

Validation of Dried Blood Spot AMH ELISA: A Convenient Alternative to Venipuncture*

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OBJECTIVE

The aim of this study was to validate the quantitation of AMH in dried blood spot samples and do a comparative analysis to the routine venipuncture method.

INTRODUCTION

Dried blood spot (DBS) technology, is a very simple, inexpensive technique for collecting drop(s) of whole blood on filter paper. DBS has been in existence for almost 50 years, but has gained interest recently due to advancement in technologies that enable sensitive and reliable methods to quantitative data from a drop of blood samples.

Dried Blood Spot AMH method is a simple blood test that measures anti-Mullerian Hormone/Mullerian inhibiting substance (AMH/MIS), a hormone which is produced by granulosa cells in ovarian follicles. The levels of AMH detected in a woman's blood are thought to reflect the growing follicles supply remaining in the woman's ovary - this has been described as the 'ovarian reserve'.

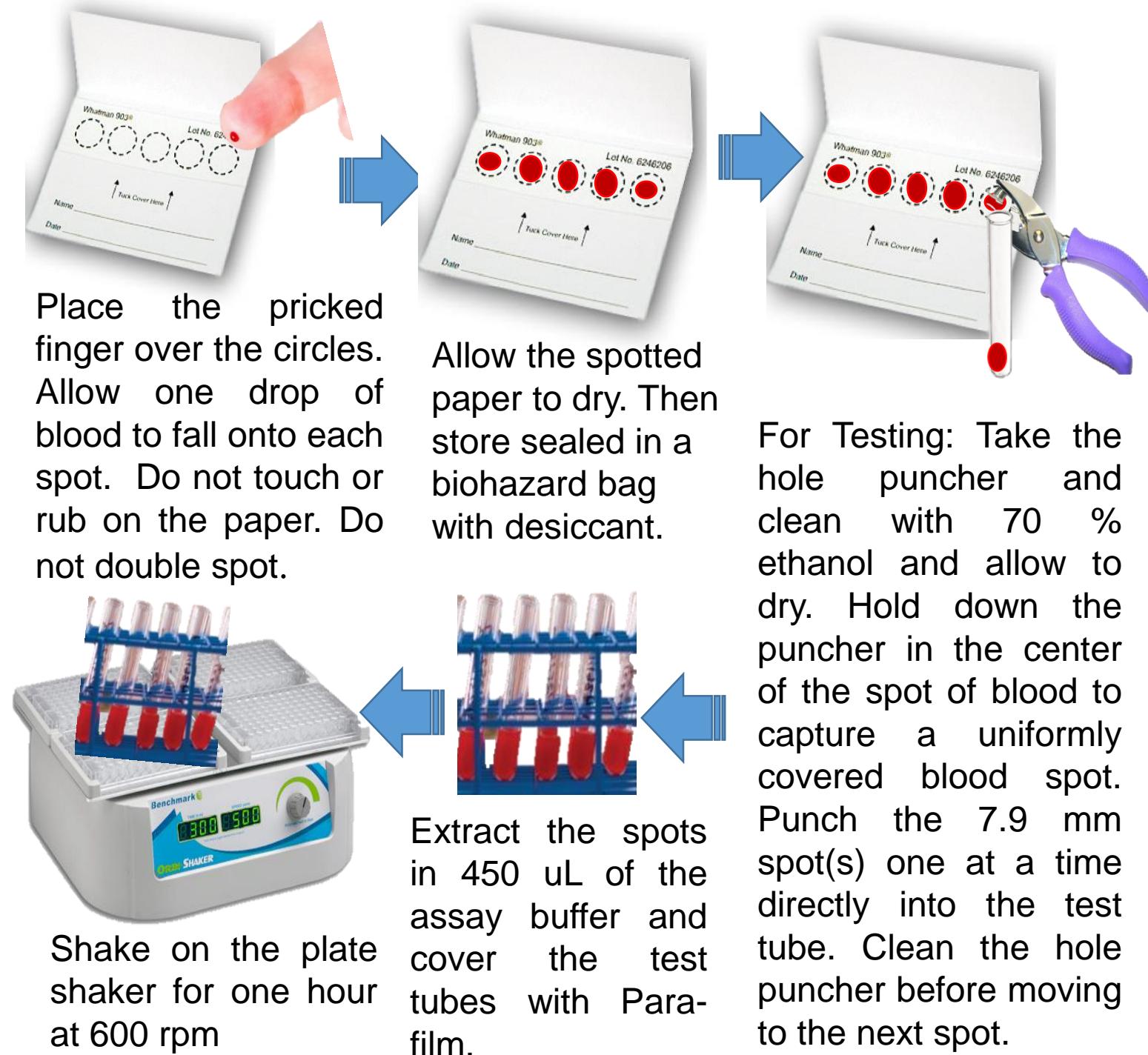
Research has shown that low AMH level indicates a low ovarian reserve and the subjects are poor responders to the drugs used in IVF clinics. On the other end of the spectrum, abnormally high levels of AMH is indicative of polycystic ovarian syndrome and can mean over-stimulation during IVF procedure. This test will be very useful in predicting the ovarian reserve for physically challenged breast cancer patients undergoing chemotherapy.

