Anti-Müllerian Hormone (serum, plasma)

## 1	Name and description of analyte

1.1	Name of analyte
Anti-Müllerian Hormone (AMH)

1.2	Alternative names
Müllerian Inhibiting Substance (MIS)
Müllerian Inhibiting Factor (MIF)

1.3	NLMC code: to follow

1.4.	Function(s) of analyte
AMH, a dimeric glycoprotein, is a member of the transforming growth factor β family of growth and differentiation factors. During normal male foetal sexual development, testicular production of AMH, secreted by Sertoli cells from the 7th week of gestation, plays a vital role in male sexual differentiation. It is responsible for the involution/regression of the female Müllerian duct while testosterone from Leydig cells stimulates differentiation of the male Wolffian ducts, the urogenital sinus and the external genitalia. In females, AMH is released from granulosa cells of preantral and small antral follicles but is not present until the 36th week of gestation, after formation of the Müllerian duct. AMH concentrations in boys are very high until puberty when they drop significantly following the increased testosterone production that downregulates AMH, probably via activation of the Sertoli cell androgen receptor. However, post pubertal concentrations of AMH in males remain significantly higher in general that those seen in adult females, although there is a significant overlap in values. Female AMH concentrations are low at birth, rise gradually and stabilise during the first few years of life and then remain relatively constant during the pubertal transition. After the mid-twenties, concentrations gradually decline, becoming undetectable after the menopause. The primary function of AMH in females is to preserve the follicular pool, mainly by resisting follicular recruitment induced by FSH.

## 2	Sample requirements and precautions

2.1	Medium in which measured
Serum (preferred); plasma (lithium heparin)

2.2	Precautions re sampling, handling etc.
Samples should be separated within 2 h of collection. Once separated, samples can be refrigerated for 2–5 days. They should be frozen at -20°C for medium term storage or at -80°C for longer term storage.

## 3	Summary of clinical uses and limitations of measurements

3.1	Uses
Differing expression between genders during childhood has led to AMH being considered as a potentially useful biomarker. In paediatric practice, AMH measurement can be useful in the investigation of disorders of sexual development, particularly in distinguishing cryptorchidism from

anorchidism and in persistent Müllerian duct syndrome, hypogonadotropic hypogonadism and as a marker of testicular function in Klinefelter syndrome (XXY). It may also be used in the pre- and post-assessment of girls and young women undergoing chemotherapy with gonadotoxic agents and in other causes of premature ovarian insufficiency (POI). AMH is also a marker of granulosa cell tumours and may be useful in the assessment of polycystic ovarian syndrome (PCOS).

In adult (post menarche – pre menopause) females, AMH is primarily used for assessment of ovarian reserve status in the field of assisted reproduction technology (ART). It is used to assess suitability for, and to guide, controlled ovarian stimulation treatment with gonadotrophins during *in vitro* fertilisation (IVF) or intra cytoplasmic sperm injection (ICSI), thus reducing the incidence of under-stimulation (no eggs) or over stimulation (leading to ovarian hyperstimulation syndrome (OHSS)). As in younger females, AMH also has a role as a marker of PCOS, in the detection and monitoring of granulosa cell tumours and to assess cancer patients undergoing treatment with gonadotoxic drugs.

3.2   Limitations
There is no support for AMH as a predictor of pregnancy outcome. The use of AMH in the diagnosis of menopause in older women is not recommended in the UK (NICE Guideline NG23). Care must be taken to consider sample storage conditions and the assay method when interpreting results.

