AnshLabs

Dried Blood Spot (DBS) AMH ELISA RUO

AL-129

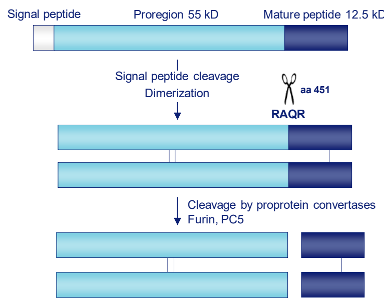
For Illustrative Purposes Only
Refer to package insert included with the product for exact specifications.

INTENDED USE

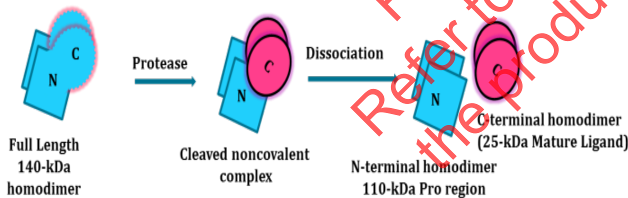
The Anti-Müllerian hormone (DBS AMH) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of AMH in human dried blood spot. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Anti-Müllerian hormone (AMH), a member of the TGFβ superfamily, is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulfide bridges. Processing of AMH is shown below.



Recent studies have shown that the AMH C-terminal homodimer is much less active than the noncovalent complex, but almost all activity can be restored by associating with the N-terminal pro-region, which reforms a complex with the mature C-terminal homodimer.[1] This finding raises the possibility that the AMH noncovalent complex is the active form of protein. It was reported that the cleaved AMH noncovalent complex binds to AMHRII and stimulates intracellular signaling, whereas full-length AMH shows only minimal activity.[2]



AMH is secreted by the Sertoli cells in males. During embryonic development, AMH is responsible for Müllerian duct regression. AMH continues to be produced by the testes until puberty and then decreases slowly to residual post-puberty values. In females, AMH is produced by the granulosa cells of small growing follicles from the 36th week of gestation onwards until menopause when levels become undetectable. Potential clinical applications of low end anti-müllerian hormone (AMH) have been published in premature ovarian insufficiency, ovarian tumors, menopause and many more.

PRINCIPLE OF THE TEST

The DBS AMH ELISA is a quantitative three-step sandwich type immunoassay.[3] In the first step Calibrators, Controls and extracted dried blood spot samples are added to AMH antibody coated microtiter wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated AMH antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of DBS AMH in the samples and calibrators.

MATERIALS SUPPLIED

CAL-129A DBS AMH Calibrator A

One vial, 1 mL, labeled DBS AMH Cal A/Sample Diluent, containing 0 ng/mL AMH in protein based buffer and Pro-Clean 400. Store unopened at 2-8°C or below until the expiration date.

CAL-129B - CAL-129F DBS AMH Calibrators B - F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 0.2 - 22 ng/mL AMH in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after run. Avoid repeated freeze thaws.

Assay Calibration:

The DBS AMH concentrations in the calibrators are traceable to the manufacturer's working calibrators. The AMH calibrator concentrations are **corrected for the DBS dilution factor and provided in the calibration card**. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-129-I & CTR-129-II DBS AMH Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high AMH concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact DBS equivalent concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after run. Avoid repeated freeze thaws.

PLT-124 AMH/MIS Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with AMH antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

EXB-129 Extraction Buffer/Sample Diluent

One bottle, 45 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

Note: Additional bottles of EXB-129, DBS AMH Extraction Buffer / Sample Diluent can be ordered if higher dilution is required.

BCR-124 picoAMH Biotin Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing biotinylated anti-AMH antibody in protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-124 picoAMH Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 2–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.
8. Tight fitting 12 x 75 mm tube racks.
9. Ahlstrom 226 or Whatman 903 (Protein Saver Card)
10. 6mm round automated puncher. For machine punching, puncher catalog number 1296-071 from PerkinElmer can be used.
11. DBS 5/16" (7.9) round puncher. For manual punching Punchline catalog number 53700 from McGill incorporated can be used.
12. If FSH and LH measurement are desired then, **AL-187 DBS FSH ELISA** and **AL-190 DBS LH ELISA** reagent kit will be needed.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

a) Follow good laboratory practice.
b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.[4]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[5] as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

Dried blood spot is the recommended sample type.

1. Use with capillary blood samples collected and dried on filter paper (Ahlstrom 226 or Whatman 903 (Protein Saver Card) according to the standard procedures established for blood collection on filter paper.
2. Wipe away the first blood drop and apply surface of the first filter paper circle to the next large drop of blood, allowing the blood to fill and completely saturate the circle.

   Note: ***Alternatively, the filter paper can be spotted by adding 60 µL of whole blood from Li-Heparin or K_2- EDTA tubes.***
3. Never use the front as well as back of the paper to fill the circle.
4. Fill at least two circles and if possible, all circles with blood.
5. After collection, dry the blood impregnated filter papers for **1- 2 hours** in a horizontal position at room temperature.
6. The dried filter paper blood spots should be stored in a low permeability re-sealable pouch at 2-8°C with a desiccant for up to 1 week or frozen at -20°C or lower for up to 3 months.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the DBS AMH ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature (23 ± 2°C) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **DBS AMH Calibrators B-F and DBS AMH Controls I & II**: Tap and reconstitute DBS AMH Calibrator B-F and DBS AMH Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
2. Note: In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.

   (a) CAL B/3: Mix 100 µL of reconstituted Cal B with 200 µL of Cal A/Sample diluent in an Eppendorf tube.
3. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.

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4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

DBS EXTRACTION PROCEDURE

Extraction of AMH from dried blood spots should be performed on the same day prior to testing.

NOTE: All blood spots should be inspected for quality.

- Do not use spots if the circle is not completely filled and impregnated with blood.
- Do not use irregular shaped spots, spots that are not impregnated throughout, or spots with multiple spotting.
- Do not use spots that have not been properly dried.

1. Label two 12 X 75 culture tubes for each unknown dried blood sample.
2. Punch out **two filter paper discs (7.9 mm) or four filter paper discs (6 mm),** impregnated with the unknown dried blood specimen, onto a clean surface and transfer the discs using clean tweezers into the corresponding tube.
3. Alternatively, punch out the paper disc directly into the culture tube using the commercially available automated punchers.
4. Add **450 µL of the AMH Extraction Buffer** to each tube, vortex well.
5. Place the tubes in a tight-fitting tube rack and incubate the tubes, shaking at a slow speed (500 - 600 rpm) at room temperature for 60 minutes.
6. Transfer the liquid from one tube into the corresponding second labeled tube. Leave the blood spot in the initial tube.
7. The blood extract is now ready for AMH measurement. *LH, FSH measurement can also be performed on the same extract when used as sample in DBS FSH ELISA (AL-187) and DBS LH ELISA (AL-190).*
8. The extracted sample (without the extracted blood spot) is stable for up to 7 days at -20°C.
9. Use the **calibrator assignment for two spots** as mentioned in the calibration card for plotting the calibration curve.

Note: *In case **only one spot is available**, add one disc in step 2 of DBS extraction procedure and follow the same steps 1-7. Use the **calibrator assignment for one spot** as mentioned in the calibration card for plotting the calibration curve.* ***Do not alter the procedure (volume of extraction buffer) to extract the dried blood spots.***

DBS EXTRACTION PROCEDURE: For Male Pediatric Samples

Extraction of AMH from dried blood spots should be performed same day prior to testing.

NOTE: All blood spots should be inspected for quality.

- Do not use spots if the circle is not completely filled and impregnated with blood.
- Do not use irregular shaped spots, spots that are not impregnated throughout, or spots with multiple spotting.
- Do not use spots that have not been properly dried.

1. Label two 12 X 75 culture tube for each unknown dried blood sample.
2. Punch out **one filter paper disc (7.9 mm)**, **or two filter paper discs (6 mm)** impregnated with the unknown dried blood specimen, onto a clean surface and transfer the disc using clean tweezers into the corresponding tube.
3. Alternatively, punch out the paper disc directly into the culture tube using the commercially available automated punchers.
4. Add **450 µL of the AMH Extraction Buffer** to each tube, vortex well.
5. Place the tubes in a tight-fitting tube rack and incubate the tubes, shaking at a slow speed (500 - 600 rpm) at room temperature for 60 minutes.
6. In the second 12 X 75 culture tube add 75 µL of the DBS AMH Extracted sample (from step 5) and add 300 µL of the DBS AMH Extraction Buffer to each tube and vortex well. The blood extract is now ready for analysis.
7. The extracted sample is stable for up to 7 days at -20°C.
8. Use the **calibrator assignment for one spot** as mentioned in the calibration card for plotting the calibration curve and apply the **dilution factor**.

Note: Multiply the results by a factor of **5X** (dilution factor).

