

# 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

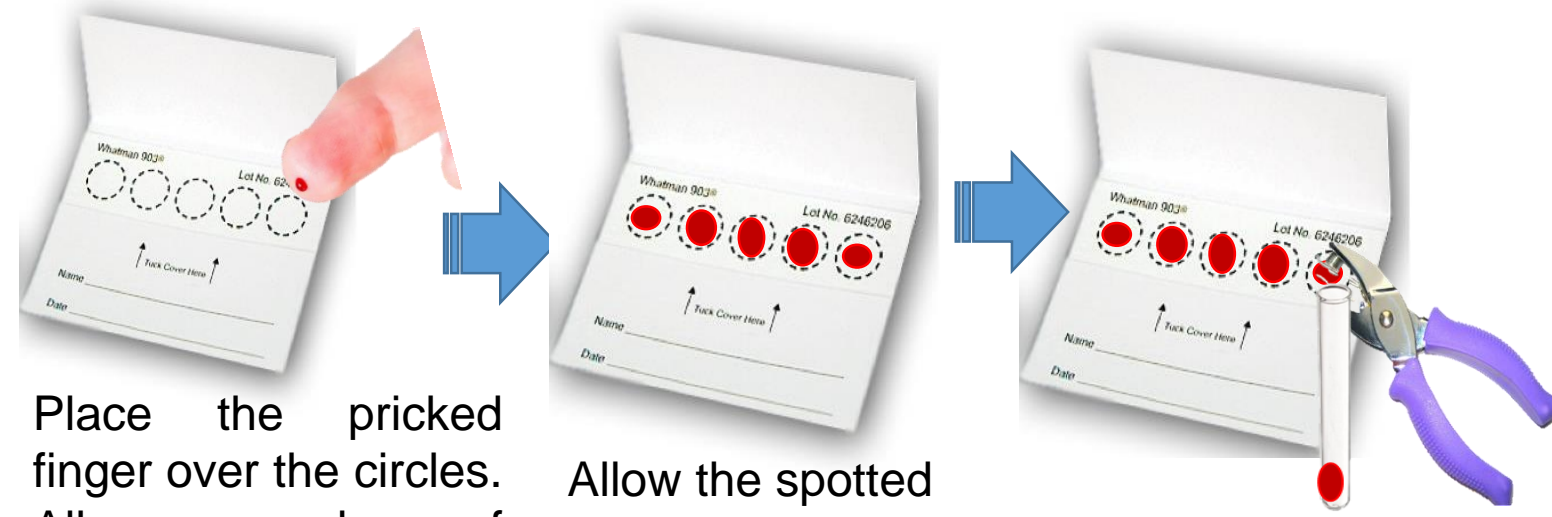
Dried blood spot (DBS) technology, is a very simple, inexpensive technique for collecting drop(s) of whole blood on filter paper. DBS has been in existence for almost 50 years, but has gained interest recently due to advancement in technologies that enable sensitive and reliable methods to quantitative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

## METHOD

### Dried Blood Spot Sample Collection and Preparation



Place the pricked finger over the circles. Allow one drop of blood to fall onto each spot. Do not touch or rub on the paper. Do not double spot.

Allow the spotted paper to dry. Then store sealed in a biohazard bag with desiccant.

