

# Novel Monoclonal Anti-Müllerian Hormone Antibodies Applicable to Sensitive Diagnostic Immunoassays

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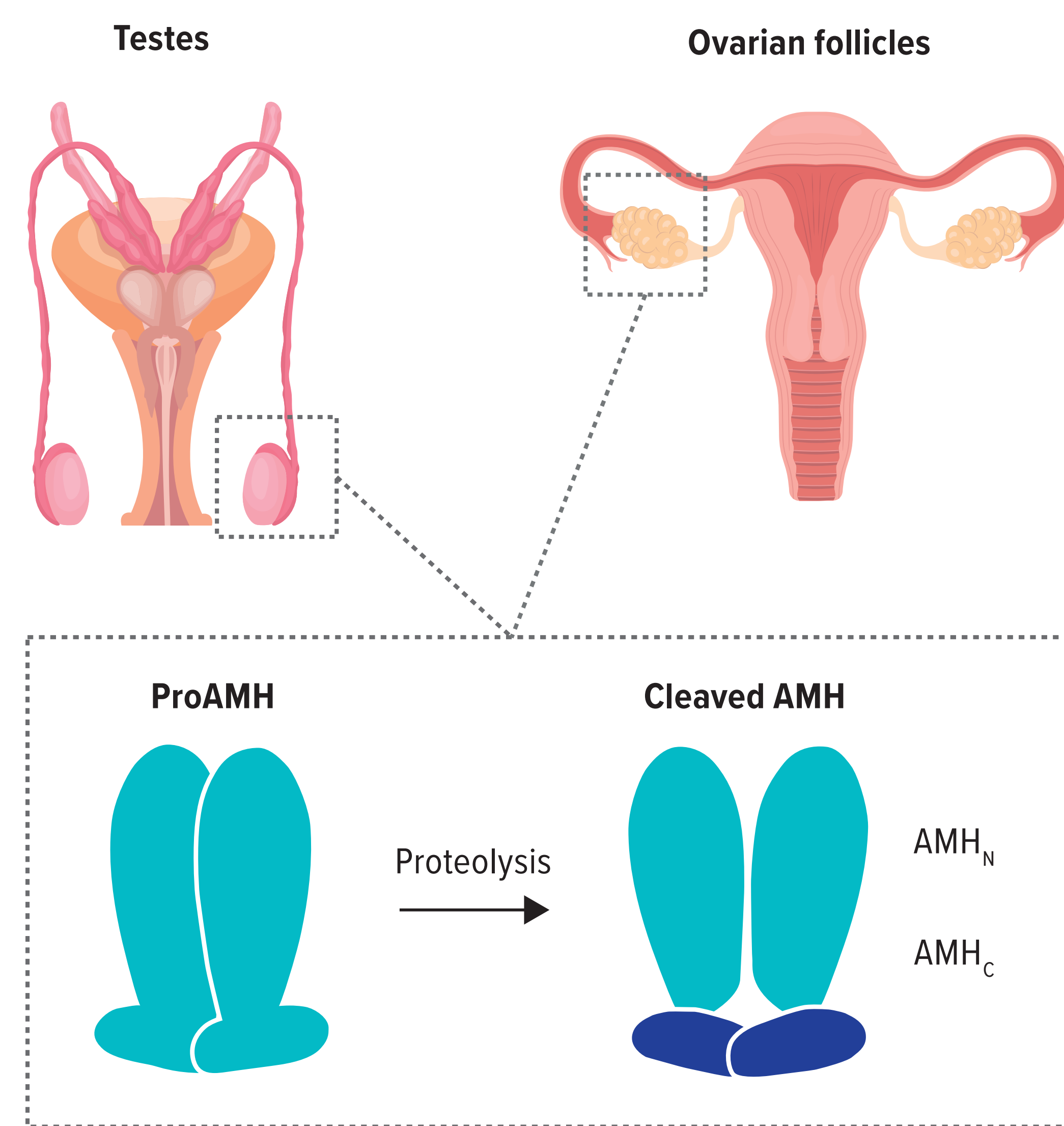
## AMH: a unique endocrine indicator of gonadal development and ovarian reserve

Anti-Müllerian hormone (AMH) is a 140-kDa glycoprotein whose embryonic expression inhibits the development of the female reproductive tract.<sup>1</sup> AMH is produced by the Sertoli cells of the testis during sexual differentiation and by the granulosa cells of the ovarian follicles.<sup>1</sup> In women, serum AMH levels peak at early adulthood and slowly decrease with age, becoming undetectable at menopause.<sup>1</sup>

AMH is synthesized as a homodimeric precursor (proAMH), which is cleaved into an N-terminal dimer (AMH<sub>N</sub>) and a C-terminal dimer (AMH<sub>C</sub>) that associate non-covalently, forming a tetrameric AMH complex (Figure 1).