PREPARATION OF SAMPLES - FOR SERUM SAMPLES

1. Serum specimens should be diluted with AMH Extraction Buffer/Sample Diluent (EXB-129) prior to testing.

2. For a 15-fold dilution level, add 10 µL of sample and 140 µL of EXB-129 in dilution plate well or culture tubes. After addition, gently shake the plate or tube for 10 minutes and transfer 100µL of the diluted sample to the designated wells on the antibody coated plate.

3. The read out (pg/mL) for diluted specimens must be corrected for the dilution factor. For example, a specimen that was diluted 15-fold prior to assay and reading 200 pg/mL will be reported as 3,000 pg/mL (i.e., 200 pg/mL x 15-fold dilution factor).

Note: For specimens where the range of AMH concentrations can be estimated, an initial dilution protocol can be employed for efficient workflow and reagent use

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

Protocol-1: For DBS Samples

NOTE: *All extracted samples reading higher than the highest calibrator should be diluted in the DBS AMH Extraction buffer prior to assay.*

1. Label the microtitration strips to be used.
2. Pipette **100 µL** of the reconstituted **Calibrators and Controls** to the appropriate wells and add **50 µL of the AMH Extraction Buffer** to **calibrators and controls wells** using a repeater pipette.
3. Pipette **150 µL** of the extracted DBS samples (see DBS extraction procedure) to the appropriate wells. *Note:* ***Do not add AMH Extraction Buffer to the extracted sample wells.***
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **3 hours** at room temperature.
5. Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.
6. Add **100 µL** of the Antibody-Biotin Conjugate RTU to each well using a repeater pipette.
7. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **1 hour** at room temperature.
8. Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
9. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
10. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature.
11. Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
12. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
13. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature.

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NOTE: *Visually monitor the color development to optimize the incubation time.*

14. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to 450 nm and 630 nm (machine blank).

    NOTE*: Zero calibrator should be programmed as "****Blank****" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at* ***450 nm*** *with background wavelength correction at* ***630 nm****.*

Protocol-2: For Serum Samples

1. Label the microtitration strips to be used.
2. Add **50 µL** of the AMH Extraction Buffer to calibrators, controls and serum specimen designated wells using a repeater pipette.
3. Pipette **100 µL** of the reconstituted Calibrator and Controls to the appropriate wells.
4. Pipette **100 µL** of 15-fold diluted serum samples (Refer Preparation of Samples- For serum samples) using precision pipette to the sample designated wells.
5. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for **3 hours** at room temperature (23 ± 2°C).
6. Aspirate and wash each strip 5 times with Wash Solution (350 µL/per well) using an automatic microplate washer.
7. Add **100 µL** of the Antibody-Biotin Conjugate RTU to each well using a repeater pipette.
8. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for **1 hour** at room temperature (23 ± 2°C).
9. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
10. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
12. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
13. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **8-12 minutes** at room temperature (23 ± 2°C).

NOTE: *Visually monitor the color development to optimize the incubation time.*

15. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm and 630 nm (machine blank).
16. IMPORTANT: All diluted serum specimens should be multiplied by the appropriate dilution factor (i.e. 15 or higher) for the final concentration using calibrator assignment for serum AMH. Alternatively, multiply the calibrators by the dilution factor (e.g., 15 or higher) and input the corrected calibrator values in the data reduction software of the spectrophotometer prior to reading the assay.

NOTE: *Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.*

Protocol for Dynex Technology (DS2)

Protocol for Dynex DS2 can be provided upon request.

RESULTS

NOTE: *The results in this package insert were calculated by plotting the data on a log vs. log scale using a cubic regression curve-fit. Other data reduction methods may give slightly different results.*

1. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
2. Calculate the mean optical density (OD) for each Calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the AMH concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the AMH concentrations of the Controls and unknowns from the recommended calibration curve by matching their mean OD readings with the corresponding AMH calibrator concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with DBS AMH extraction buffer and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. **Multiply the value by a dilution factor if required.**

LIMITATIONS

The reagents supplied in this kit are optimized to measure AMH levels in human Dried Blood Spot and other biological fluids. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[6]

The DBS AMH ELISA assay has been optimized for use with 7.9 mm and 6 mm filter paper disc. Deviation to 7.9mm and 6 mm disc size may give different results.