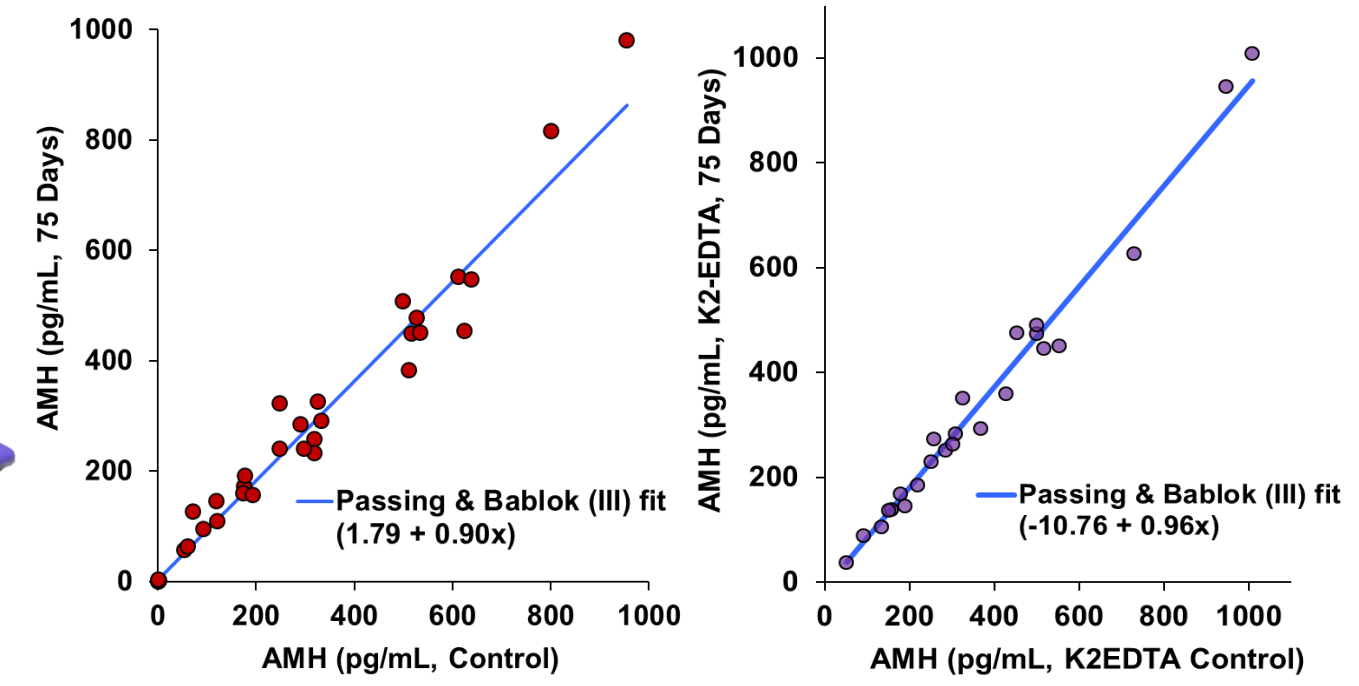
For Testing: Take the hole puncher and clean with 70 % ethanol and allow to dry. Hold down the puncher in the center of the spot of blood to capture a uniformly covered blood spot. Punch the 7.9 mm spot(s) one at a time directly into the test tube. Clean the hole puncher before moving to the next spot.



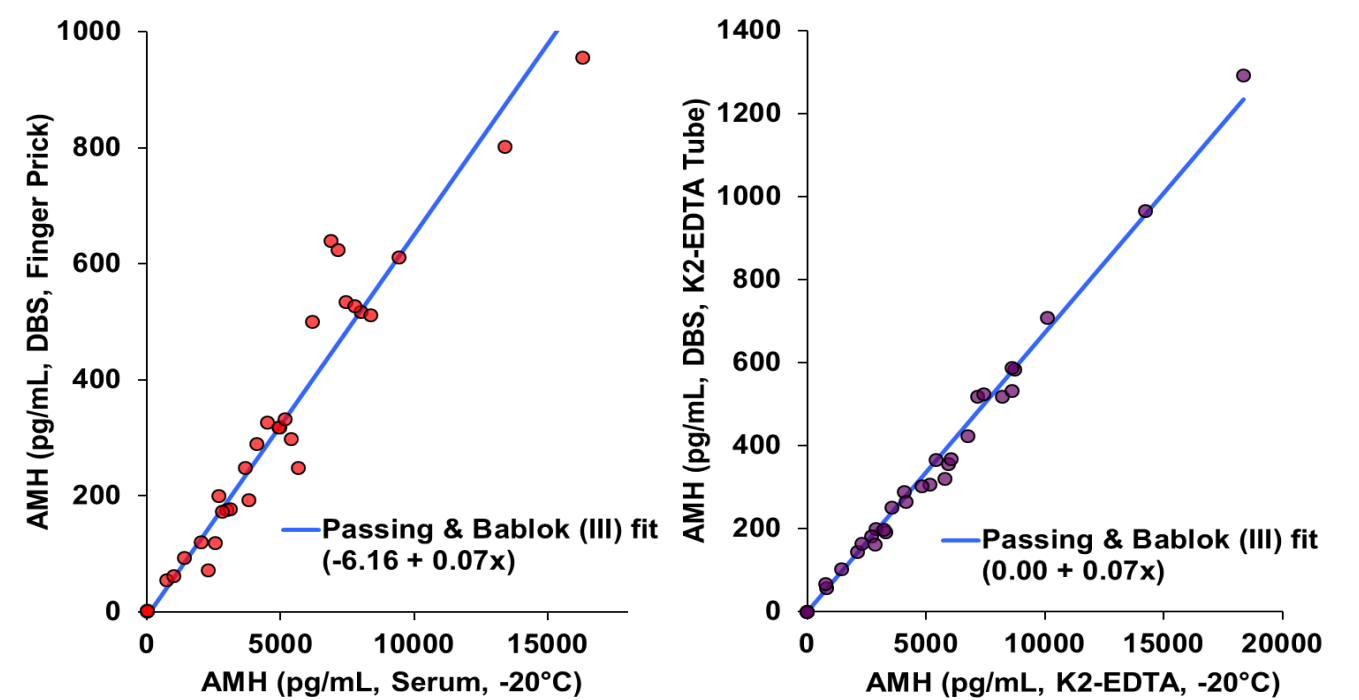
Shake on the plate shaker for one hour at 600 rpm

