Cardiometabolic Risk Screening using Simple and Convenient Dried Blood Spot

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Abstract

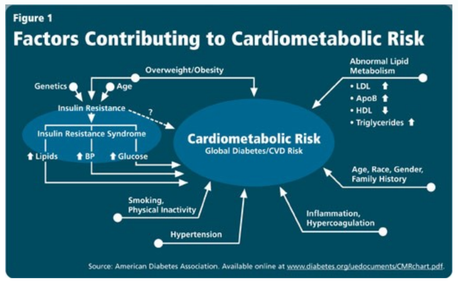
Background: Cardiovascular and metabolic risk factors are gaining more attention as potential targets of intervention to combat against increased mortality due to cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Insulin resistance, elevated triglycerides, raised hemoglobin A1c (HbA1c) and inflammatory markers like C-reactive protein (CRP), in addition to other factors have been clearly shown to relate to these conditions. New tools must be made available to clinicians to formulate optimal treatment strategies for effective management of such risk conditions.

Methods: Blood spots obtained from finger sticks on a filter paper were air-dried for 4 hours at room temperature after collection. 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 . HbA1c was tested using an immunoturbidimetric assay.

Results: The blood spot CRP values demonstrated good correlation with the serum CRP values (R=0.98. 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 (R^2= 0.99) and non-fasting (R^2= 0.93) blood spot and serum samples, respectively. The correlation coefficient was found to be 0.96 for HbA1c measurements in serum and bloodspot samples.

Conclusion: We have developed rapid, reliable and accurate methods for fasting blood insulin, high-sensitivity CRP, HbA1c and triglyceride detection in dried blood spot samples. Our preliminary results have also shown the automation feasibility of this assay for high throughput screening studies. This wellness panel can be helpful as a first step in comprehensive prevention and treatment approach for patients at cardiometabolic risks (CVD, T2DM, or metabolic syndrome).

Background



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. Serum and rehydrated blood spots were tested in parallel for insulin using the commercial ELISA kits from DRG.

Bloodspot hs-CRP

Serum and dried blood spot samples from volunteers were collected and stored as explained above. One 6.0 mm disk of dried bloodspot was punched and 200 µL of extraction buffer was added and incubated at room temperature for two hours. A 20 µL of extracted sample and 100 µL of CRP enzyme conjugate from the assay kit was incubated at room temperature for 45 minutes in a 96 well plate. After washing with distilled water,100 µL of substrate TMB solution was added and incubated 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

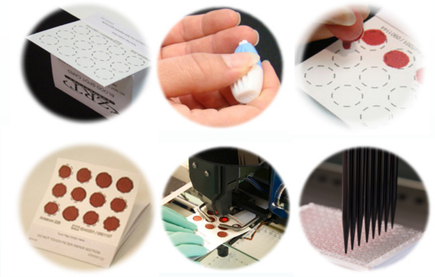
Bloodspot extract was prepared by punching out two 6.0 mm disks from bloodspots and 200 µL of methanol was added to each well. The plate was then incubated for 2 hours at 37°C. Triglycerides were measured by modifying the method of Qurashi et al. 50 µL of sample, standards, controls or blanks were added 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.

Hemoglobin A1c

The method is based on the interaction between antigen and antibody to directly determine the HbA1c in extracted blood from paper disks. HbA1c in blood is known to bind to latex particles. Therefore, a latex-HbA1c-mouse anti-human HbA1c antibody complex is formed when mouse anti-human HbA1c monoclonal antibody is added. Further, addition of goat anti-mouse IgG polyclonal antibody forms an agglutination by interacting with the monoclonal antibody. The concentration of HbA1c is proportional to the total amount of agglutination. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

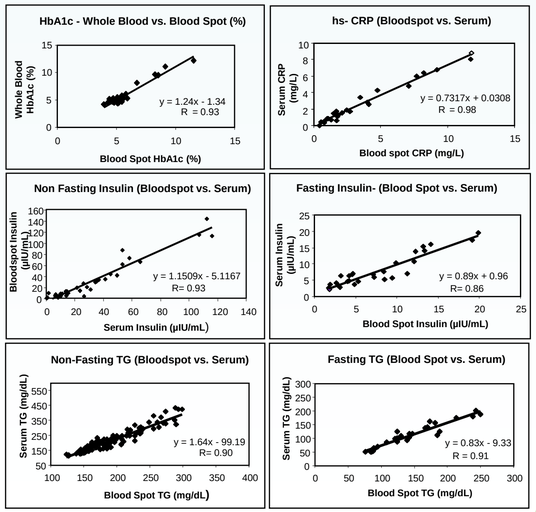
One large spot was punched using the multipuncher and 500 µl of lysis buffer was added. The extraction plate was incubated for 2 hours at RT. Twenty microliters of this hemolysate was incubated with anti-human HbA1c monoclonal antibody for five minutes at 37°C followed by addition of 60 uL goat anti-mouse IgG polyclonal antibody. Microtiter plate was incubated at 37°C for five minutes and read at 620 nm.

Bloodspot Collection



Results

Analyte	Inter-assay CV (%)	Intra-assay CV (%)	Recovery (%)	Lower Limit of Detection
Hs-CRP	7.91±2.3	7.26±1.2	105.6±8.5	0.114 mg/L
Triglycerides	8.93±2.2	4.89±1.4	110.02±1.2	15.4mg/dL
Insulin	14.39±3.6	7.57±2.7	98.83±14.	1.5µIU/mL
HbA1c	9.15± 2.9	5.87± 2.5	99.9± 8.8	2.0%



Discussion

Cardiovascular and metabolic factors are gaining attention as potential targets of intervention in the prevention and treatment of cardiovascular disease and type 2 diabetes mellitus. New tools will enable clinicians formulate treatment strategies for effective prevention and management of cardiovascular and metabolic conditions. In this study, we present an improved high throughput screening method for estimation of insulin, high sensitivity CRP, triglycerides and hemoglobin A1c in 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. The role of triglycerides as risk factor in cardiovascular and coronary artery disease has been stressed by several investigators. ADA has suggested that HbA1c is a better screening tool for diabetes and prediabetes than oral glucose.

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 dried blood assays in our laboratory have shown improvements in terms of precision, accuracy and ease of high throughput screening of these biomarkers.

Conclusion

The stability, efficient recovery, and excellent correlation between levels of these markers in dried bloodspot and serum make these assays a reliable and convenient tool for screening of cardiometabolic risk factors. A bloodspot panel consisting of fasting insulin, hs-CRP, HbA1c and triglycerides along with blood pressure and cholesterol level assessments is recommended for a population at risk and also for those with diagnosed cardiovascular disease or type 2 diabetes mellitus.

References

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