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.

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.

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 binds to AMHRII and stimulates intracellular signaling, whereas full-length AMH shows only minimal activity.

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/MS 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).

METHOD

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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