4   Analytical considerations

4.1   Analytical methods
AMH is measured by enzymatic immunoassay methods. Benchtop or semi-automatic enzyme linked immunosorbent assays (ELISAs) were the first diagnostic methods for AMH released by commercial manufacturers. First generation kits in widespread use in the UK were those from Diagnostic Systems Laboratories (DSL) and Immunotech (IOT), which gave quantitatively different results. Beckman Coulter then amalgamated these two methodologies into a second generation kit, the Beckman Coulter Gen II AMH ELISA, which utilises the AMH antibodies from the DSL kit and the AMH calibrators from the IOT kit. After initial problems, purportedly due to interference from complement in the sample, a pre-dilution step was introduced into the protocol resulting in more reliable results. Of the manual/semi-automatic ELISAs in clinical use in the UK the Beckman Coulter Gen II AMH ELISA is the most common. It is an enzymatically amplified two-site immunoassay using an anti-AMH capture antibody and a secondary horseradish peroxidase conjugated antibody, allowing for detection with tetramethylbenzidine (TMB) substrate, which can be measured at 450nm. The measured absorbance is directly related to the AMH concentration in the original sample. This assay has a limit of detection (LOD) of 0.57 pmol/L and a limit of quantitation (LOQ) of 1.14 pmol/L (at 20% total imprecision). Calibrators are quantified in terms of mass i.e. ng/mL but serum values are normally reported as concentrations i.e. pmol/L (1ng/mL = 7.14 pmol/L). A more sensitive ELISA kit, used mainly for research, is also available from Ansh laboratories. The LOD and LOQ values for this assay are 0.16pmol/L and

0.43 pmol/L respectively. This method utilises the same assay technology as the Gen II but uses recombinant human AMH protein for the calibrators rather than the bovine derived calibrators used in the Gen II kit. Not surprisingly, these two ELISAs give quantitatively different values.

Since 2014, some of the larger diagnostics companies have developed autoanalyser methods for the detection of AMH. These are derived from the Gen II assay but have been adapted to autoanalyser signal detection methods thus enabling a higher throughput and real time, rather than batch, analysis. The two major autoanalyser methods in use in the UK are the Beckman Access assay and the Roche Elecsys AMH assays, both of which use the same antibodies as the Beckman Coulter Gen II ELISA. The manufacturers quote the following for the Beckman Access AMH assay which takes 39 minutes to run: LOD 0.02 pmol/L and LOQ 0.036 pmol/L (at 20% total imprecision). The Roche Elecsys assay has a sample run time of 18 minutes with the following parameters: LOD 0.071 pmol/L and LOQ 0.21 pmol/L.

4.2   Reference method
A definitive reference method for the measurement of AMH has yet to be defined. Autoanalyser methods are calibrated to the Beckman Coulter Gen II AMH ELISA (which was in turn calibrated to the Immunotech calibrators).

4.3   Reference materials
There is currently no International Reference Preparation for human AMH. In 2014, a recommendation was made to produce a first WHO international reference material for AMH (WHO/BS/2014.2242). This is an ongoing project being implemented by the National Institute for Biological Standards and Control in the UK. Reference material for current autoanalyser assays is considered to be the working calibrator of the Gen II AMH ELISA assay.

4.4   Interfering substances
The degree and type of interference is method specific. Readers should refer to individual method instructions for use (IFU) for details. Interference from heterophilic antibodies in the sample is possible. Interference from haemolysis is much lower in the Elecsys AMH method compared to the Beckman Coulter Gen II ELISA method (>10 g/L v. >2 g/L respectively), while lipaemia <10 g/L and icterus <0.6 g/L do not interfere in either assay. Biotin (>30 µg/L) may interfere in the Roche Elecsys AMH assay.

4.5   Sources of error
Past studies have shown significant variation in results obtained using different assay methods, so care must be taken in considering or comparing results in the literature or from different laboratories.

5   Reference intervals and variance

5..1   Reference interval (adults)
Reference intervals are age, gender, method and laboratory dependent. For illustration the current Roche Elecsys AMH ranges given in pmol/L for

a Caucasian population are shown. Values are: median (2.5th – 97.5th percentile).

Healthy males   34.2 (5.5–103.0)
Healthy females
20–24y   28.6 (8.71–83.6)
25–29y   23.6 (6.35–70.3)
30–34y   20.0 (4.11–58.0)
35–39y   14.2 (1.05–53.5)
40–44y   6.29 (0.193–39.1)
45–50y   1.39 (0.071–19.3)
PCOS females 48.6 (13.3–135.0)

5..2   Reference intervals (others)
- AMH in assisted reproduction
The predominant use of AMH measurement is in the investigation of ovarian reserve status in programmes of assisted reproduction technology (ART). The current NICE guideline for fertility (CG156) gives advice on probable response to controlled ovarian gonadotrophin stimulation. An AMH of ≤5.4 pmol/L predicts a low response to stimulation and an AMH of ≥25.0 pmol/L predicts a high response to stimulation. These values are based on historical correlations of circulating AMH to antral follicle count (AFC) measured by trans-vaginal ultrasound (TVUS). It must be noted that the assays used to derive these AMH values are no longer in widespread use in the UK and new ranges for outcome in ART should be set up using the modern AMH assays in individual laboratories and IVF units.

