# Anti-Müllerian Hormone: Results from the RCPAQAP Pilot Program

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Anti-Müllerian hormone (AMH) is a useful test to assist with the evaluation of ovarian function and follicular reserve in women seeking fertility treatment. It can also be elevated with polycystic ovarian syndrome and granulosa cell tumours. Accordingly, AMH is now offered by an increasing number of laboratories on different platforms.

There is currently no Certified Reference Material for AMH. Previous studies have highlighted the presence of inter-laboratory variability[1,2]. Factors contributing to assay imprecision include sample storage conditions and the time between collection and analysis. Interference from complement has also been identified in commercial assays[3].

Following requests to develop an EQA for AMH[1], the RCPAQAP undertook a pilot study in 2017 in collaboration with the Prince of Wales Hospital Clinical Chemistry Department and Australian Scientific Enterprise.

## Method

Serum was sourced from discarded placentas of male newborns and analysed by the Prince of Wales Hospital Clinical Chemistry Department to confirm presence of AMH. The material was then spiked into a human serum pool, then diluted out to produce six linearly related levels. A concentration range between 3 to 70 pmol/L was specified. The material was then aliquoted, lyophilised, packaged and shipped to RCPAQAP for distribution.

Twelve laboratories were invited to participate in the pilot program. A diverse mix of participants were selected, with pathology laboratories from both public and private sectors and a fertility clinic chosen. Eleven laboratories were from Australia and 1 from New Zealand. Every state in Australia was represented.

Participants were sent 6 samples (one of each level) to be run over a course of 3 weeks. Information regarding method (analytical principle, instrument and reagent) was requested. Results were returned and preliminary statistical analysis was conducted. The data was then further assessed by ACEN to determine the Allowable Limits of Performance (ALP). This involved reviewing existing data on assay performance and biological variation.

## Results

- There were 6 Beckman Coulter and 6 Roche Diagnostics users participating in the pilot
  - 10 participants submitted results for every survey
- The ALP was set at +/- 1.0 to 6.0 pmol/L, and +/- 15% for > 6.0 pmol/L
- Overall inter-assay CV ranged from 6.5 to 13.7%.
- The within laboratory CV at the 50th percentile was 2.6%
- Roche Diagnostics inter-assay CV ranged from 2 to 7%
- Beckman Coulter inter-assay CV ranged from 9 to 18%
- The median high and low for both Roche Diagnostics and Beckman Coutler users were comparable
- The material was deemed sufficiently linear with R2 = 0.9993

### Figure 1a.
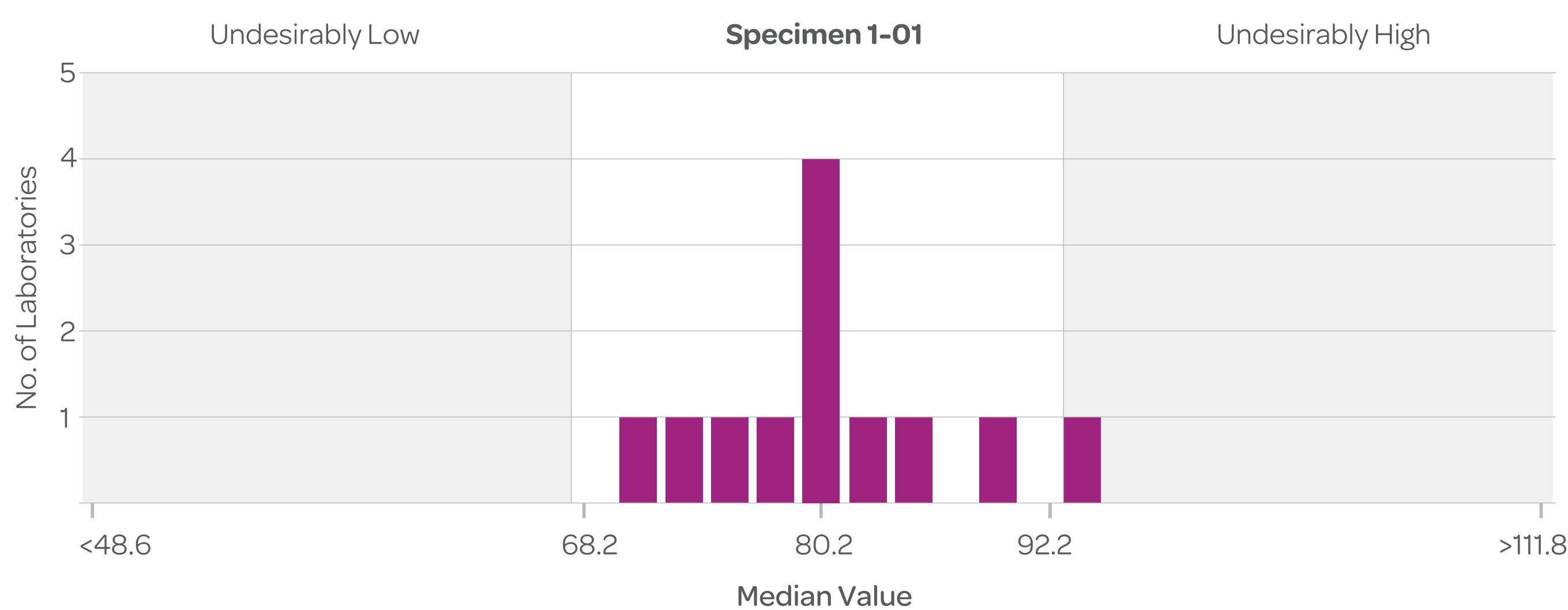Distribution of results for the high concentration sample in pmol/L



