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, 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

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

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.

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 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.

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
1. 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.
2. The sensitivity of the Dried Blood Spot AMH ELISA (12.5 pg/mL) makes it well suited for ovarian reserve testing for all ages.
3. The specimen stability, low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

*Research Use Only.