SAT-475

Novel Comparative Analysis of Dried Blood Spot and Serum ELISAs for AMH, FSH, and LH in Patients Seeking IVF Treatment

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OBJECTIVE

- Many studies have shown that the low serum AMH concentrations, low LH concentrations, and high FSH concentrations in subjects seeking IVF treatment are indicative of a low ovarian reserve. These subjects are poor responders to the drugs used in IVF clinics.
- On the other end of the spectrum, abnormally high levels of serum AMH concentrations and high LH/FSH ratio are indicative of polycystic ovarian syndrome and can mean over-stimulation during the IVF procedure.
- The aim of this study is to validate the hormone measurements using Dried Blood Spot technology as an alternative to serum or plasma measurements and compare the correlation matrix between AMH, LH, FSH, Inhibin B, AFC, Age, and BMI in patients seeking infertility workup.

INTRODUCTION

- The advancement in ELISA and CLIA methodologies have enabled extremely sensitive and reliable methods to quantitate blood hormone concentrations from a drop of blood.
- Innovations in the field of micro-sampling devices that can collect volumetrically identical multiple spots have revolutionized the capillary blood collection method.
- Dried Blood Spot technology has many advantages over conventional serum/plasma blood collection. DBS inactivates pathogens, lowers the biohazard risk and is stable at room temperature for a week. This unique advantage enables the DBS to be transported across the globe by regular mail and minimizes the shipping and handling costs.
- The most challenging part of the DBS technology is to find a well-validated, easy to implement ELISA or CLIA method for clinical labs.
- Most of the commercial ELISA and CLIA methods have been well validated for serum and plasma samples and have negligible information on DBS sampling methods, DBS analytical characteristics, and clinical expected ranges.

MATERIALS AND METHODS

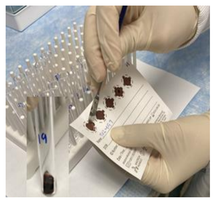
- Matched dried blood spot and plasma specimens were collected on day 3 of the mensuration cycle from 100 subjects between the ages of 21 and 49 years. These subjects were visiting the clinic for initial fertility workup. The subjects on medications for ovarian suppression were excluded from the study.
- Baseline Antral Follicle Counts by transvaginal ultrasound, BMI, and age were collected at the clinic.
- DBS and K_2EDTA plasma specimens were kept refrigerated at -20°C until tested in

1. Ansh Labs DBS AMH ELISA (AL-129)
2. DBS FSH ELISA (AL-187)
3. DBS LH ELISA (AL-190)
4. picoAMH ELISA (C-N terminus, AL-124-r)
5. PCOCheck AMH ELISA (N-N terminus, AL-196)
6. US Inhibin B (AL-195) ELISA.

- Regression analysis using Passing-Bablok fit, and Spearman rank correlations were used to calculate the slope and correlation coefficient between hormone measurements between the plasma and DBS specimens.
- Regression analysis using Passing-Bablok fit, and Spearman rank correlations were used to calculate the slope and correlation coefficient between AMH, Inhibin B, FSH, LH, AFC, Age, and BMI.

Enzyme-Linked Immunosorbent Assay Method

Human Anti-Mullerian Hormone, Luteinizing hormone, Follicle stimulating hormone present in the DBS specimen is sandwiched between the two anti-AMH antibodies and quantitatively measured.



Sample Preparation DBS Extraction

450ul of DBS Extracted Sample in each tube

DBS AMH ELISA **(150uL)**

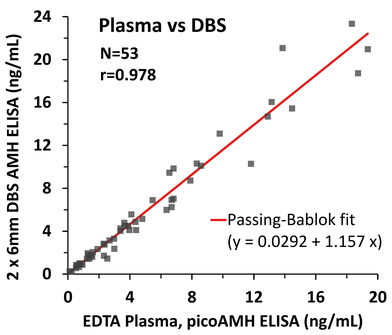
DBS LH ELISA (**60uL**)

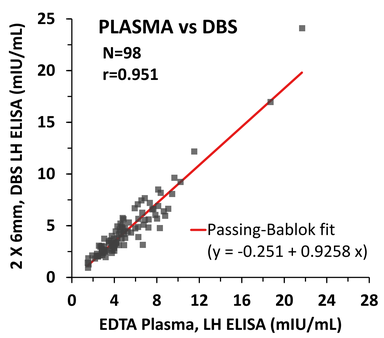
DBS FSH ELISA (**100uL**)