Extract the spots in 450 uL of the assay buffer and cover the test tubes with Parafilm.

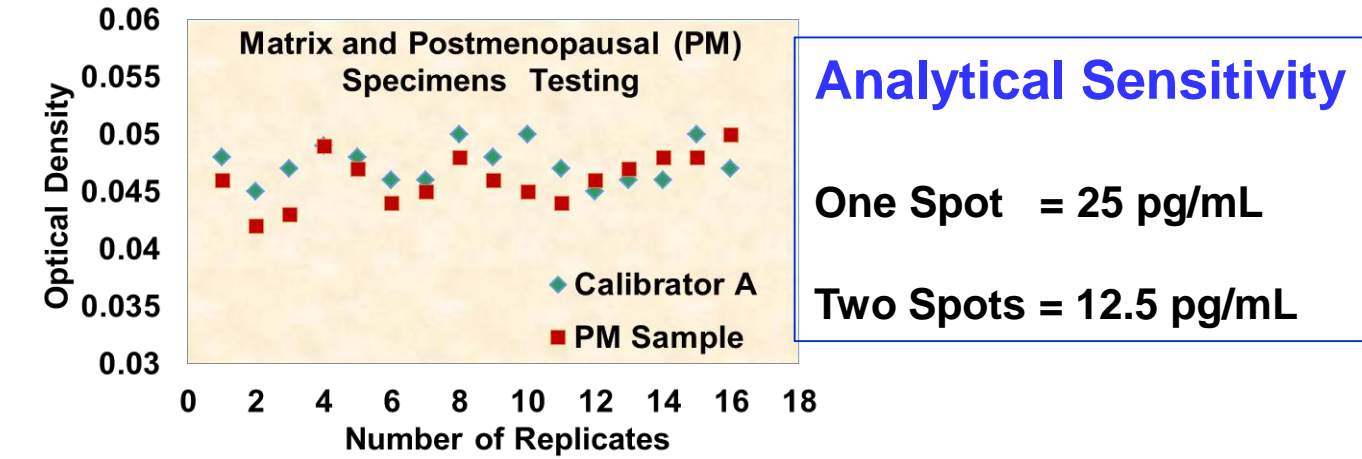
### Dried Blood Spot Stability at 2-8°C After 75 Days



