovery	Lower Limit of Detection
14.39 3.61	7.57 2.74	98.83 14.3	1.5μIU/mL

Bloodspot C-Reactive Protein



Inter-assay Variation (%CV)	Intra-assay Variation (%CV)	Percent Recovery	Lower Limit of Detection
7.91 2.39	7.26 1.24	105.6 8.5	0.114 mg/L

Bloodspot Triglycerides



Inter-assay Variation (%CV)	Intra-assay Variation (%CV)	Percent Recovery	Lower Limit of Detection
8.93 2.24	4.89 1.41	110.02 1.3	15.4 mg/dL

Discussion & Conclusion

In this study, we present an improved high throughput screening method for estimation of insulin, high sensitivity CRP and triglycerides in dried blood spot to provide a preliminary tool to help patients and physicians develop a better management option for cardiometabolic risk factors. The blood spot methods have been used by several investigators because of the low cost, ease of sample collection, storage and transport. In addition, patients and clinicians prefer these methods due to small sample requirements and minimal invasiveness as compared to venipuncture. A few of the studies have earlier reported laboratory protocols for insulin, c-reactive protein and triglycerides in dried blood spot. The protocols in our laboratory have shown improvements in terms of precision, accuracy and ease of high throughput screening of these biomarkers when extracted from dried blood spot. As insulin resistance and CRP levels have been shown to be correlated in adults, measurement of CRP levels along with insulin may help in the assessment of potential cardiovascular risk. In addition, the role of triglycerides as risk factor in cardiovascular and coronary artery disease has been stressed by several investigators.
We conclude that the stability, efficient recovery and a good correlation of concentration of these markers in dried blood spot and serum make these assays a reliable tool for significant medical interventions in terms of getting better handle on risk factors posing serious health risks to patients with cardiometabolic problems. A wellness panel in dried blood spot consisting of fasting insulin, hs-CRP, triglycerides, hemoglobin A1c along with blood pressure and cholesterol level assessments is recommended for population at risk and also for those with pre-diagnosed conditions of cardiovascular disease or type 2 diabetes mellitus.

References

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2. McDade TW, Burhop J, Dohnal J. High sensitivity enzyme immunoassay for c-reactive protein in dried blood spots. Clin Chem 2004; 3: 652- 654.
3. Ridker PM, Furing JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome and the risk of incident cardiovascular events. Circulation 2003; 107: 391- 397.