Advantages of Dried Blood Spot Technology

Features	DBS Method	Conventional Method
Medically Trained Professional	Minimally invasive technique and can be self collected by finger prick.	Invasive and collected at clinics by phlebotomist by venipuncture.
Biohazard Comparison	Dried blood inactivates pathogens and lowers the biohazard risk.	Potentially infectious and prone to bacterial contamination.
Sample Processing	No centrifugation required.	Centrifugation required for serum/plasma separation.
Sample Volume	Few drops (μ L) of blood. Easy to split spots between sites. Ideal method for lab animals.	Multiple tubes and 5-10mL/tube type. Not ideal for splitting between sites.
Shipping	Small size and can be stacked and shipped by regular mail.	Special handling. Expensive cold chain requirement.
Stability & Storage	Stable at ambient temperature. Limited refrigerator space.	Stable Frozen. Large freezer space required.

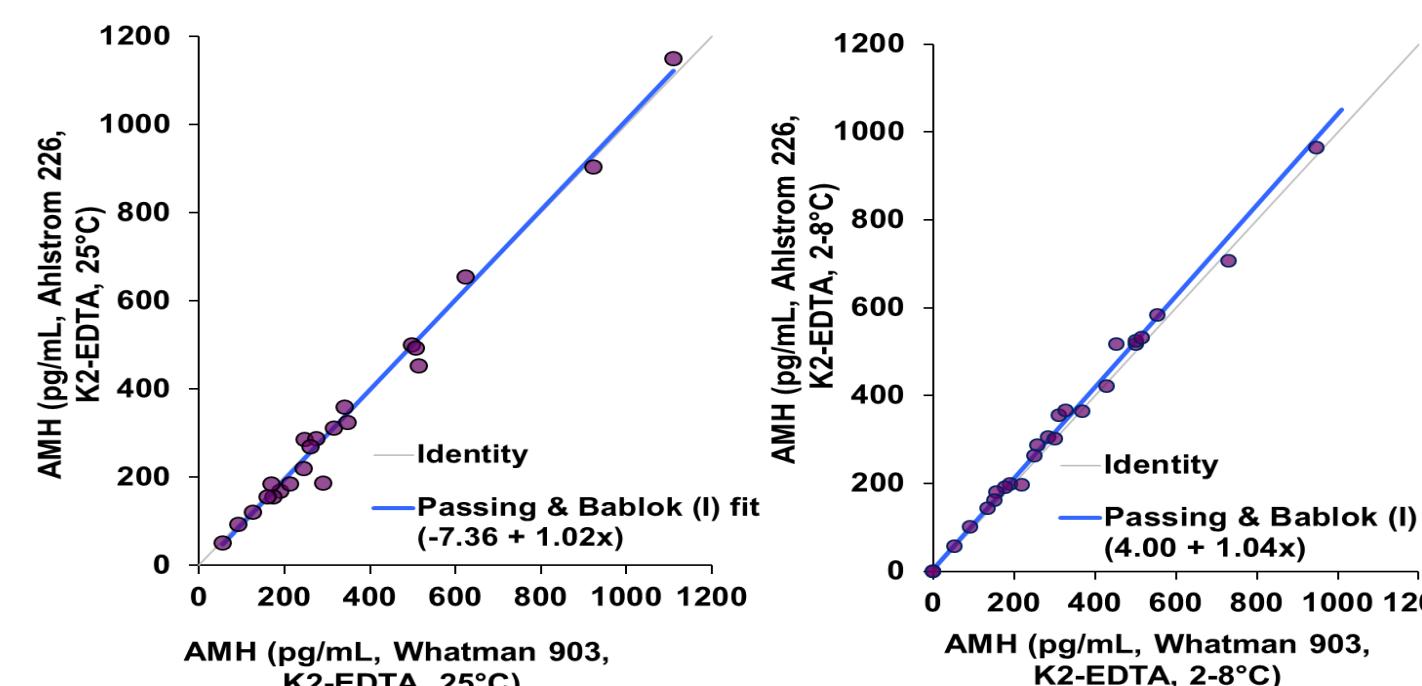
METHOD

Dried Blood Spot Sample Collection and Preparation

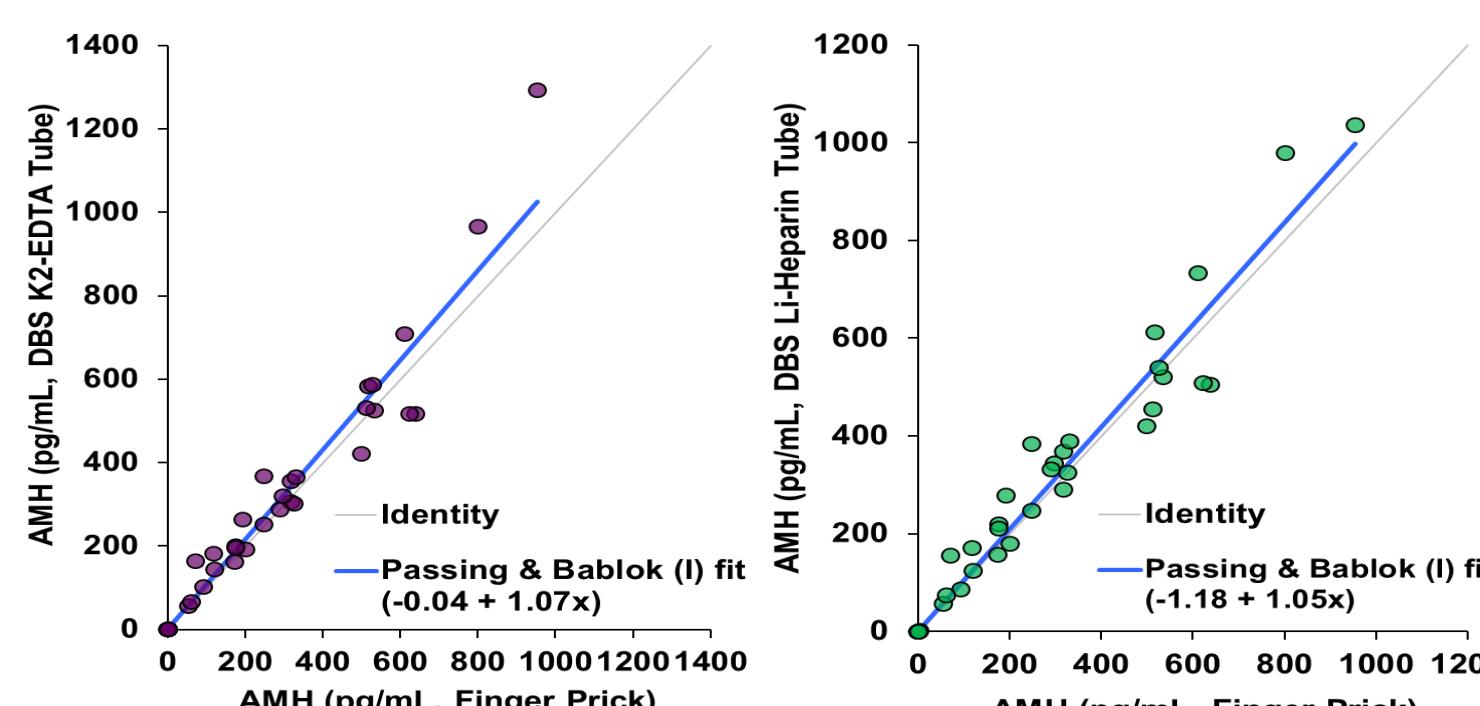


RESULTS

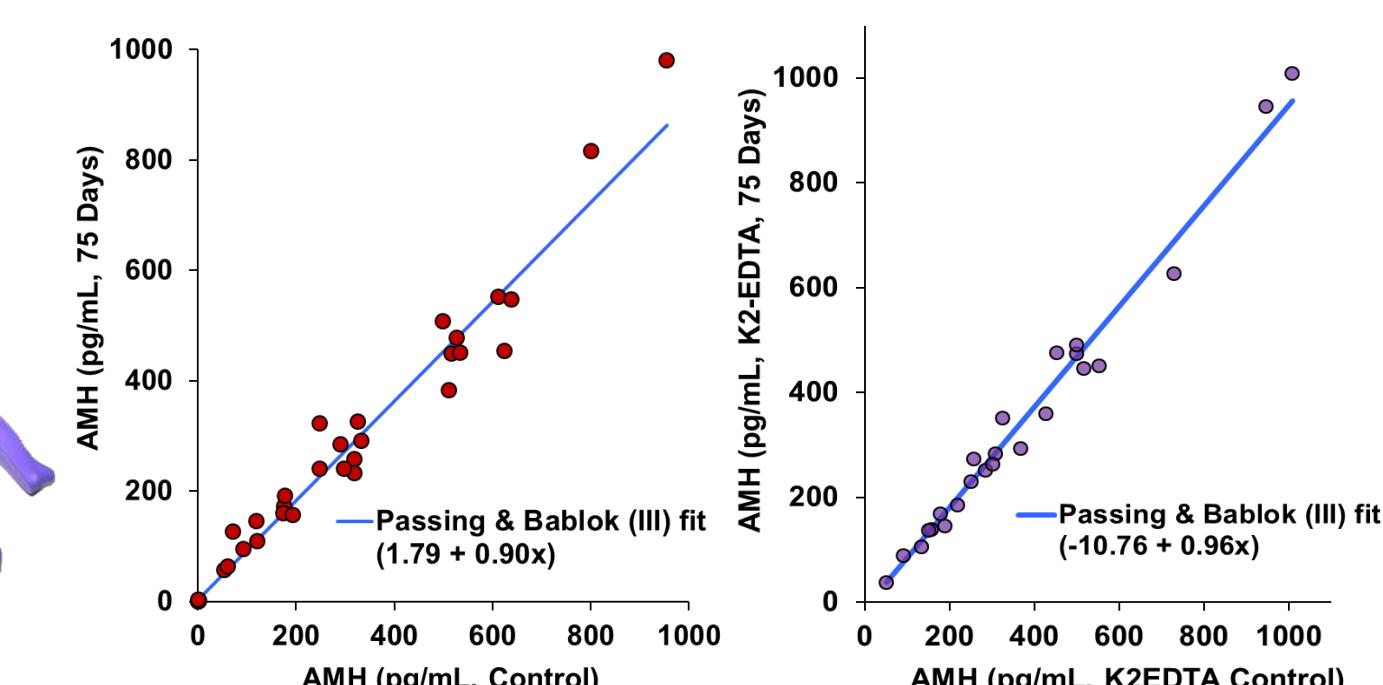
Comparison of FDA Approved Dried Blood Spot Cards



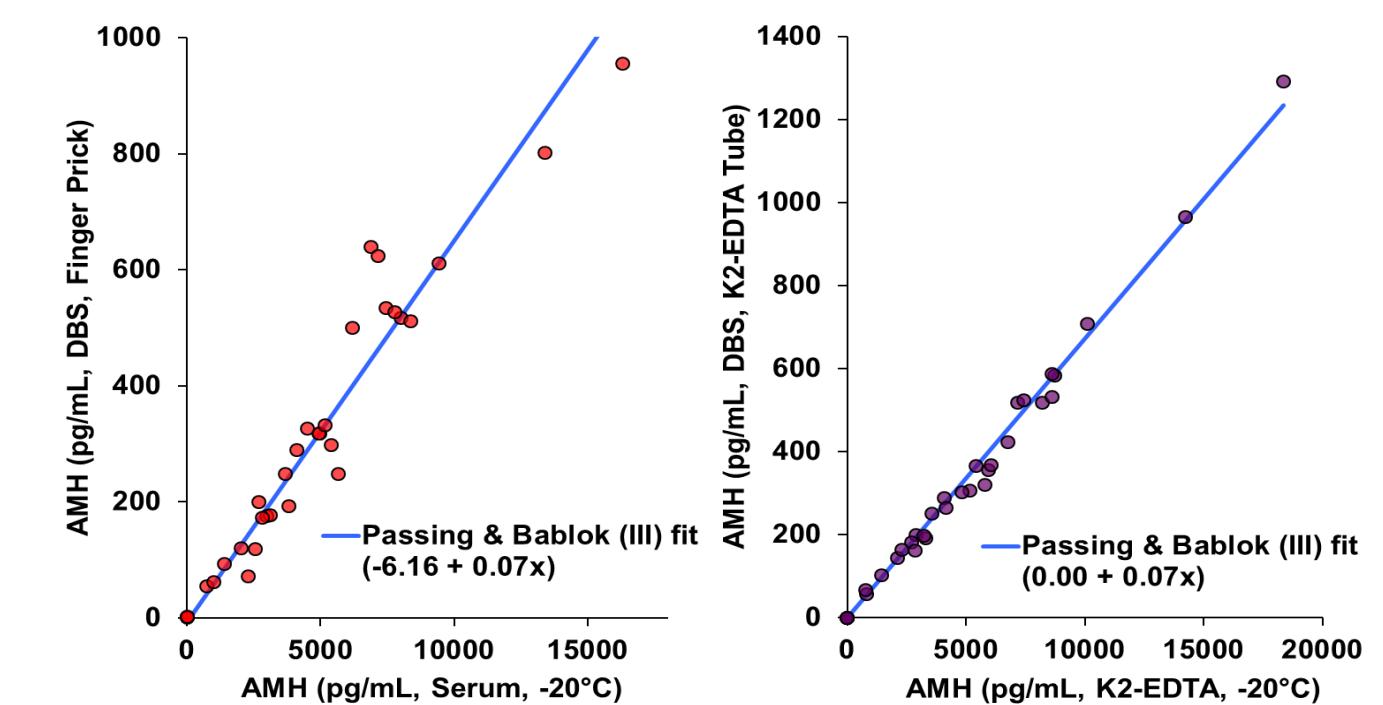
Finger vs Venous Dried Blood Spots Comparison