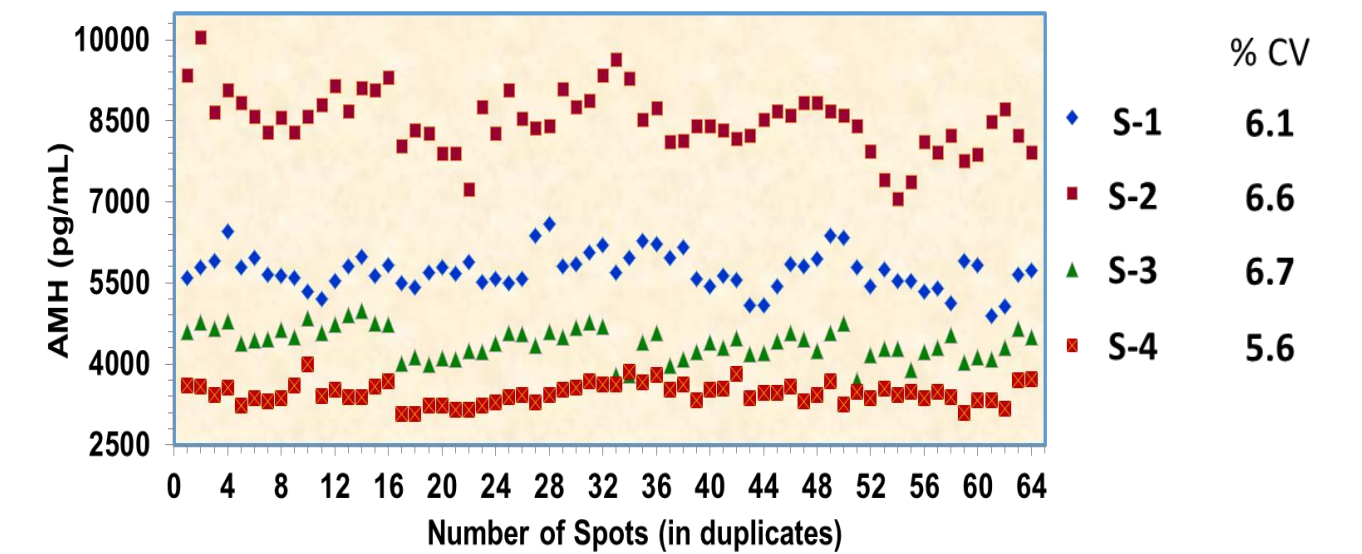
### Serum and Plasma to DBS AMH Equivalence



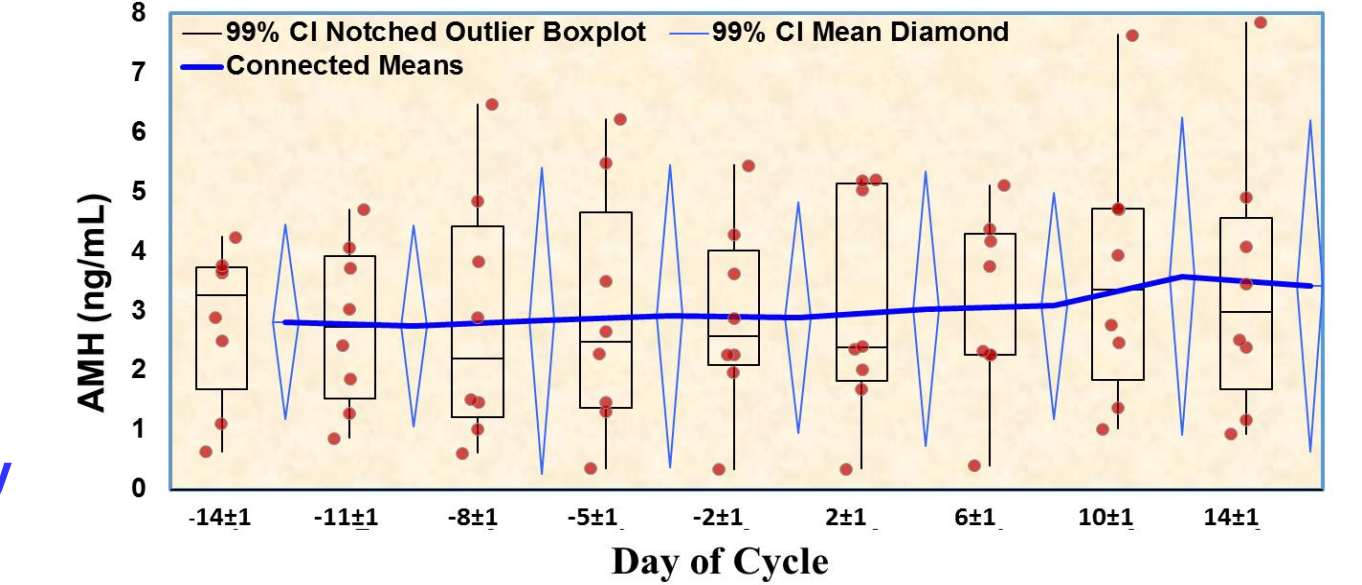
### Analytical Sensitivity and Matrix Accuracy