- AMH in Paediatrics.
The use of AMH in paediatric medicine is of increasing interest; however, the establishment of normal ranges for neonates and young people of each gender are yet to be fully established using current assay methods.

5..3   Extent of variation
5..3.1 Interindividual CV – no data
5..3.2 Intraindividual CV
        Variability in the level of AMH in circulation has been reported to be 10–20% across the menstrual cycle (Rustamov et al, 2014), although this is reflective of both biological and analytical variability and appeared to be method dependent. A recent study found an average total intraindividual AMH variability of 20%, with the majority of this being biological (Hadlow et al, 2016).
5.3.3   Index of individuality – no data
5.3.4   CV of method – typically < 5% for the automated methods.
5.3.5   Critical difference – no data

5.4   Sources of variation
        AMH concentration varies widely with gender and age and less so with circadian rhythm and stage in the menstrual cycle.

6   Clinical uses of measurement and interpretation of results

6.1   Indications and interpretation

- Adults

The prime use of AMH analysis in adults is for the assessment of ovarian reserve in several clinical situations including fertility status prior to ART, to assess damage from exposure to gonadotoxic agents and premature ovarian failure or insufficiency. In females it can also be used to detect and monitor PCOS and granulosa cell tumours and it has been used to detect chemotherapy-induced testicular toxicity in male cancer patients.

- Paediatrics

Prepubertal and pubertal males with hypogonadotropic hypogonadism are likely to have low AMH concentrations owing to decreased FSH stimulation, although AMH will increase in response to FSH treatment. Patients with constitutional delayed puberty, or with defective androgen production or sensitivity, maintain AMH at high prepubertal concentrations. Theoretically therefore, AMH should be valuable as a tool for discrimination of hypogonadotrophic hypogonadism and congenital delay of puberty but its value remains largely unproven clinically. AMH is of value and widely advocated as a clear discriminator between cryptorchidism, anorchia, 46XY complete gonadal dysgenesis and cases of persistent Müllerian duct syndrome (PMDS). Boys with Klinefelter syndrome (47, XXY) have normal AMH concentrations until puberty. After the expected pubertal decline, AMH concentrations fall to subnormal values owing to the progressive destruction of the testes in these patients

AMH is also a marker of ovarian reserve in the pre-pubertal population and can be used to assess patients who have undergone cranial irradiation or treatment with gonadotoxic agents as part of cancer therapy. AMH has also shown potential as a marker for PCOS in adolescents, showing a raised concentration compared to controls, which correlates with ovarian volume and androgen level. AMH can be used to detect premature ovarian failure/insufficiency e.g. in Turner's Syndrome.

6.2   Confounding factors

- Adults

AMH concentrations in older females with PCOS can often return to normal as the ovarian reserve declines. In some instances, the presence of a granulosa cell tumour on a background of PCOS may be difficult to diagnose; however, AMH concentrations in granulosa cell tumour tend to be much higher than those seen in PCOS. External factors such as the oral contraceptive pill, smoking status and obesity can lead to reduced concentrations of AMH and should be considered.

- Paediatrics

The primary method of testicular evaluation in prepubertal children is to administer human chorionic gonadotrophin (hCG) and see if there is a testosterone response, detecting both the presence of functioning testicular tissue and testosterone biosynthetic defects. As such, in routine XY DSD cases a persistently low AMH may have a high predictive value for a low hCG-stimulated testosterone, but a normal AMH concentration has a low predictive value for a normal hCG-stimulated testosterone.

7   Causes of abnormal results

7.1   High values
7.1.1   Causes
In the paediatric population high values are to be expected in male neonates and infants; however a high value in a female indicates further investigation is required for either the presence of testicular tissue, or a granulosa cell tumour. In postpubertal females, high AMH values are seen in PCOS or in granulosa cell tumours.

7.1.2   Investigation
AMH can be used as an adjunct to androgen, FSH and antral follicle count (AFC) in the investigation of PCOS. AMH can be used instead of or in conjunction with inhibin B to investigate granulosa cell tumour.