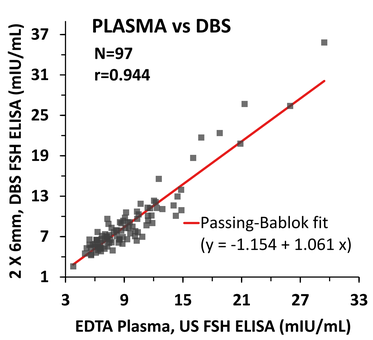
AMH, LH, & FSH Results (Same Extracted Spots)

RESULTS

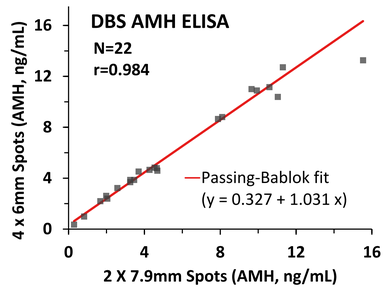
METHOD COMPARISON
K_2EDTA Plasma vs Dried Blood Spot Specimens

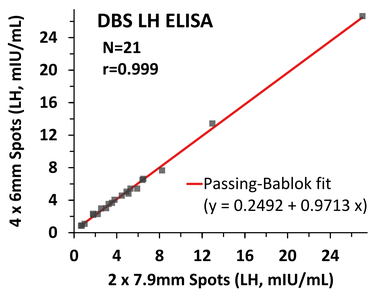


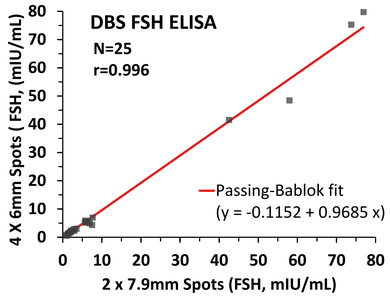




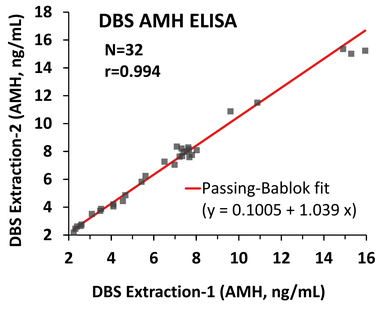
MANUAL VS AUTOMATED DBS CARD PUNCHER
Optimized DBS Punch Size

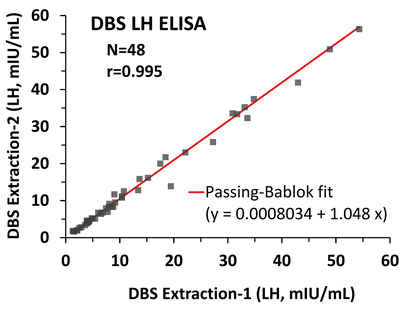


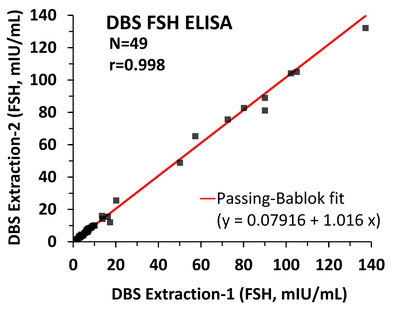




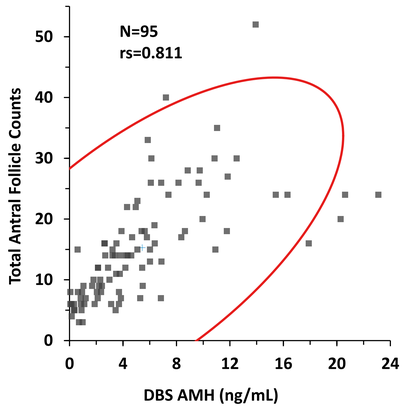
DRIED BLOOD SPOTS REPRODUCIBILITY
Effect of Spot-to-Spot Variability In AMH, LH And FSH Measurements