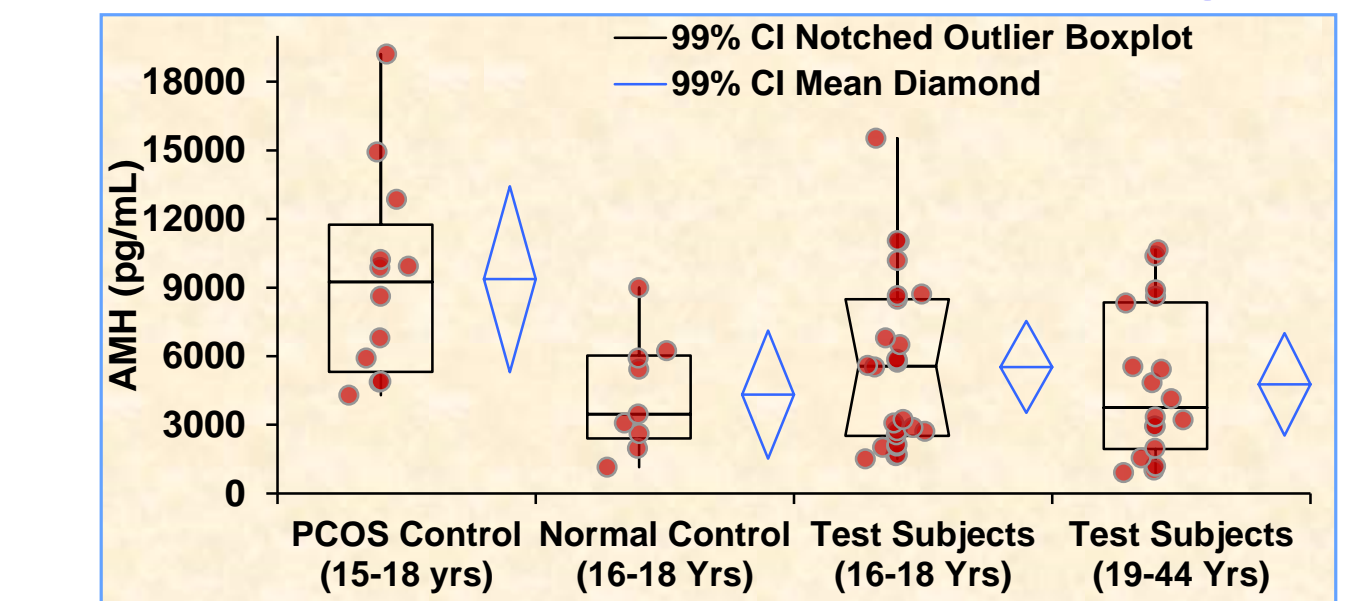
### Imprecision: 32 Spots in Duplicates over 5 Runs