7.2   Low values
7.2.1   Causes
Low AMH concentrations in males are seen in hypogonadotropic hypogonadism or can signify an absence or loss of Sertoli cell function (e.g. loss of testicular tissue in postpubertal boys with Klinefelter syndrome). Poor ovarian reserve, premature ovarian failure or insufficiency and the post-menopausal phase in women all lead to low or undetectable AMH concentrations.

7.2.2   Investigation
AMH is used as an adjunct to gonadotrophin and testosterone measurement in cases of hypogonadotropic hypogonadism, ambiguous genitalia and other DSD in infants. It is useful in the investigation of premature ovarian insufficiency (e.g. in Turner syndrome) and in the assessment of ovarian reserve both in women wishing to conceive and pre-/post- gonadotoxic cancer therapy.

8   Performance

8.1   Sensitivity, specificity etc. for individual conditions
When AMH has been used to investigate cases of bilateral anorchia and complete gonadal dysgenesis an undetectable AMH result has provided a high enough diagnostic value to avoid the need for invasive surgical exploration (Ahmed, 2016; see Guidelines).
In a meta-analysis by Iliodromiti *et al*, 2013 (see below) the sensitivity and specificity in diagnosing PCOS was 79.4% and 82.8% respectively for an AMH cut-off of >33.6 pmol/L.

9   Systematic reviews and guidelines

9.1   Systematic reviews

Paediatrics
Josso N, Rey RA Picard JY. Anti-müllerian hormone: a valuable addition to the toolbox of the paediatric endocrinologist. *Int J Endocrinol* 2013; 2013 article id 674105.

Lindhart Johansen M, Hagen CP, Johannsen TH *et al*. Anti-müllerian hormone and its clinical use in paediatrics with special emphasis on disorders of sex development. *Int J Endocrinol* 2013; 2013 article id 198698.

Assisted reproduction
Iliodromiti S, Kelsey TW, Wu O *et al*. The predictive accuracy of Anti-müllerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. *Hum Reprod Update* 2013; 20(4):560–70.

Tal R, Tal O, Seifer BJ, Seifer DB. Anti-müllerian hormone as a predictor of implantation and clinical pregnancy after assisted conception: a systematic review and meta-analysis. *Fertil Steril* 2015;103(1):119–130.

PCOS
Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013; 98(8):3332–40.

Dewailly D. Diagnostic criteria for PCOS: Is there a need for a rethink? *Best Pract Res Clin Obstet Gynaecol* 2016; Nov. 37:5-11.

Cancer therapy
Peigné M, Decanter C. Serum AMH level as a marker of acute and long term effects of chemotherapy on the ovarian follicular content: a systematic review. *Reprod Biol Endocrinol*. 2014; Volume 12: Article 26 (10 pages).

Dunlop CE, Anderson RA. Uses of anti-müllerian hormone (AMH) measurement before and after cancer treatment in women. *Maturitas* 2015; 80(3): 245–50.

9.2   Guidelines
Ahmed SF, Achermann JC, Arlt W *et al*. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015) *Clin Endoc (Oxf)* 2016; 84(5): 771–88.

NICE CG 156: Fertility problems: assessment and treatment. Published February 2013, Updated Aug 2016. London: National Institute for Health and Clinical Excellence.

NICE Guideline NG23: Menopause: diagnosis and management. Published November, 2015. London: National Institute for Health and Clinical Excellence.

9.3   Recommendations

None

10   Links

10.1   Related analytes
Inhibin B is a Sertoli cell marker and a granulosa cell marker that can assist in the detection of functioning prepubertal testicular tissue in the former and granulosa cell tumour in the latter.

10.2   Related tests
FSH, androgens, hCG stimulation test.

Additional references
Rustamov O, Smith A, Roberts SA *et al*. The measurement of anti–müllerian hormone: a critical appraisal. *J Clin Endocrinol Metab* 2014; 99–(3):723-732.

Hadlow N, Brown SJ, Habib A *et al*. Quantifying the intraindividual variation of antimüllerian hormone in the ovarian cycle. *Fertil Steril* 2016;106(5): 1230–1237.

Levi M, Hasky N, Stemmer SM *et al.* Anti-Müllerian hormone is a marker for chemotherapy-induced testicular toxicity. *Endocrinology* 2015; 156(10):3818––27.

Authors: Dr Allen Yates and Dr Helen Jopling

Date Completed: 1.2017
Date Revised: