POSTER BOARD #SUN-0013

Development of stable picoAnti-Müllerian Hormone ELISA: Sensitive, Reliable and Reproducible results*

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OBJECTIVE

To develop a well characterized human AMH ELISA that can accurately measure circulating levels of AMH in women with diminished ovarian reserve.

INTRODUCTION

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.

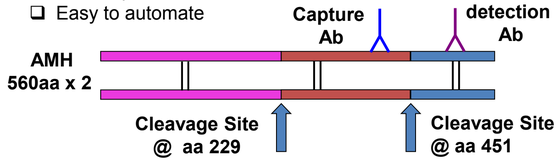
Recent studies have shown that the AMH C-terminal homodimer is much less active than the noncovalent complex, but almost all activity can be restored by associating with the N-terminal pro-region, which reforms a complex with the mature C-terminal homodimer. This finding raises the possibility that the AMH noncovalent complex is the active form of the protein. Use of characterized antibody pairs with linear epitopes are essential towards understanding the circulating forms, sensitivity and stability of AMH.

Serum AMH levels have been reported to be strongly associated with age, ovarian antral follicle counts (AFC), serum FSH and are generally thought to be relatively stable over the menstrual cycle compared to other markers of ovarian reserve. 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.

Serum AMH has been a marker of interest in young breast cancer survivors as pre-chemotherapy AMH levels may predict post-chemotherapy ovarian function, and a rise in levels after chemotherapy may reflect ovarian recovery. Several studies have also used serum AMH to model age at menopause. However, due to limited sensitivity of previous assays, it has not been possible to detect AMH five years prior to menopause until present.

METHOD

- ❑ Three-step sandwich ELISA
- ❑ Standardized human recombinant Calibrators
- ❑ Sample Type : Serum, Lithium-Heparin, EDTA, Acid Citrate Dextrose Plasma
- ❑ No complement Interference
- ❑ Easy to automate



Antibody clone / Isotype	AMH Epitope	Affinity (Region)
AMH Capture (24) $IgG2_b$	358- 369	+++ (Midpro)
AMH Detection (32) IgG1	491-502	+++ (Mature)

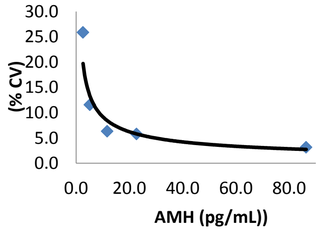
Antibody Selection: Multiple serum samples were tested on region specific AMH antibody pairs and compared to a commercial assay. The antibody pair was selected based on:

- ❑ Linear epitopes for capture and detection Antibodies in the stable midpro-region and mature region, respectively.
- ❑ Specific to human AMH.
- ❑ Clinically relevant form.
- ❑ Parallelism between native and recombinant human AMH.

RESULTS

Limit of Detection: The lowest amount of AMH in a sample that can be detected with a 95% probability (45 runs in duplicate, n=90) was **1.2 pg/mL**. The value was determined by processing four serum samples in the range of 1.7pg/mL to 11.6pg/mL following CLSI EP17 guidelines.

Limit of Quantitation: The estimated minimum dose achieved at 20% total imprecision is **3.9 pg/mL**. The value was determined by processing seven samples in the range of 1.7 -371pg/mL (45 runs in duplicates, n=90) following CLSI EP17 guidelines.



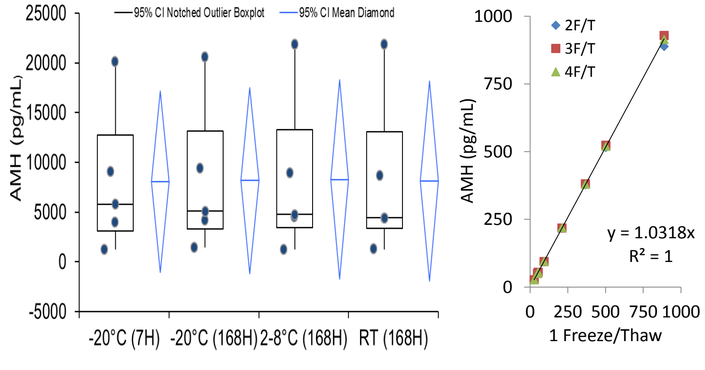
Imprecision: Reproducibility of the picoAMH ELISA assay was determined in a study using two kit controls and three serum pools. The study included a total of 40 assays, two replicates of each per assay (n=80). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the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

Sample	Conc. (pg/mL)	Within run		Between run		Total	
		SD	CV	SD	CV	SD	CV
Control I	64.1	1.8	2.9%	2.0	3.2%	2.7	4.3%
Control II	186.4	6.5	3.5%	5.8	3.1%	8.7	4.7%
QC1	22.6	0.8	3.7%	1.0	4.5%	1.3	5.8%
QC2	86.5	1.9	2.2%	1.9	2.2%	2.7	3.1%
QC3	373.2	7.7	2.1%	14.2	3.8%	16.2	4.3%

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

Cross-Reactant	Concentration	% Cross-reactivity
Rodent/Bovine/Equine/Canine AMH	130ng/mL	0.00
Full-Length AMH dimer	1000pg/mL	100
Human mature AMH (>99.9%)	600pg/mL	0.00
pro-mature AMH dimer (>90%)	610pg/mL	91.3

Sample Stability



Linearity of Dilution: Serum samples and recombinant human AMH antigen were diluted in calibrator A/Sample diluent.

Sample	Dilution Factor	Expected AMH (pg/mL)	Observed AMH (pg/mL)	% Recovery
1	Neat	449.2	NA	NA
	1:2	224.6	211.8	94%
	1:4	112.3	105.7	94%
	1:8	56.1	55.4	99%
	1:16	28.1	27.1	97%
2	1:10	558.7	Neat	NA
	1:20	279.3	298.7	107%
	1:40	139.7	142.5	102%
	1:80	69.8	67.0	96%
	1:160	34.9	33.1	95%
Human AMH Antigen	Neat	746.0	NA	NA
	1:2	373.0	377.2	101%
	1:4	186.5	179.9	96%
	1:8	93.2	88.0	94%
	1:16	46.6	44.7	96%
	1:32	23.3	23.1	99%

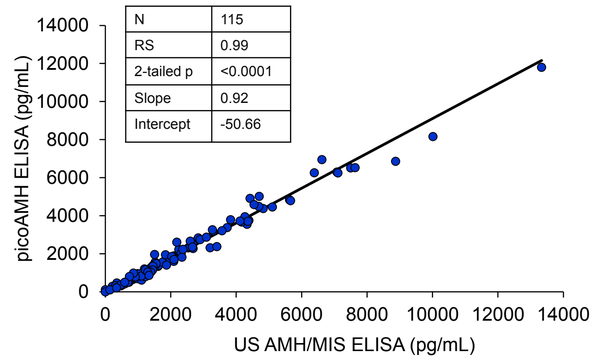
Spike Recovery: Serum samples were spiked with varying concentrations of recombinant human AMH and % recovery was calculated.

Sample ID	Endogenous Value in pg/mL	Expected in pg/mL	Observed in pg/mL	% Recovery
1	121.15	150.9	154.5	102%
		178.0	174.9	98%
		202.6	201.5	99%
2	130.11	159.4	159.1	100%
		186.1	182.4	98%
		210.4	218.9	104%
3	113.45	143.6	139.0	97%
		170.9	169.9	99%
		196.0	199.1	102%

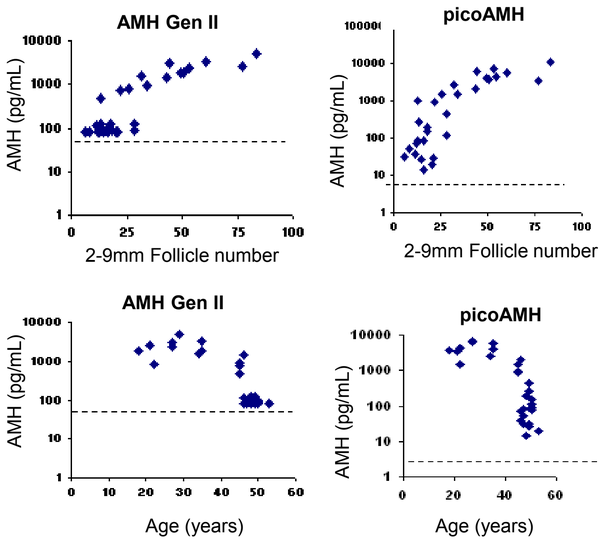
Reference Range: Serum samples were pre-diluted 1:10 or higher prior to assay. Female samples over 45 years were run neat.

Sample	Median Age	Median AMH (pg/mL)	Range (pg/mL)
Female (n=33) Age 25-40 Years	34	2560	190-9130
Female (n=30) Age 41-45 Years	43.5	275.9	4.2-3300
Female (n=36) Age 46-50 Years	48	150.4	4.03-539.9
Female (n=47) Age 51-55 Years	53	18.04	6.7-29.4
Post-Menopausal (n=38) Age 53-70 Years	61	Non Detectable	Non Detectable

Method Comparison: Serum samples were pre-diluted 1:10 or 1:20 prior to picoAMH ELISA. Matched serum samples were run undiluted in USAMH/MIS ELISA.



AMH and AFC: Relationship between matched serum AMH to number of 2-9mm follicles (top) and female age (bottom) between picoAMH ELISA and AMH Gen II assays. *Horizontal dashed lines shows ELISA sensitivities.*



CONCLUSIONS

A well-characterized, linear epitope, clinically relevant, highly sensitive, reliable and easy-to-perform human specific AMH assay has been developed. The assay is suitable for research use to detect and monitor AMH levels in

- ❑ Women in menopausal transition.
- ❑ Pre and post-chemotherapy ovarian function.
- ❑ Primary ovarian insufficiency.
- ❑ Polycystic ovary syndrome.
- ❑ Granulosa cell tumor.

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