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QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- DBS AMH ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for DBS AMH controls are printed on the **Calibration card**.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean Absorbance	Conc. (ng/mL) Two DBS	Conc. (pg/mL) Serum
A1, A2	A	0.022 (Blank)	0	0
B1, B2	B	0.024	0.074	4.4
C1, C2	C	0.108	0.38	22.8
D1, D2	D	0.472	1.9	114
E1, E2	E	1.511	6.5	389.9
F1, F2	F	3.346	16.5	989.8

NOTE: Conversion factor from two DBS to serum is 16.67.
[Two DBS (ng/mL) / 16.67] *1000 = Serum (pg/mL)
CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.

Analytical Characteristics

The performance characteristic results are reported in ng/mL and can be converted to pmol/L using the conversion factor below.

1 ng/ml = 7.14 pmol/L

Analytical Sensitivity

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 16 replicates of calibrator A (0 ng/mL) and calibrator B (0.07 ng/mL) is 0.008 ng/mL. The analytical sensitivity calculated for one DBS is 0.025 ng/mL and two DBS are 0.0125ng/mL

Analytical Specificity:

This monoclonal antibody pair used in the assay is specific for human AMH and does not detect rat, mouse, porcine, equine, bovine, canine and ovine AMH. The antibody pair chosen does not cross react to Inhibin A, Inhibin B, Activin A, Activin B and Activin AB.

Precision:

Reproducibility of the DBS AMH ELISA assay was determined in a study using 128 DBS samples from four subjects. The study included a total of 4 assays, 2 replicates of each DBS (n=64 for each sample). Representative data were calculated and are presented in table below.

Sample	Mean Conc.	Within Run		Between Spots		Total	
	ng/mL	SD	%CV	SD	%CV	SD	%CV
Sample-1	8.40	0.21	2.4%	0.45	5.3%	0.49	5.9%
Sample-2	12.26	0.36	2.9%	0.70	5.7%	0.78	6.4%
Sample-3	6.55	0.18	2.7%	0.37	5.7%	0.41	6.3%
Sample-4	5.23	0.16	3.0%	0.22	4.3%	0.27	5.3%

Interference:

When potential interferents (hemoglobin, triglycerides, and bilirubin) were added at least at two times their physiological concentration to control sample, AMH concentration were within ± 10% of the control.

Linearity:

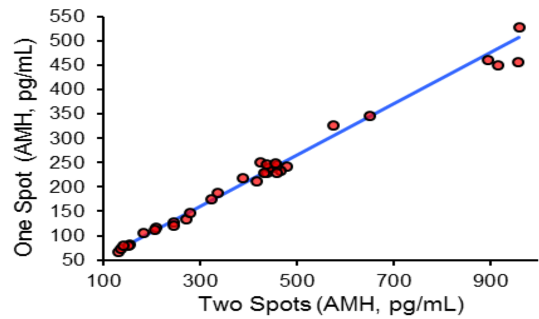
Based on NCCLS EP-6-P, multiple dilutions of the three dried blood samples and antigen containing various AMH/MIS levels were diluted with extraction buffer/sample diluent. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	Neat	11.967	NA	NA
	1:2	5.984	5.701	95%
	1:4	2.992	3.084	103%
	1:8	1.496	1.566	105%
	1:16	0.748	0.746	100%
2	Neat	7.066	NA	NA
	1:2	3.533	3.384	96%
	1:4	1.767	1.768	100%
	1:8	0.883	0.813	92%
	1:16	0.442	0.419	95%
3	Neat	5.101	NA	NA
	1:2	2.551	2.321	91%
	1:4	1.275	1.17	92%
	1:8	0.638	0.586	92%
	1:16	0.319	0.318	100%
Antigen	Neat	22.1	NA	NA
	1:2	11.05	11.183	101%
	1:4	5.525	5.64	102%
	1:8	2.763	2.927	106%
	1:16	1.381	1.559	113%

Method Comparison:

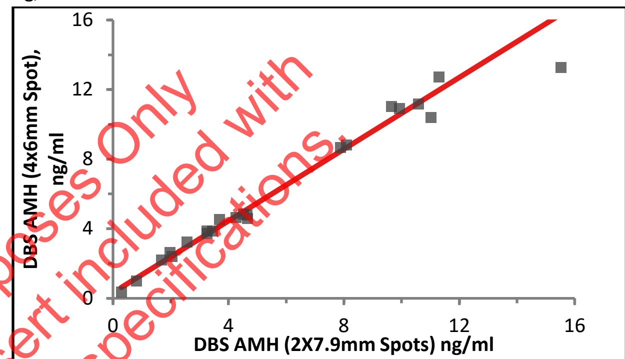
One versus Two Dried Blood Spots: Extraction was performed using matched one versus two dried blood spots. Passing Bablok analysis of the results yielded the following Regression:

