MON-527

Development of a Well Characterized Ultra-Sensitive Human Anti-Mullerian Hormone Chemiluminescence Assay: Evaluation of Potential Clinical Applications.

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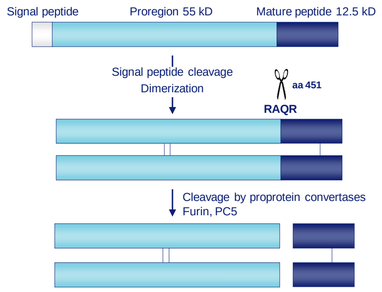
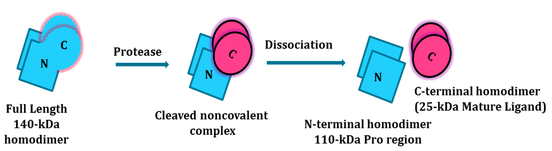
INTRODUCTION

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. In males, AMH is secreted by the Sertoli cells. 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 anti-Müllerian hormone (AMH) have been published in in-vitro fertilization, polycystic ovary syndrome, primary ovarian insufficiency, granulosa cell tumors, menopause and many more.

Processing of human AMH



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 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.



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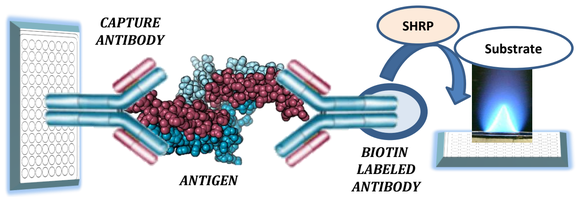
METHOD

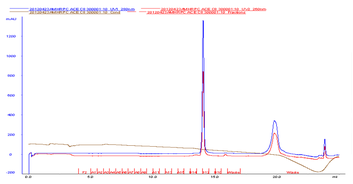


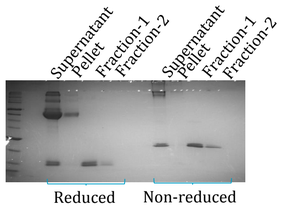
RESULTS

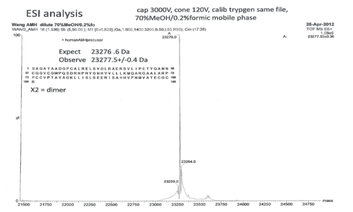
Antibody Selection: Multiple serum samples were tested on over 20 optimized antibody pairs and compared to a commercial AMH assay. The antibody pair was selected based on

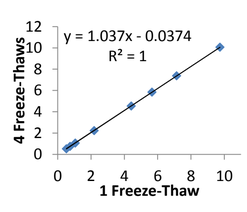
- ❑ Binding in the stable pro-region and mature region.
- ❑ Linear epitopes
- ❑ Specific to human AMH

Assay Calibration: The recombinant AMH concentrations in calibrators are standardized to purified mature AMH preparation that is characterized by mass spectroscopy and optical density at 280nm. The calibrators are stable upon reconstitution at -20°C or below and up to four freeze thaw cycles.

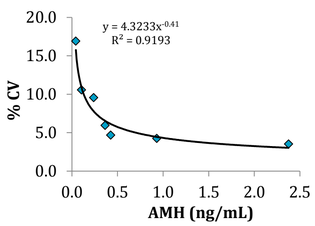








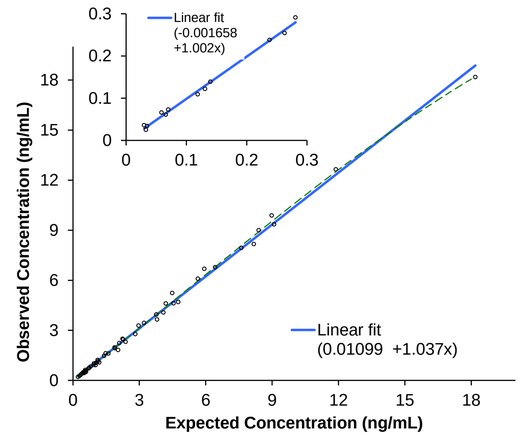
Limit of Quantitation: The estimated minimum dose achieved at 20% total imprecision is 0.012 ng/mL. The value was determined by processing six samples in the range of 0.043-2.38 ng/mL over twelve runs and two days in duplicates (n=24) following CLSI EP17 guidelines



Imprecision: Reproducibility of the USAMH/MIS CLIA assay was determined in a study using three serum pools. The study included a total of 12 assays, four replicates of each per assay (n=48).

Sample	Mean Conc.	Within Run		Between Run		Total	
	(ng/mL)	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
Pool-1	0.424	0.015	3.55	0.013	3.16	0.02	4.75
Pool-2	1.058	0.032	3.02	0.015	1.43	0.35	3.34
Pool-3	2.376	0.031	1.29	0.081	3.41	0.087	3.65

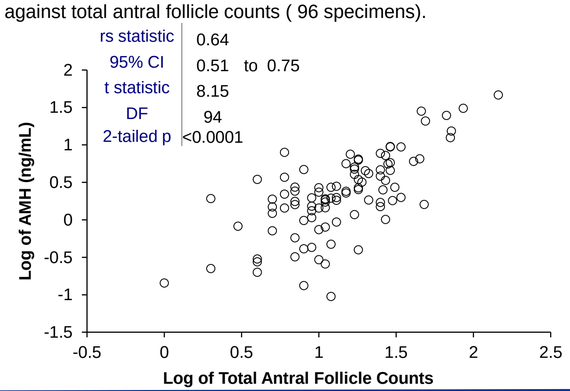
Linearity of Dilution: Multiple dilutions of the four serum samples containing various AMH/MIS levels were diluted with Calibrator A/sample diluent. The linearity plot is represented below.



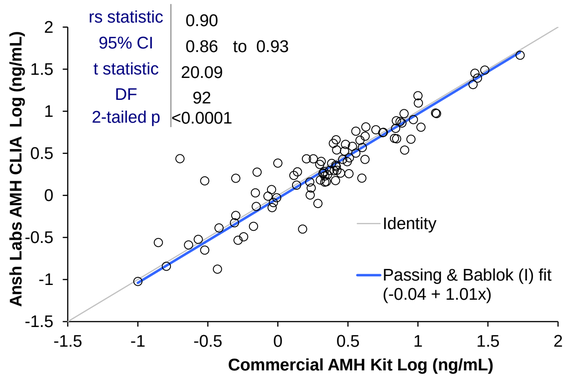
Cross Reactivity and Interference:

Analytes	Concentration	% Cross-reactivity/interference
Human AMH (Pro-Mature)	44 ng / ml	100%
hAMH(Pro >95% purity))	300 ng/mL	0.24
Mature AMH (>90% purity)	120 ng/mL	< 1.0
Inhibin A, Inhibin B	I0 ng/mL	ND
Activin A, Activin B, Activin AB	50 ng/mL	ND
Alpha-2 Macroglobulin	50 ng/mL	ND
FSH, LH	500 mIU/mL	ND
Estradiol, Progesterone	100 ng/mL	ND
Hemoglobin	1.35 mg/mL	< 1.0
Triglygerides	5.0mg/mL	< 2.9
Bilirubin	0.6mg/mL	< 3.3

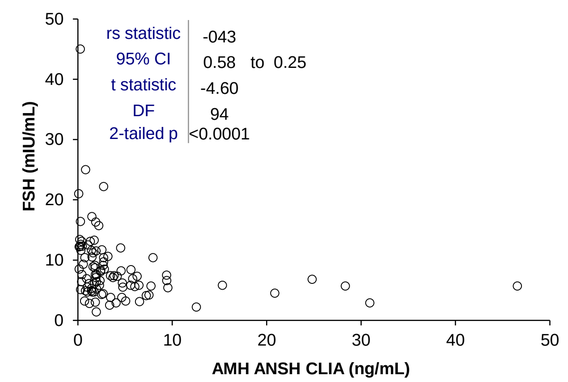
Method Comparison: AnshLabs USAMH CLIA, was compared against total antral follicle counts (96 specimens).



Method Comparison: AnshLabs USAMH CLIA, was compared against commercial kit using 96 serum samples of patients visiting the infertility clinic in the range of 0.1-46 ng/mL



Method Comparison: AnshLabs USAMH CLIA was compared against Follicle stimulating Hormone (96 specimens).



CONCLUSIONS

- ❑ A sensitive, reliable and easy-to-run microplate AMH assay has been developed to measure AMH in serum and other biological fluids.
- ❑ The approximate median AMH levels found in healthy population can be measured within <5% CV using this assay.
- ❑ The assay has shown excellent analytical performance and is suitable for studies in the area of in-vitro fertilization, polycystic ovary syndrome, primary ovarian insufficiency, granulosa cell tumors, menopause , etc.

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