### DBS AMH and Intra Cycle Variability (N=8)



### DBS AMH and Ovarian Reserve Testing

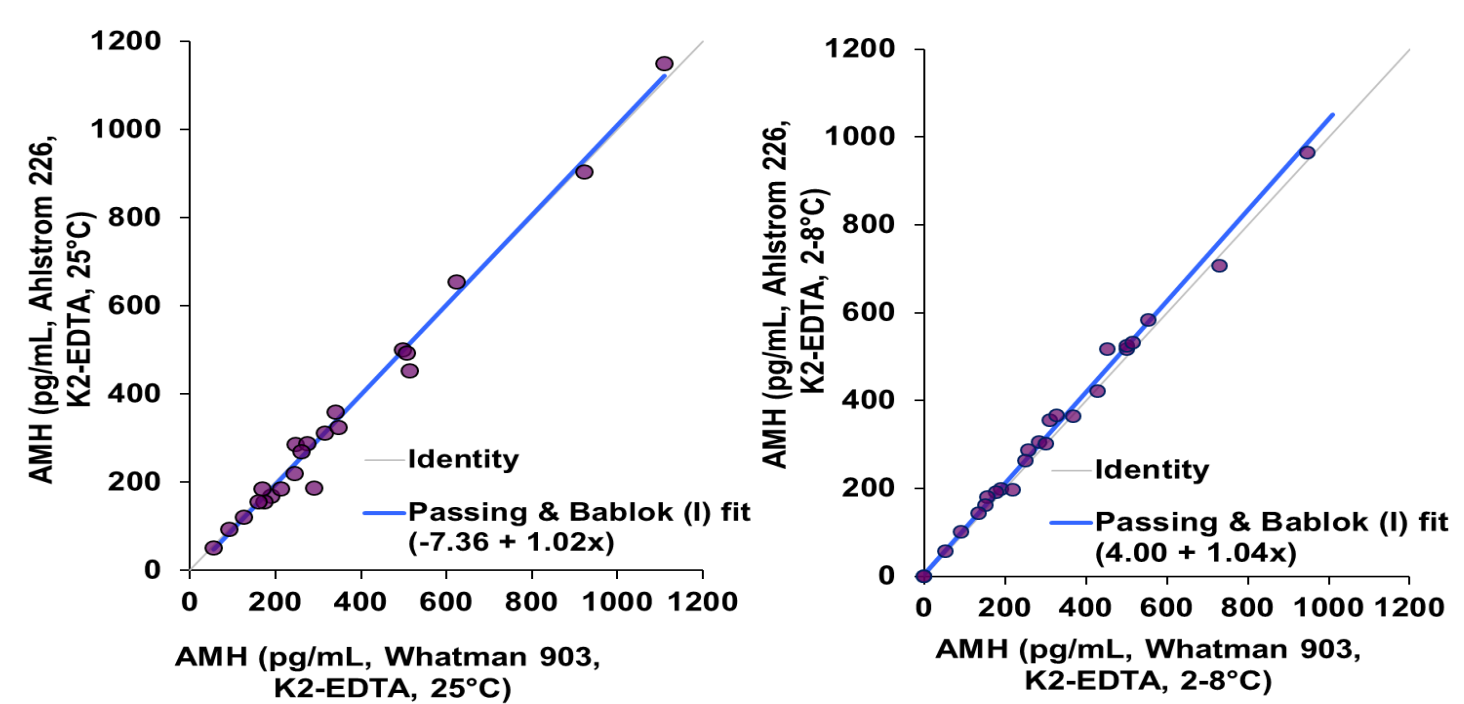


### Advantages of Dried Blood Spot Technology

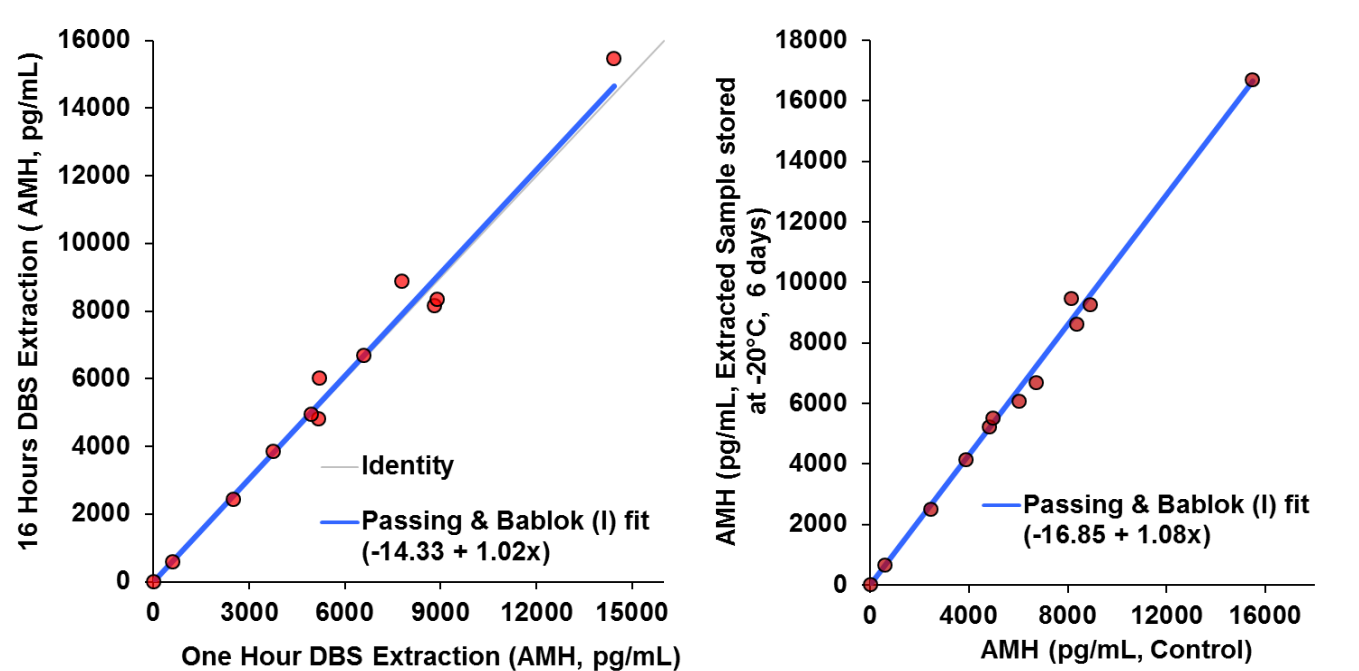
| Features                              | DBS Method  | Conventional Method   |
|---------------------------------------|---|---|
| <b>Medically Trained Professional</b> | Minimally invasive technique and can be self collected by finger prick.                   | Invasive and collected at clinics by phlebotomist by venipuncture.          |
| <b>Biohazard Comparison</b>           | Dried blood inactivates pathogens and lowers the biohazard risk.                          | Potentially infectious and prone to bacterial contamination.                |
| <b>Sample Processing</b>              | No centrifugation required.   | Centrifugation required for serum/plasma separation.                        |
| <b>Sample Volume</b>                  | Few drops (uL) 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. |
| <b>Shipping</b>                       | Small size and can be stacked and shipped by regular mail.                                | Special handling. Expensive cold chain requirement.                         |
| <b>Stability &amp; Storage</b>        | Stable at ambient temperature. Limited refrigerator space.                                | Stable Frozen. Large freezer space required.                                |

