



# Filter Paper- Dried Blood Spot Assay of Insulin Measurement

David Zava, Sanjay Kapur, Zahra M Kashi  
ZRT Laboratory, Beaverton, Oregon



## Abstract

**Objective:** The objective of this study was to assess the feasibility of using blood spots collected by fingerpricks and dried on filter paper for measuring insulin. Blood spot testing offers advantages of convenience of sample collection at any time outside the clinical setting and simplicity of sample processing, which may allow for expanding the scope of endocrinological studies to previously unreachable subjects and study sites.

**Methods:** Twelve fasting and fifty non-fasting healthy subjects contributed a blood spot sample from a finger-stick and a blood sample through venipuncture. The blood spots obtained from finger sticks were air-dried at room temperature after collection and stored at -200C within 24 hr of collection. Serum was stored frozen at -200C. For blood spot testing, 6.4 mm discs were punched from the dried blood spots and rehydrated in assay buffer. 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 same commercial ELISA kits.

**Results:** 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 mean of fasting insulin levels were 7.3 IU/ml (range 1.4-17.1) for serum and 7.9 IU/ml (range 2.1-17.8) for blood spots. The mean of non-fasting insulin levels were 29.9 IU/ml (range 1.8- 116.19) for serum and 31.6 IU/ml (range 1.5- 143.9) for blood spots.

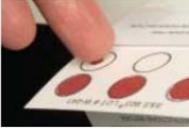
**Conclusion:** We conclude that insulin levels obtained from both fasting and non-fasting blood spot samples correlate well with values obtained from standard serum insulin assays.

## Objectives

- ❖ The objective of this study was to assess the feasibility of using blood spots collected by fingerpricks and dried on filter paper for measuring insulin.
- ❖ Blood spot methods are used in various research applications including cross-sectional studies; longitudinal, population-based epidemiologic studies; anthropological and disease association studies; hematological and forensic studies.
- ❖ Blood spot testing offers advantages of convenience of sample collection at any time outside the clinical setting.
- ❖ Blood spot testing offers simplicity of sample processing (no phlebotomist, centrifugation, or refrigeration of sample necessary), analyte stability, low invasiveness, reduction of sample volume, and ease of sample storage for repeat or additional testing.

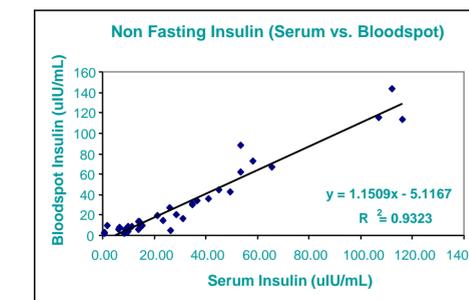
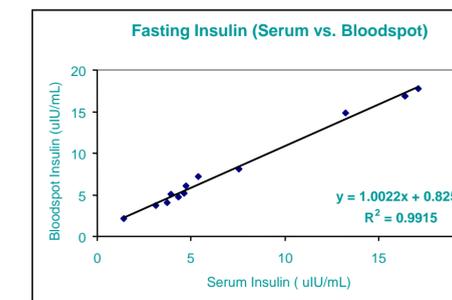
## Methods

### Blood Collection Procedure

1. Wash hands then prepare Blood Sample Card for collection 
2. Prepare the lancet 
3. Twist the orange tab off and discard 
4. Wipe fingertip with alcohol prep pad provided 
5. Prick finger with lancet as shown 
6. Wipe away first drop 
7. Collect blood on the filter paper 
8. One drop per circle 
9. Let spots air dry 

- ❖ Twelve fasting and fifty non-fasting healthy subjects with no history of diabetes contributed a blood spot sample from a finger-stick and a blood sample through venipuncture.
- ❖ 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.
- ❖ For blood spot testing, 6.4 mm discs were punched from the dried blood spots and rehydrated in assay buffer.
- ❖ 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 same commercial kits based on a direct sandwich ELISA technique.

## Results



- ❖ 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 mean of fasting insulin levels were 7.3  $\mu$ U/ml (range 1.4-17.1) for serum and 7.9  $\mu$ U/ml (range 2.1-17.8) for blood spots.
- ❖ The mean of non-fasting insulin levels were 29.9  $\mu$ U/ml (range 1.8- 116.19) for serum and 31.6  $\mu$ U/ml (range 1.5- 143.9) for blood spots.

## Conclusions

- ❖ We conclude that insulin levels obtained from both fasting and non-fasting blood spot samples correlate well with values obtained from standard serum insulin assays.
- ❖ Blood spot testing represents a less invasive technology than venipuncture that may allow expanding the scope of endocrinological studies to previously unreachable subjects and study sites.
- ❖ The design of the assay and the nature of the samples also make this method suitable for screening programs that consist of self-sampling.

## References

- ❖ Buttler N, et al. Development of a bloodspot assay for insulin. Clinica Chimica Acta., 2001; 310: 141-150.
- ❖ Dowlati. B, Dunhardt. P et. Al. Quantification of insulin in dried blood spots. J. Lab. Clin. Med., 1998; 131: 370.
- ❖ Laakso M. How good a marker is insulin level for insulin resistance? Am. J. Epidemiol., 1993; 137: 959- 965.