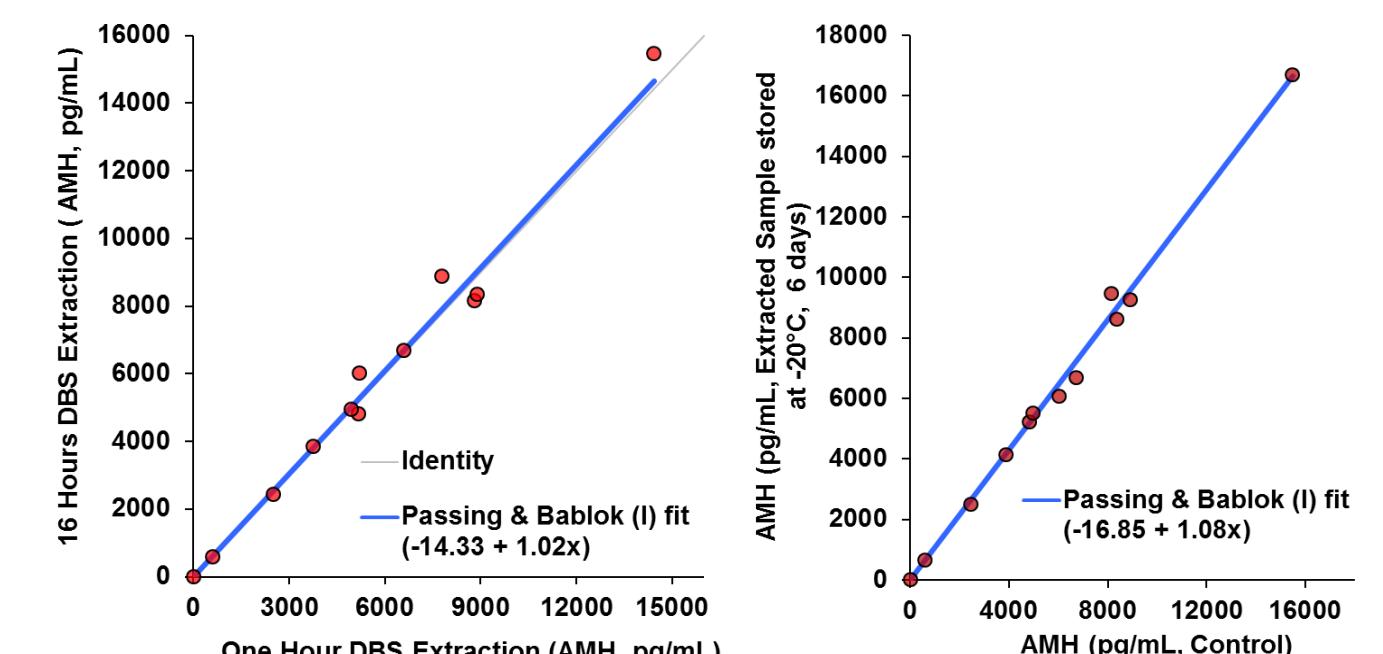
Dried Blood Spot Stability at 2-8°C After 75 Days