## RESULTS

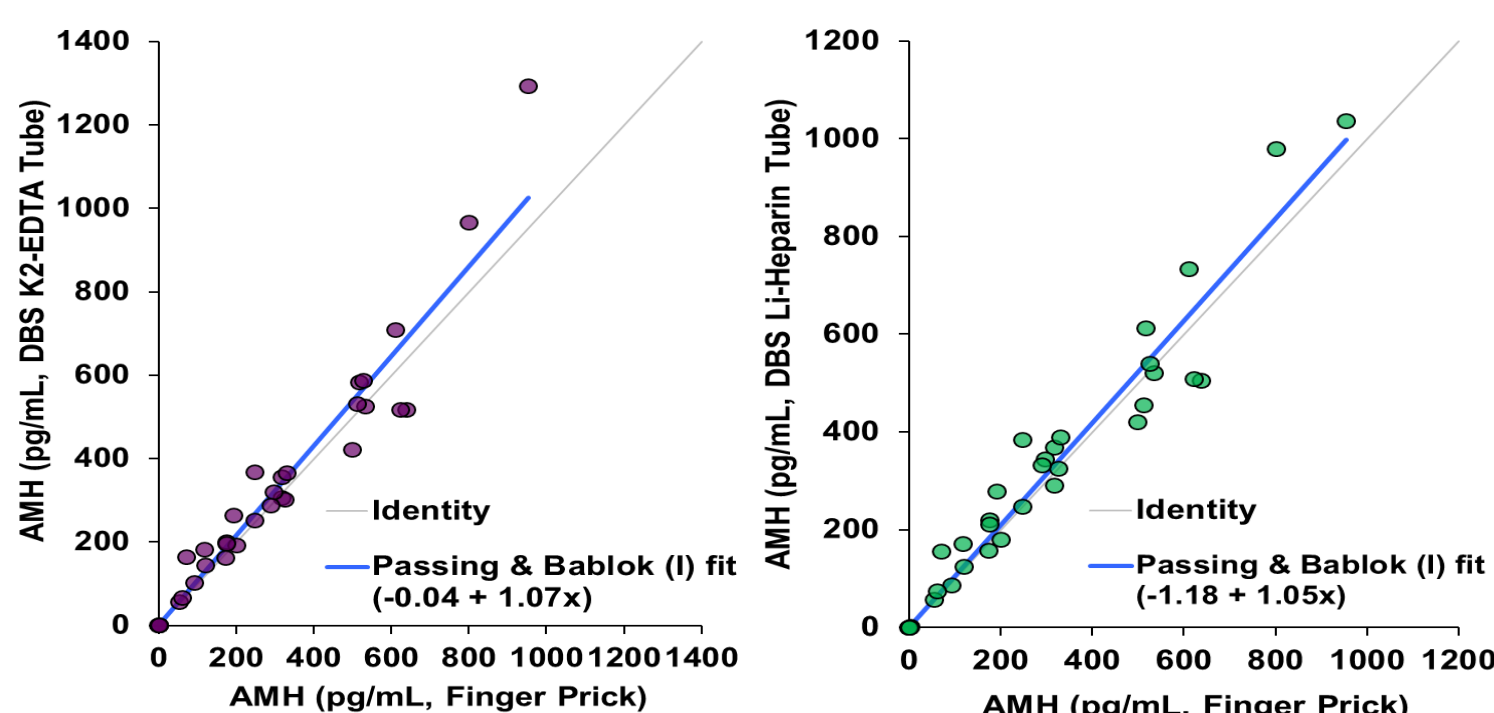
### Comparison of FDA Approved Dried Blood Spot Cards