Serum AMH concentration reflects the size of the follicle pool and the likelihood of pregnancy – independent of the menstrual cycle – and is utilized to assess responsiveness to ovarian stimulation in *in vitro* fertilization (IVF)<sup>1,3,4</sup> and to assess both ovarian dysfunction and the impact of gonadotoxic treatments.<sup>1</sup> In men, serum AMH has traditionally been used as a biomarker for gonadal development.<sup>2</sup>

Both proAMH and AMH<sub>N</sub>/AMH<sub>C</sub> can be found in circulation and target organs, challenging the development of specific, high-affinity AMH antibodies. We have developed six novel monoclonal antibodies (mAbs) that recognize AMH<sub>N</sub>, exhibiting high sensitivity and applicability to diagnostic purposes. In this study, AMH mAb binding properties were evaluated in fluorescence immunoassays (FIA) on purified recombinant AMH<sub>N</sub>, native AMH, and clinical sera with varying AMH concentrations.



**Figure 1.** Anti-Müllerian hormone (AMH) is synthesized as a precursor (proAMH) in the testis and ovarian follicles. The full-length proAMH homodimer is proteolytically cleaved into an N-terminal (AMH<sub>N</sub>) and C-terminal dimer (AMH<sub>C</sub>), forming a tetrameric AMH complex.

## Materials & Methods

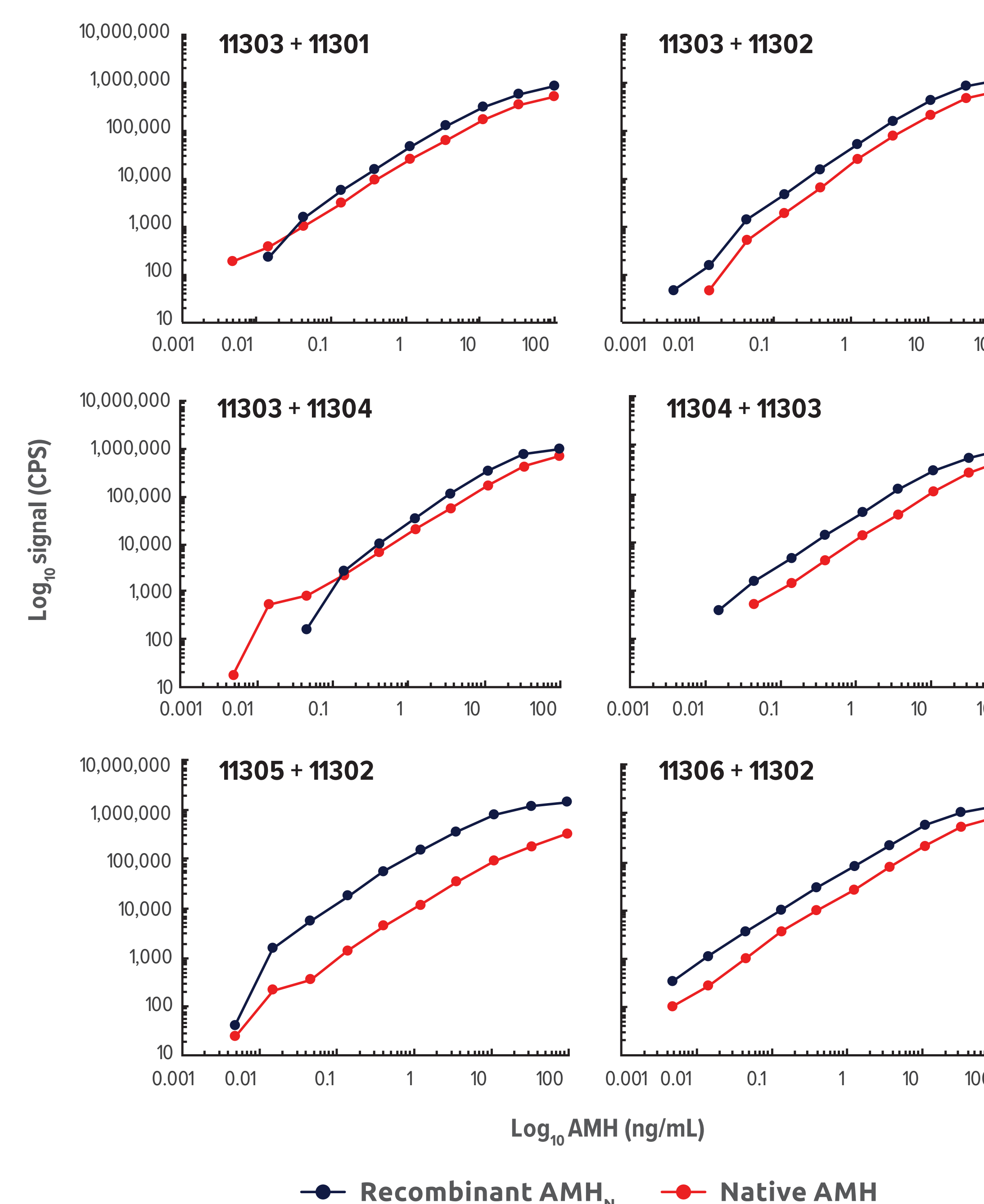
Six anti-human AMH mAbs, 11301 (#100756), 11302 (#100757), 11303 (#100758), 11304 (#100759), 11305 (#100760), and 11306 (not yet commercially available; all Medix Biochemica), were assessed for their AMH antigen binding properties. The optimal mAb pairs were determined using commercial controls [Serorm Immunoassay Liq L-1 (#207005), L-2 (#207105), and L-3 (#207205); all SERO], with reported AMH levels of 5.1, 3.7, and 1.6 ng/mL, respectively (data not shown). Standard curves were generated using sandwich FIA for selected mAb pairs with purified recombinant AMH<sub>N</sub> in a buffer solution and native AMH in serum at concentrations ranging from 0.005 to 100 ng/mL. Biotin-conjugated antibodies and europium-labeled streptavidin were used for detection.