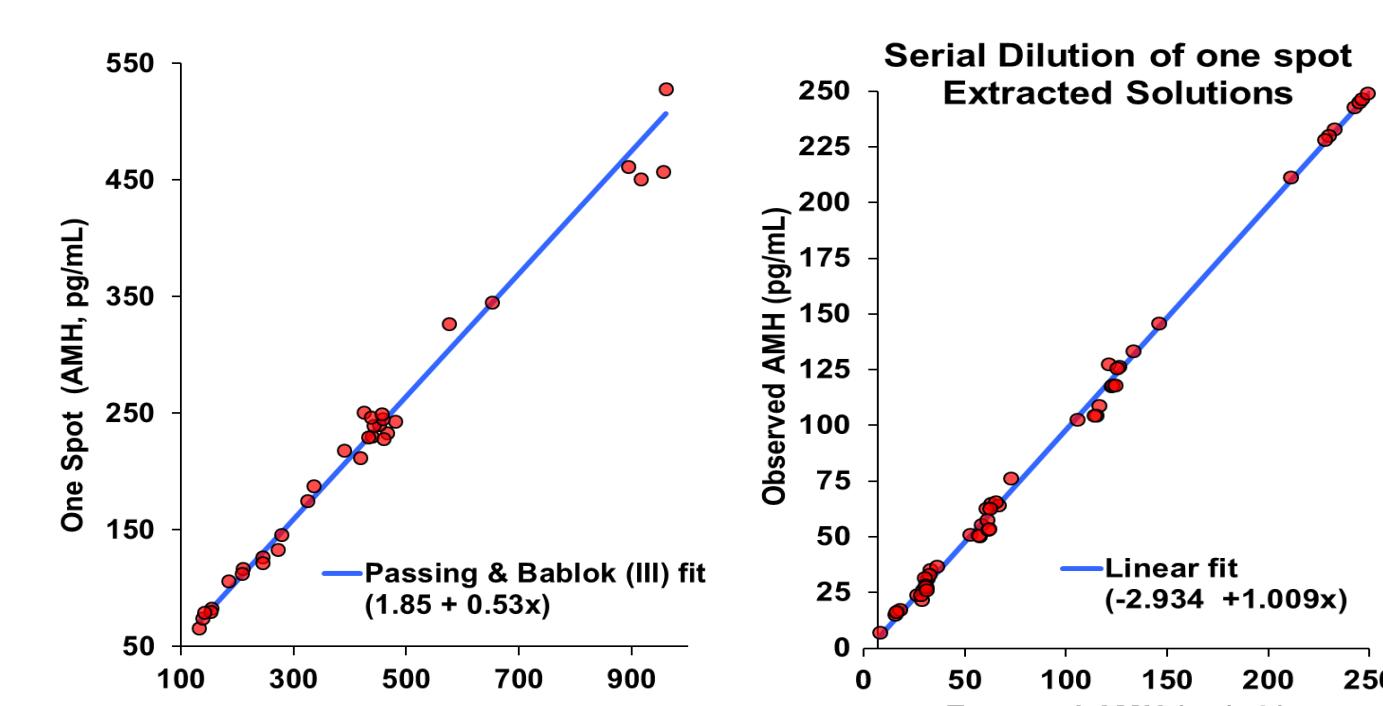
Serum and Plasma to DBS AMH Equivalence



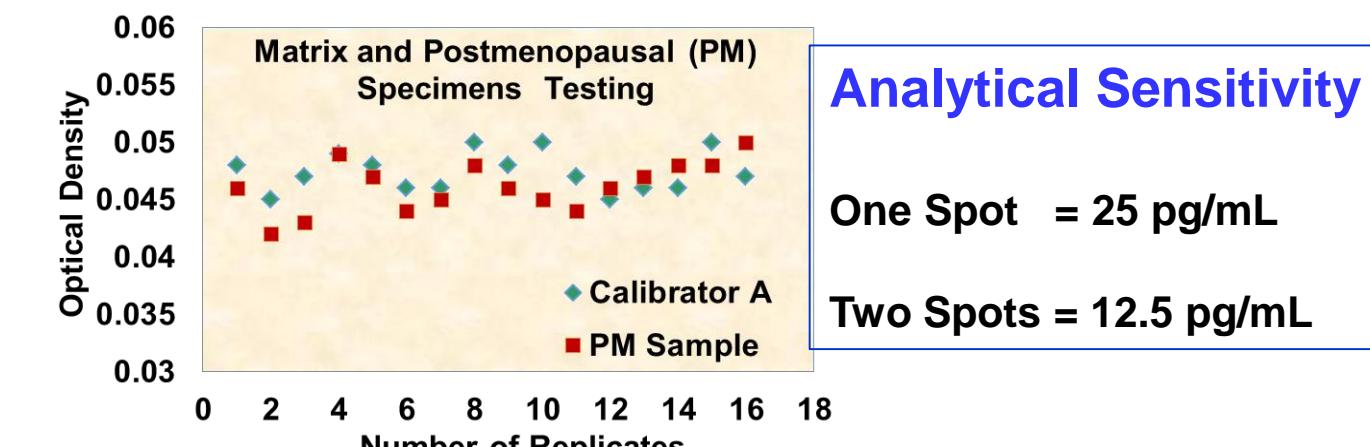
Extraction Efficiency and Extracted Sample Stability