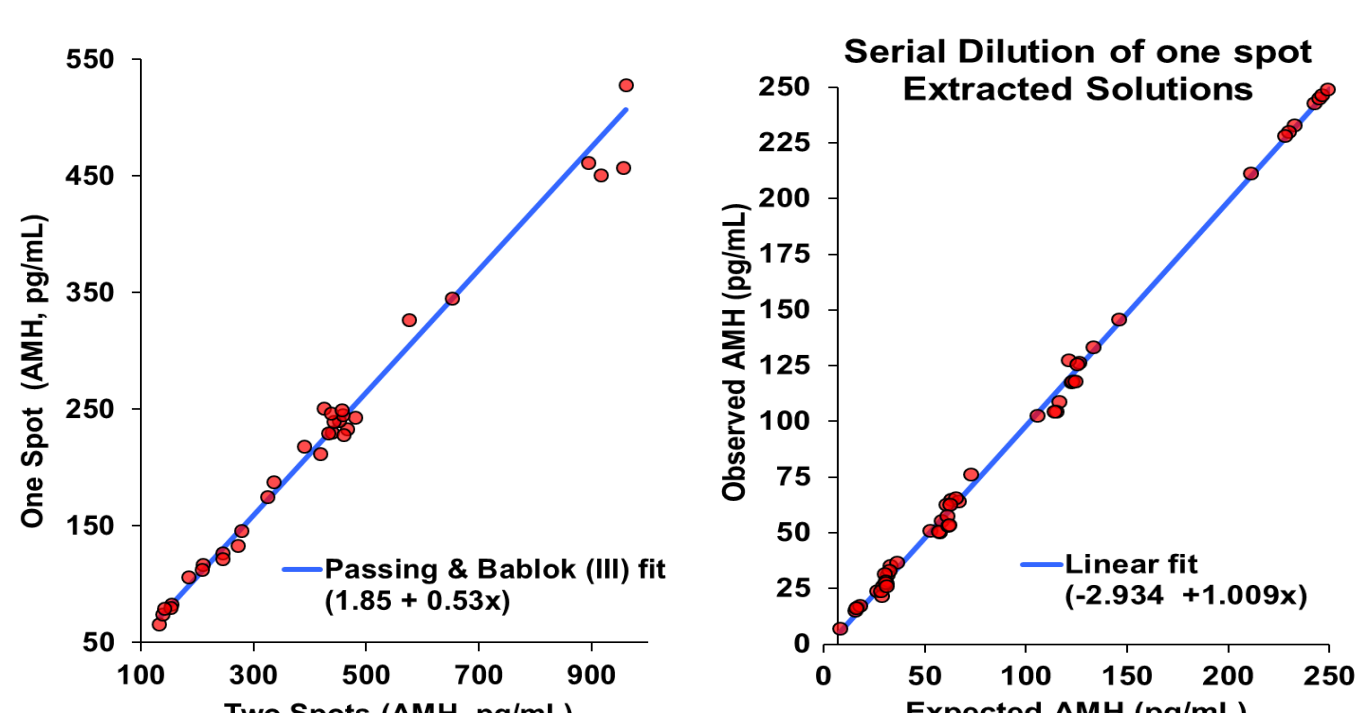
### Extraction Efficiency and Extracted Sample Stability



### Finger vs Venous Dried Blood Spots Comparison



### One vs Two Spots Extraction & Linearity of Dilution



## 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.