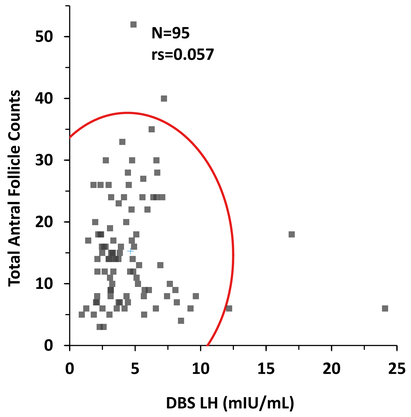


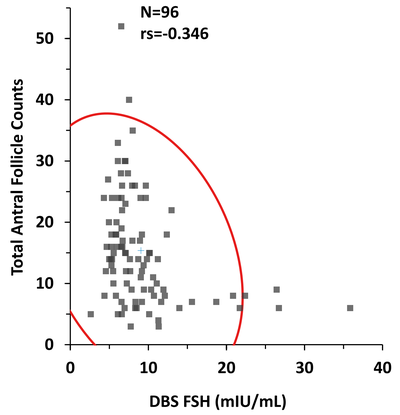




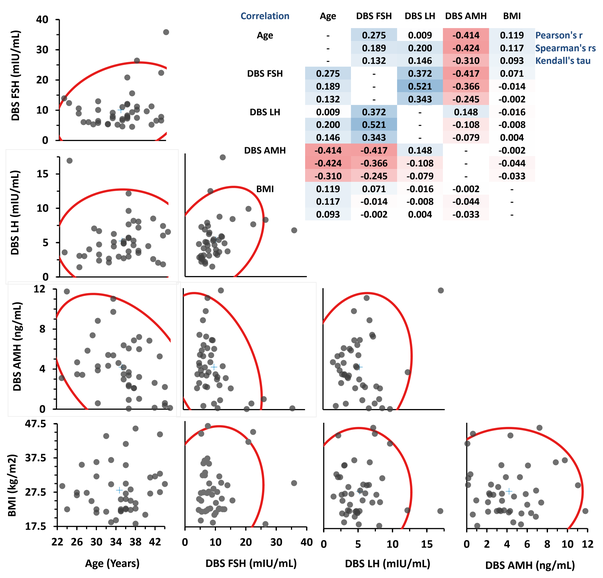
CLINICAL DIFFERENTIATION
Comparison Of DBS AMH, LH, FSH Concentrations To Antral Follicle Counts



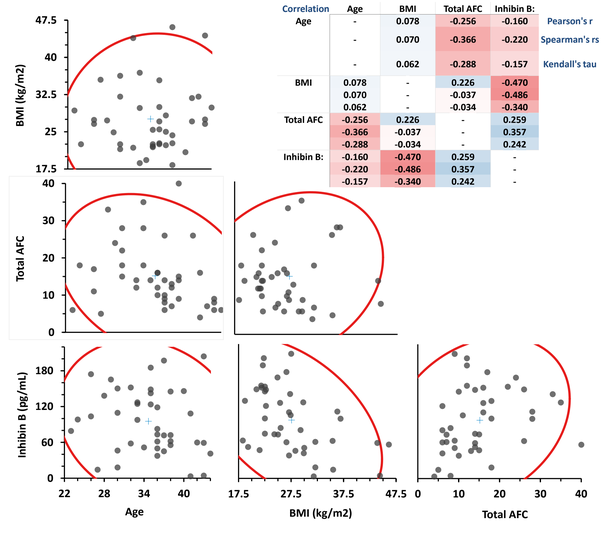




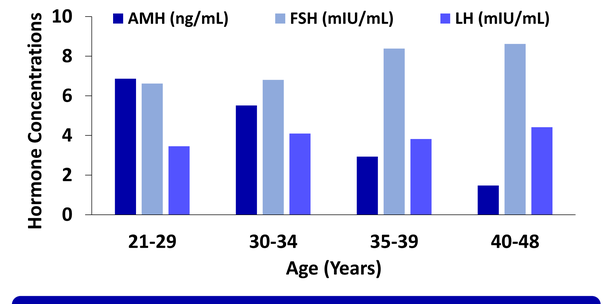
Correlation Matrix: Age, BMI, AMH, LH, & FSH



Correlation Matrix: Age, BMI, AFC, & Inhibin B



DBS AMH, LH, & FSH Expected Ranges



CONCLUSIONS

1) The plasma and Dried Blood Spot concentrations were highly correlated therefore, both the methods can be used in clinical labs.
2) DBS AMH is highly correlated to antral follicle counts and can be used as an alternative to vaginal ultrasound in estimating AFC.
3) Inhibin B concentrations were consistently low in subjects with less than three AFC and can be used as an additional marker to confirm low AFC and AMH results.
4) The Dried Blood Spot AMH, LH and FSH ELISA are specific, sensitive and reproducible and are well suited for ovarian reserve testing.
5) DBS specimens and DBS extracted samples are stable and can be stored frozen for long-term use, opening new avenues for research testing.