**ABSTRACT**

**Objective:** The aim of this study was to develop a highly sensitive and simple dried blood spot human AMH ELISA to assess ovarian reserve.

**Relevance:** AMH has been reported to be strongly associated with age, antral follicle counts (AFC), FSH, and has emerged as a clinically useful biomarker of ovarian reserve. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. Dried blood spot specimens stability makes it a practicable alternative to venous blood. It opens new possibilities in AMH testing, such as comparison of historical to current patient results; simplified blood sampling for patients in remote locations or who are homebound. Instead of traveling to a clinic to get blood drawn, a blood spot sample can be taken at a convenient site and mailed to a laboratory. This technology will be especially useful for monitoring ovarian function of physically challenged cancer patients undergoing chemotherapy.

**Methodology:** A three-step, sandwich-type enzymatic microplate assay has been developed to measure AMH levels in two 7.9 mm dried blood spot discs in less than 6 hours. The assay measures human AMH and uses stabilized recombinant human AMH as calibrators (7-1000 pg/mL). This method uses a drop of whole blood collected on filter paper from a simple finger stick. The sample is eluted from the dried blood spot in an extraction solution and is added directly to the well. The assay measures the bio-essential AMH and does not exhibit interference by hemocytin in the extracted spot.

**Validation:** Ansh Labs DBS AMH ELISA (AL-129), when compared to Ansh Labs US AMH ELISA (AL-105) using 56 matched serum and dried blood spot samples in the range of 62-18443 pg/mL, yielded a correlation coefficient of 0.98 (p < 0.0001) and a slope of 0.96 with an intercept of -0.01 pg/mL. DBS AMH ELISA (AL-129) when compared to Ansh Labs picoAMH ELISA (AL-124) using 65 matched serum and dried blood spot samples in the range of 5-5240 pg/mL, yielded a correlation coefficient of 0.99 (p<0.0001) and a slope of 1.02 with an intercept of -4.7 pg/mL. Serial dilution of seven extracted dried blood specimens (5000-11000 pg/mL) in the sample diluent showed an average recovery of 87-105%. Total imprecision, calculated on 3 controls over 40 runs, 2 replicates per run, was 5.84% at 22.58 pg/mL, 3.15% at 86.51 pg/mL and 4.34% at 373.18 pg/mL. The functional sensitivity of the assay calculated at 20% CV was 3.9 pg/mL.

**Conclusions:** A highly simplified, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age. The DBS results are comparable to serum-based assays. The specimen stability, ease and low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

**INTRODUCTION**

Measurement of AMH in community-based studies, or studies requiring multiple blood draws at present requires venous blood. Venipuncture blood draws are costly, invasive and must be performed by a trained phlebotomist in close proximity to a facility where blood samples can be centrifuged, separated and frozen. Dried blood spots (DBS)—drops of whole blood collected on filter paper following a simple finger stick—represent a minimally invasive alternative. The participant’s finger is cleaned, pricked with a sterile, disposable lancet of the type commonly used to monitor blood glucose, and up to five drops of whole blood are applied to the paper. Samples are allowed to dry, and then stacked and stored in plastic bags prior to shipment to the laboratory.

A major advantage of DBS sampling is that it is relatively painless and noninvasive, low cost, and can be implemented by non-medically trained personnel in the participant’s home or other non-clinical setting. The test will help researchers monitor the ovarian function of patients with polycystic ovary syndrome, granulosa cell tumor subjects undergoing chemotherapy, etc.

**METHOD**

- Ansh Labs Dried Blood Spot (DBS) AMH sandwich ELISA (AL-129)
- Standardized to recombinant human AMH Calibrators.
- Can be measured reproducibly on Ahistrom 226 and Whatman 903 filter paper.
- Human specific, linear epitope capture and detection Abs in the stable midpro-region and mature region of AMH.

**Sample Preparation**

- Label two 12 X 75 culture tubes for each unknown dried blood sample.
- Punch out two filter paper disc (7.9 mm), impregnated with the unknown dried blood specimen, onto a clean surface and transfer the disc using clean tweezers into the corresponding tube.
- Add 450 μL of the DBS AMH Extraction Buffer to each tube, vortex well.
- Place the tubes in a tight fitting tube rack and incubate the tubes, shaking at a slow speed (400-450 rpm) at room temperature for 60 minutes.
- Transfer the liquid from one tube into the corresponding second labeled tube leaving the blood spot in the initial tube. The extracted sample is ready to be analyzed in AL-129 AMH ELISA.

**AL-129 DBS AMH ELISA Procedure**

The DBS AMH ELISA is a quantitative three-step sandwich type immunoassay.
- Add 150 μL of calibrators and extracted dried blood spot samples to AMH antibody coated microtiter wells, incubated for 3 hrs and wash.
- Add 100 μL of biotinylated AMH antibody solution, incubate for 1 hr and wash.
- Add 100 μL of SHRP conjugate solution, incubate for 30 mins and wash.
- Add 100 μL of TMB solution, incubate for 10 mins and add 100 μL of acidic stopping solution.
- Measure the optical density and calculate the AMH concentrations against the calibration curve.

**RESULTS**

**Limit of Detection:** The lowest amount of AMH in a serum that can be detected with a 95% probability (45 runs in duplicate, n=90) is 1.2 pg/mL and the corresponding Dried Blood Spot equivalent is 35 pg/mL.

**Cross-Reactivity:** Recombinant and native AMH antigens were run as unknown in the assay and the % cross-reactivity was calculated.

**Linearity:** Multiple dilutions of the three dried blood samples and calibrator F containing various AMH/MIS levels were diluted with extraction buffer/sample diluent. The % recovery on individual samples is represented in

**Imprecision:** Reproducibility of the DBS AMH ELISA assay was determined in a study using two kit controls and three serum pools. The study included a total of 40 assays, two replicates of each per assay (n=80). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

**Method Comparison**

The DBS AMH ELISA has been compared to Ultra-Sensitive AMH/MIS ELISA using 56 matched serum and dried blood spot samples in the range of 0.6 - 18.5 ng/mL. Passing-Bablok analysis of the results yielded the following Regression:

**CONCLUSIONS**

- The DBS AMH ELISA method is a well-characterized, sensitive human-specific, easy-to-perform, and reliable immunoassay.
- The dried blood spot sample results are comparable to serum-based assays.
- DBS sampling is noninvasive and can be implemented by non-medically trained personnel in the participant’s home or other non-clinical setting.
- The specimen stability, ease, and low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.