One vs Two Spots Extraction & Linearity of Dilution



Analytical Sensitivity and Matrix Accuracy

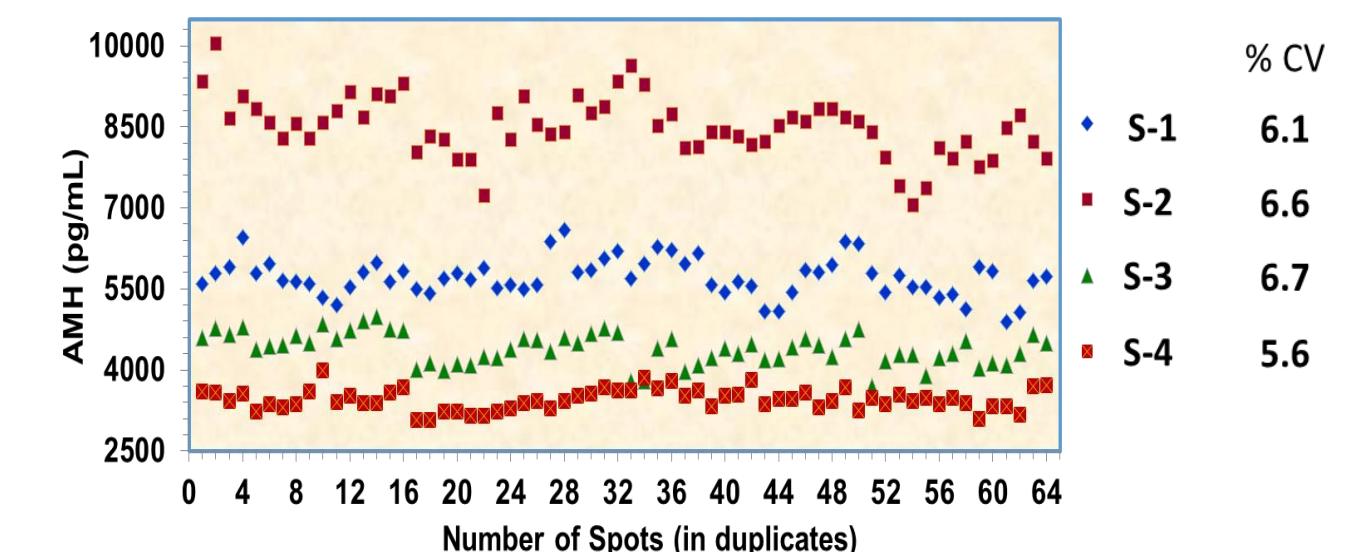


Analytical Sensitivity

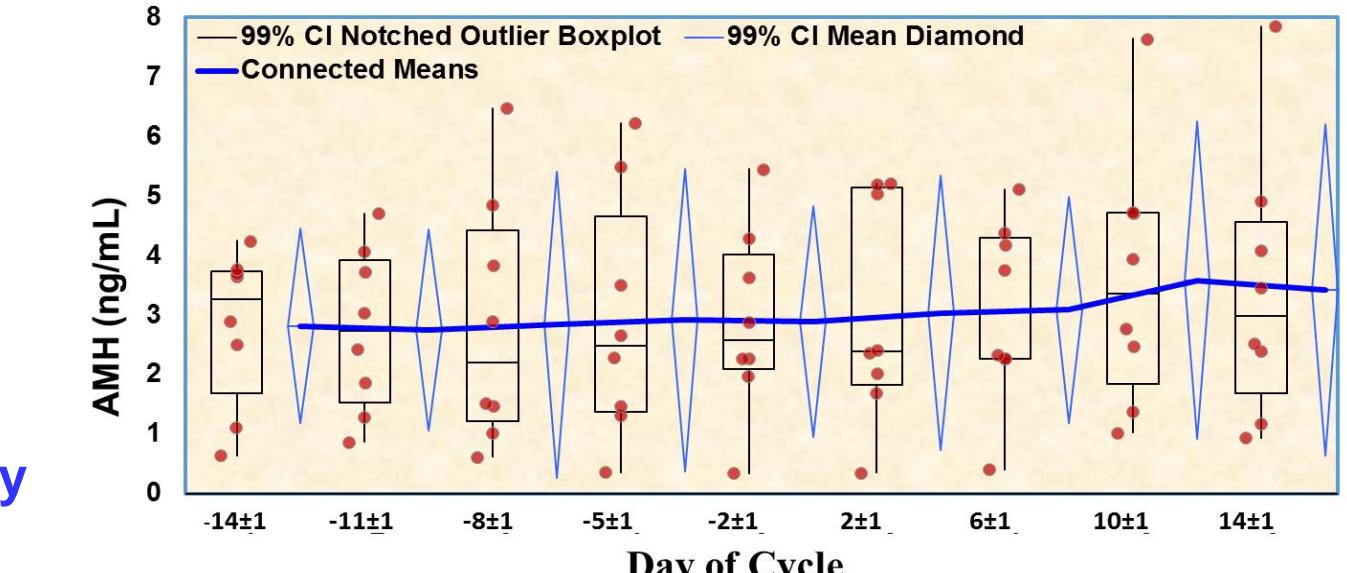
One Spot = 25 pg/mL

Two Spots = 12.5 pg/mL

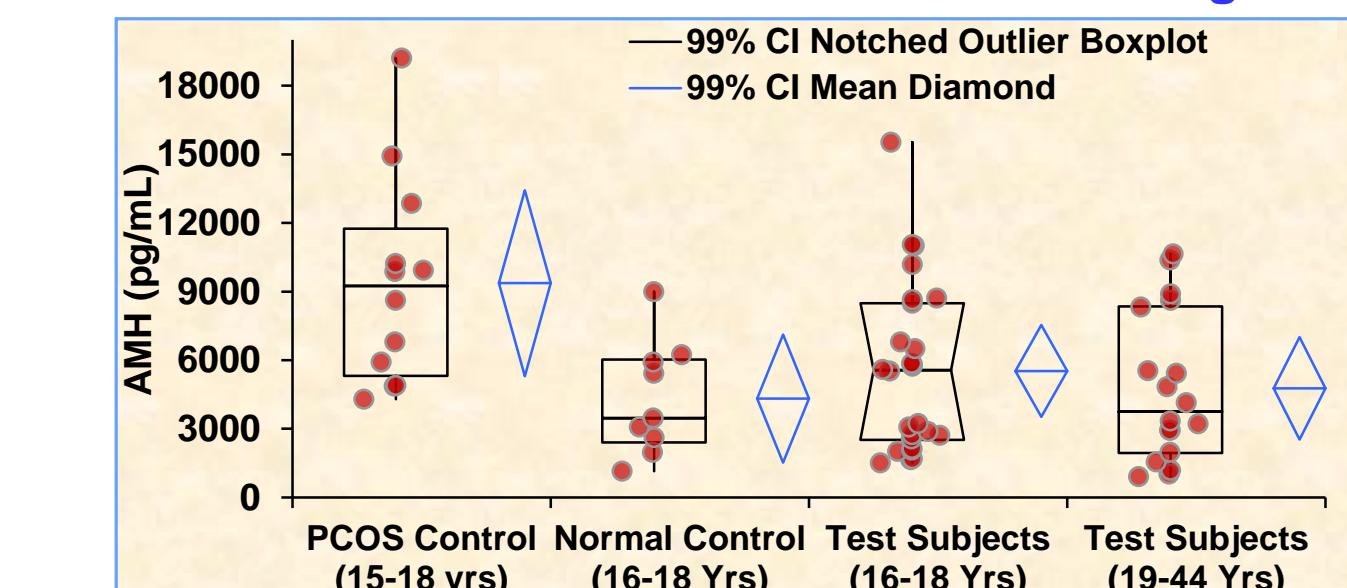
Imprecision: 32 Spots in Duplicates over 5 Runs



DBS AMH and Intra Cycle Variability (N=8)



DBS AMH and Ovarian Reserve Testing



CONCLUSIONS

- The correlation coefficient between serum and Dried Blood Spot AMH ELISA was > 0.98 with a coefficient of variation of $<7\%$. The method can be used as a better alternative to venipuncture.
- The sensitivity of the Dried Blood Spot AMH ELISA (12.5 pg/mL) makes it well suited for ovarian reserve testing for all ages.
- The specimen stability, low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

