# Gender Differences in High-Sensitivity C-Reactive Protein as Determined by Dried Blood Spot Assay

Sonia Kapur, Sanjay Kapur and David Zava
ZRT Laboratory, Beaverton, OR, United States

## Background & Objective

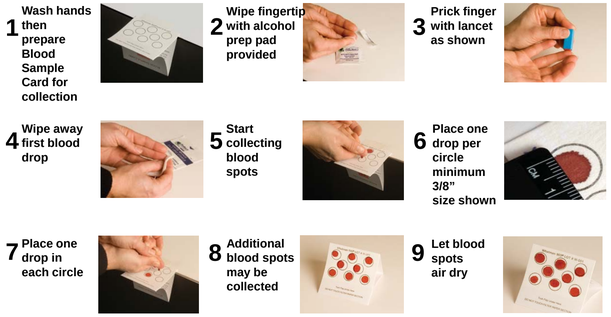
***Background:*** **Individuals with metabolic syndrome have been shown to be at a high risk for cardiovascular problems, but most cross-sectional studies of C-reactive protein (CRP) and metabolic syndrome have been carried out either in males or females only. Few studies have shown that CRP levels are indicative of the onset of metabolic syndrome and diabetes in women. There are some other reports showing strong correlation of several markers of inflammation to insulin resistance and metabolic syndrome in women. Significant race and gender differences in the distribution of CRP levels as determined by conventional serum assays, have been reported. A dried blood spot assay for measurement of high sensitivity CRP (hs-CRP) was used in this study. This technology provides convenience of sample collection and the possibility of large-scale cross-sectional studies in different population groups.**

***Objective:*** **To study gender differences in hs-CRP levels using a dried blood spot assay.**

## Methods

**We measured hs-CRP in 115 men and 143 women with a rapid and sensitive dried blood spot method, irrespective of age or race. In addition to normal venipuncture, the subjects were asked to provide finger stick blood samples on special filter paper cards, as shown below, which were dried and later used for hs-CRP analysis. The dried blood spots were rehydrated in extraction buffer only for two hours as compared to longer extraction procedures reported earlier and were utilized for hs-CRP ELISA assay available commercially. Standards and controls were also prepared in a similar fashion. The high concentration standard (5 µg/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. The color was developed using 100 µL of substrate TMB 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.**

## Blood Collection Procedure




## Results

**The CRP concentration ranges varied from 0.113- 4.03 mg/L in men to 0.34- 11.71 mg/L in women. It was observed that women had higher levels of hs-CRP than men with a median of 2.36 and 1.58 mg/L respectively (Fig 1). This gender difference in CRP levels was highly significant with p =0.0002. A good correlation was observed in hs-CRP levels in dried blood spots when compared with their respective serum values.**

***Accuracy:*** **CRP concentrations in blood spot samples collected from volunteers were compared with their corresponding serum CRP values. The comparison provided the following data as shown in figure 2 (Correlation coefficient = 0.9776; Slope = 0.7317; Intercept = 0.031). The minimum detectable concentration of the CRP ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.114 mg/L. Additionally, the functional sensitivity was determined to be 0.114 mg/L (as determined with interassay %CV < 20%). Lower limit of hsCRP ELISA is approximately 0.114 mg/L; upper limit = 15 mg/L.**

**Figure 1** CRP Levels in Males vs. Females

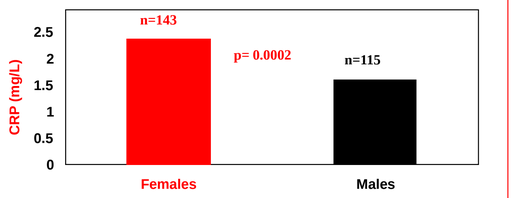
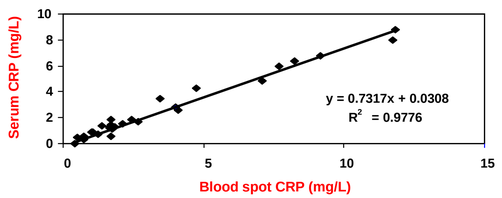



**Figure 2** hs-CRP (Bloodspot vs. Serum)




| 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 |

***Precision, Recovery and Linearity:*** **Within-run precision was determined by replicate determinations of five different blood spot samples. Between-run precision was determined by replicate measurements of six different blood spot samples over a series of individually calibrated assays. Various blood spot samples of known CRP levels were combined and assayed in duplicate. The mean recovery was 105.6%. Three blood spot samples were serially diluted to determine linearity. The mean recovery was 103.99%**

## Discussion & Conclusion

**Khera and colleagues, in addition to few other reports have confirmed race and gender differences in C-reactive protein levels and the relevance of CRP levels in terms of cardiovascular risk assessments. Several studies have indicated that, in addition to infection and inflammation, high levels of CRP could indicate risk of cardiovascular disease in patients with type-1 diabetes, metabolic syndrome or the onset of type 2 diabetes. Measurement of CRP has been recommended by the American Heart Association and CDC, but more research in larger population is required before the clinical utility in epidemiological and community based studies is established. The potential difficulties in measurement of CRP in such population based studies has been stressed earlier, especially in terms of blood collection through venipuncture. The dried blood spot assay for CRP used in this study may add significant value to ease of sample collection and transport for assessing the overall cardiovascular risks in both men and women of different origin. Using our fast and improved dried bloodspot assay, we have shown a significant difference in hs-CRP concentrations among men and women. This may help explain greater association of diabetes in women with conditions like dyslipidemia and cardiovascular problems. The rapid and convenient blood spot method will be helpful in studying demographic correlation of hs-CRP levels in both men and women, with measures of insulin resistance and components of metabolic syndrome.**

## References

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