The optimal mAb pairs were then utilized for the FIA-based quantification of AMH in 48 clinical sera with AMH concentrations varying from 0.03 to 23.8 ng/mL. Samples 1–27 were analyzed on the UniCel Dxl 800 Access Immunoassay System (Beckman Coulter) and samples 28–48 on cobas® 8000 modular analyzer (Roche). Both of these assays contain AMH mAbs recognizing the AMH<sub>N</sub> and AMH<sub>C</sub> subunits.

## Sensitive binding of recombinant and native antigens by AMH mAb pairs

In this study, we identified several well-functioning mAb pairs with sensitive detection of AMH in a wide concentration range (Figure 2).

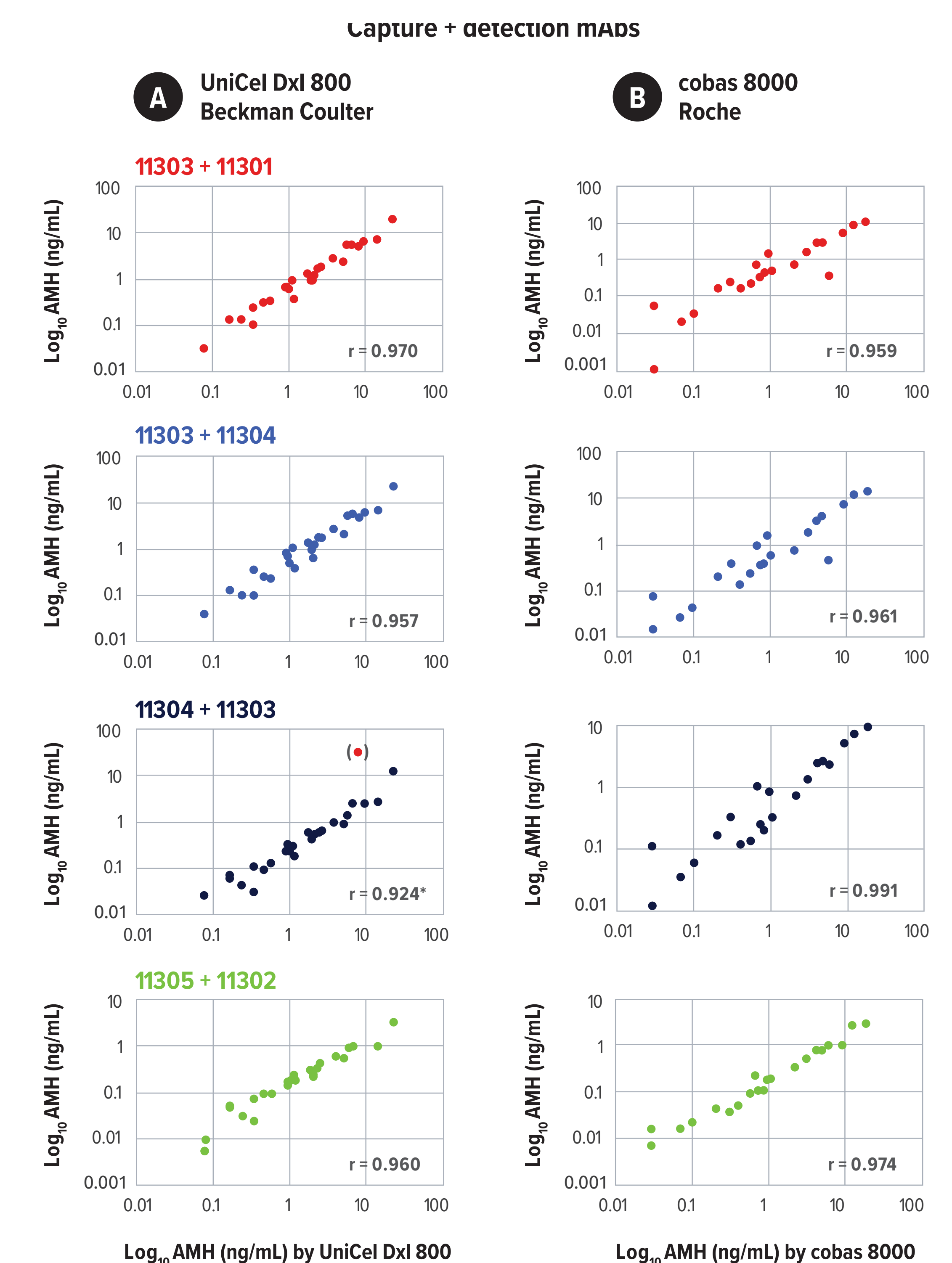
Several mAb pairs exhibited a linear assay performance with an excellent agreement in the detection of the recombinant and native AMH antigens.



**Figure 2.** The binding properties of AMH mAb pairs as determined by sandwich FIA. Purified recombinant AMH<sub>N</sub> (turquoise) and native AMH present in serum (magenta) were used as assay antigens. Capture mAb is mentioned first, and detection mAb (biotin-conjugated) second in the title of each graph. CPS, counts per second.

## Accurate AMH detection from clinical sera

AMH mAb pairs exhibited consistent performance in AMH detection from clinical sera. Serum AMH concentrations quantified by FIA were well in line with the AMH levels obtained on two gold-standard assays, UniCel Dxl 800 (Figure 3A) and cobas® 8000 (Figure 3B), with correlations reaching 0.991.



**Figure 3.** Detection of native AMH from clinical sera. A) Samples 1–27 were analyzed on a Dxl 800 and B) samples 28–48 on a cobas® 8000. Biotin-conjugated mAbs were used as detection antibodies. \*,  $r = 0.510$  with the outlier (data point in red) included in the statistical analysis.  $r$ , Pearson's correlation coefficient.

## Conclusions

This study demonstrates sensitive binding of the novel AMH mAbs to a biochemically challenging antigen – both as recombinant AMH<sub>N</sub> and native AMH. We identified several well-performing mAb pairs for clinical AMH detection, exhibiting an excellent inter-assay correlation with two gold-standard assays. Our AMH mAbs can be utilized for the development of sensitive diagnostic assays for the evaluation of gonadal function in a clinical setting.

## Acknowledgements

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## References

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