### Figure 1b.
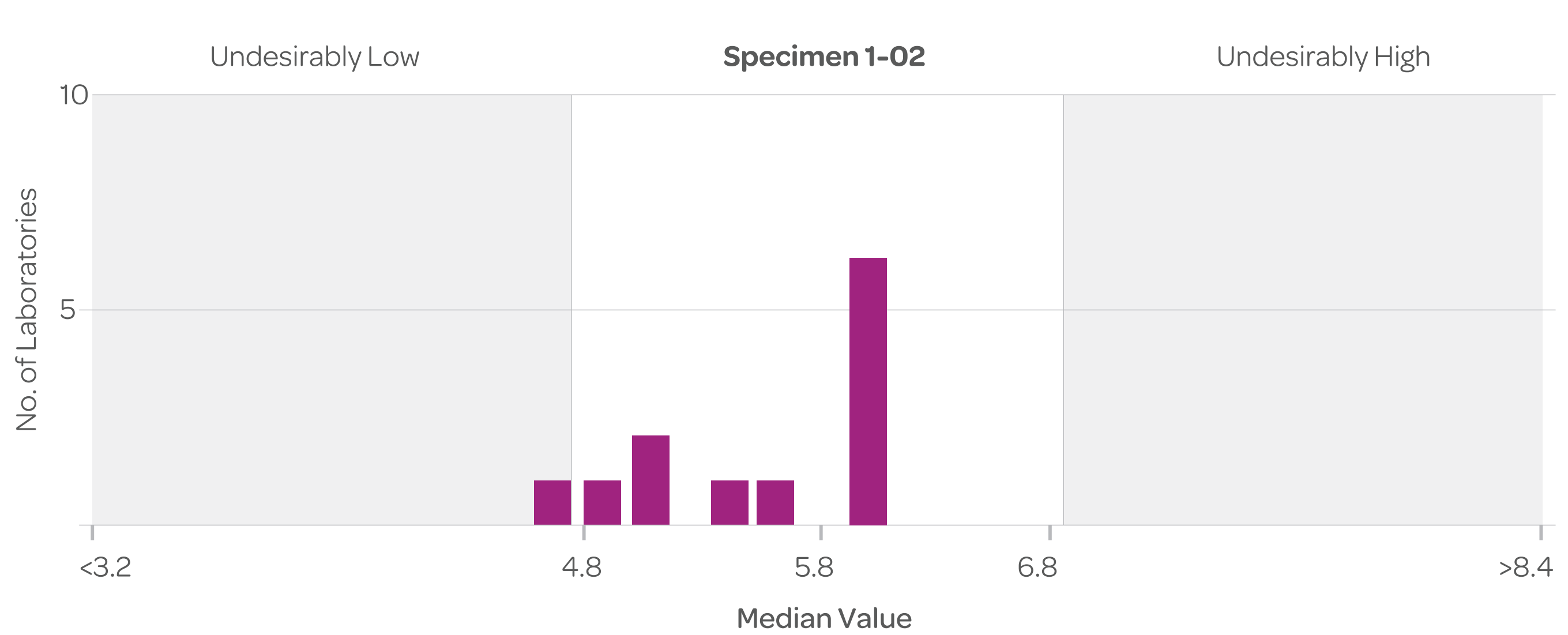Distribution of results for the low concentration sample in pmol/L



### Table 1a.
Summary of results for all Roche Diagnostic users (Cobas e411, e170, e601/602)

| Level | 1 | 2 | 3 | 4 | 5 | 6 | Avg. |
|---|---|---|---|---|---|---|---|
| Median (pmol/L) | 5.9 | 20.0 | 34.1 | 49.0 | 64.1 | 80.2 | |
| S.D. | 0.4 | 0.7 | 0.9 | 1.0 | 1.6 | 1.7 | 1.0 |
| %CV | 7.0 | 3.6 | 2.6 | 2.0 | 2.5 | 2.1 | 3.3 |
| Number | 6 | 5 | 5 | 5 | 5 | 6 | |

### Table 1b.
Summary of results for Beckman Coulter users (Access/Access2)

| Level | 1 | 2 | 3 | 4 | 5 | 6 | Avg. |
|---|---|---|---|---|---|---|---|
| Median (pmol/L) | 5.5 | 18.0 | 37.1 | 45.4 | 73.0 | 79.5 | |
| S.D. | 0.7 | 1.9 | 6.8 | 4.2 | 9.8 | 9.4 | 5.5 |
| %CV | 11.9 | 10.2 | 18.0 | 9.0 | 14.2 | 11.5 | 12.5 |
| Number | 6 | 5 | 5 | 5 | 5 | 6 | |

## Conclusion

Although only a small number of laboratories were surveyed, the results demonstrate good overall analytical agreement between methods for both high and low levels of AMH. The pilot was considered successful and a full EQA program commenced in January 2018.

References
1. Ho et al. Anti-Mullerian Hormone (AMH) Survey Results from the AACB Western Australia Quality Assurance Group (WAQAG). 2016
2. Zuvela E; Walls M; Matson P. Within-laboratory and between-laboratory variability in the measurement of anti-müllerian hormone determined within an external quality assurance scheme. Reprod Biol. 2013; 13(3):255-7
3. Gassner; Jung. First fully automated immunoassay for anti-Müllerian hormone. Clin Chem Lab Med. 2014; 52(8):1143-1152