One DB Spot Extracted = 0.53 (Two DB Spots Extracted) + 1.85



Four Dried Blood Spots (6mm) versus Two Dried Blood Spots (7.9mm): Extraction was performed using matched four (6mm) versus two (7.9mm) dried blood spots using calibration concentration specified in the calibration card (CRD-129). Passing Bablok analysis of the results yielded the following Regression:

DBS AMH (4x6mm Spots), ng/ml = 0.327 + 1.031 DBS AMH (2X7.9mm Spots) ng/ml



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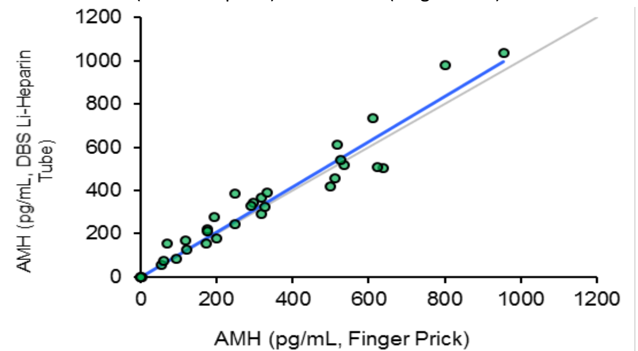
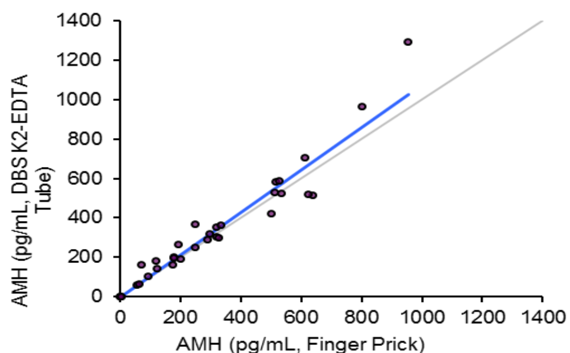
Sample Type:

Matched whole blood from Li-Heparin and K_2-EDTA plasma tubes were spotted and compared to whole blood spots from finger prick in DBS AMH ELISA assay (AL-129). Passing Bablok analysis of the results yielded the following

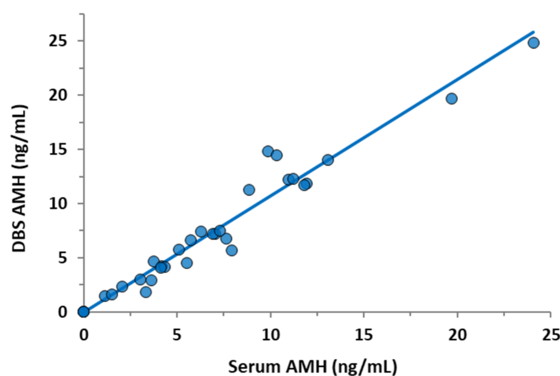
AMH (DBS Li-Heparin) = 1.05 AMH (Finger Prick) – 1.18



AMH (DBS K_2-EDTA Tube) = 1.07 AMH (Finger Prick) - 0.04



Serum and Dried Blood Spot (DBS) samples were compared using 33 matched samples in the range of 0 – 25 ng/mL. Passing Bablok analysis of the results yielded the following regression
DBS AMH = 0 + 1.072 Serum AMH
(r=0.975)



EXPECTED RESULTS

The expected results were determined by testing 20,738 specimens from a total of 13,555 healthy subjects whose blood was collected at multiple study sites in the US and Europe. Because AMH declines as ovarian reserve diminishes with age the values obtained using the AL-124-i ELISA are age stratified. AMH measurements are in pg/mL.

Age Range	Specimens Tested*	Mean	Mean SE	Lower Limit (2.5%)	Upper Limit (97.5%)
20-25	234	5018	331	740	17356
26-30	542	4218	151	444	12774
31-35	985	3424	106	249	12213
36-40	1436	2375	67	36	8874
41-45	2723	881	21	<2	3866
46-50	5931	258	6	<2	1718
51-55	4546	46	2	<2	390
56-60	1972	6	3	<2	12
61-65	2369	2	<2	<2	3

* These 20,738 specimens were from a total of 13,555 individual women

Note*: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.*

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