Practical Laboratory Medicine 41 (2024) e00419

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Registered Report Stage II

Analytical performances of a novel fluorescent immunoassay of anti-Müllerian hormone and establishment of the reference intervals in Chinese children

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ARTICLE INFO

Keywords:
AMH
Fluorescent immunoassay
Analytical performance
Pediatrics
Reference interval

ABSTRACT

Background: AMH is important in child growth and the concentrations change with age and gender. This study aimed to evaluate the performance of the Pylon AMH assays and establish pediatric reference intervals.

Methods: The experiments on imprecision, sensitivity, linearity, reportable range, interference and comparison were carried out to evaluate the analytical performance. The AMH reference ranges were calculated in 238 females and 346 males aged 0–18 years using robust methods.

Results: The repeatability and the within-laboratory imprecision CVs of the assay were 3.7 % and 6.4 % at 2.25 ng/mL, and 4.6 % and 6.4 % at 15.49 ng/mL, respectively. The sensitivity (LoB = 0.05 ng/mL, LoD = 0.1 ng/mL and LoQ = 0.3 ng/mL) was verified. The linearity was 0.1–19.55 ng/mL and report up to 391 ng/mL with 20x pre-dilution. There was no significant interference from hemoglobin (500 mg/dL), triglyceride (500 mg/dL), bilirubin (10 mg/dL), cholesterol (800 mg/dL) and biotin (3000 ng/mL). The AMH measured by the Pylon assays correlated to those measured by the Elecsys assays. In males, the AMH levels were high at birth (0 d-1 m: median 95.10 ng/mL) and increased to a peak (7 m-1y: median 158.80 ng/mL) before they decreased with age (15–18 y: median 6.31 ng/mL). In females, the AMH concentrations were low at birth (0 d-1 m: median 0.20 ng/mL) and increased with age (15–18 y: median 3.03 ng/mL).

Conclusion: The Pylon AMH assays showed good analytical performance and the AMH reference intervals in chinese children determined may provide a basis in clinical diagnosis and treatment of related diseases.

1. Introduction

Anti-Müllerian Hormone (AMH) is a dimeric glycoprotein of transforming growth factor β family whose members play important roles in tissue growth and differentiation [1]. AMH is synthesized by the Sertoli cells of the testes in males, and by the granulosa cells of ovaries in females. The hormone first goes through glycosylation and dimerization to be secreted as a ~140 kDa prohormone precursor, proAMH, which consists of two identical 70 kDa subunits connected by disulfide bonds. ProAMH is activated by protease cleavage in the gonads to form a non-covalently bound complex (AMHN, C) that binds to its receptor AMHR and triggers intracellular

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https://doi.org/10.1016/j.plabm.2024.e00419
Received 21 February 2024; Received in revised form 29 June 2024; Accepted 15 July 2024
Available online 16 July 2024

signaling pathways. Both prohormone and active forms of AMH are found in the circulation [2].

In fetuses, AMH is involved in the development and differentiation of the reproductive system [3,4]. The Sertoli cells of the male fetus start to produce AMH as early as the 7th week of gestation, and AMH induces the degeneration of the Müllerian duct to inhibit the development of female sexual organs. In the female fetus, AMH is secreted only in the late gestation at very low levels. Due to the lack of AMH, the Müllerian duct develop into the fallopian tubes, the uterus, the cervical and the upper part of the vagina in the female fetus. After birth, AMH continues to regulate the development and maturation of gonads and the fertility in females. It plays an important role in the selection of dominant follicles and follicular development by inhibiting the recruitment of primordial follicles into growing follicles and reduces the sensitivity of growing follicles to follicle stimulating hormone (FSH) to prevent excessive follicle consumption [5]. Thus, AMH has a gonadal specific expression pattern, and its serum concentrations showed sexual dimorphism.

AMH is helpful for the diagnosis and/or treatment of disorders of sex development (DSD) in children, including ambiguous genitalia, anorchia, cryptorchidism, hypogonadism and hermaphroditism, as well as for the management of precocious puberty and delayed puberty. In girls, AMH can be used as an ovarian reserve marker to evaluate fertility and select protection measures for those undergoing cancer treatments. The concentrations of AMH vary greatly between boys and girls of different ages and are significantly different from those of adults. Therefore, it is important to determine the pediatric reference ranges for the clinical application of AMH assays in children. Although several studies reported pediatric reference intervals in the Caucasian [6,7], there exist substantial differences of AMH levels between ethnicities [8]. There was limited data on the age-specific reference intervals of Chinese boys and girls. Further, AMH immunoassays were lack of standardization [9], so assay-specific reference ranges are necessary for optimal clinical use. Based on the requirements of ISO15189:2003 (E) standard and clinical requirements, the laboratory must provide a reliable reference range for local testing items for clinical practice. In this study, the performances of a novel AMH assay, the Pylon AMH assays based on the cycle enhanced fluorescence immunoassays (CEFIA) were evaluated according to the CLSI guidelines, and the age-specific reference intervals were determined using the Pylon assays in Chinese children between 0 and 18 years old.

2. Materials and methods

2.1. Sample collection

Remnant serum samples from routine clinical laboratory tests were collected in Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. To determine the reference intervals in children, remnant samples of phenotypical males and females between 0 and 18 years old were included, while those from the children suspected with DSD and endocrine diseases were excluded. All samples were frozen and stored in - 80 °C before measured in batches. All procedures were performed in compliance with the institutional guidelines and approved by the institutional ethics committee (XHEC-D-2023-189).

2.2. AMH measurements

AMH in serum samples were measured by the Pylon AMH assays using the Pylon IRIS analyzer (ET Healthcare Inc., China). The Pylon AMH assays can be traced to Beckman AMH assays. The reagent kit has two AMH Calibratiors (high value and low value) and two AMH Quality Control Materials (high value and low value). Calibration is required: when 1) using new lot of kits, 2) every 28 days. Quality Control is required when using new lot of kits. The Pylon AMH assays are based on the CEFIA method. A description of the CEFIA assay procedures was available elsewhere [10]. In brief, the assay format was a unitized test strip containing wells with pre-dispensed reagents. A quartz-glass probe coated with capture antibodies on its tip moved between the wells to capture AMH in the sample which later formed an immune complex with detection antibodies. Then, fluorescent polymers bound to the immune complexes through biotin and streptavidin interaction and fluorescence was measured to calculate the AMH concentration using a calibration curve. The whole analysis process was automatically completed by the Pylon IRIS analyzer in about 10 min.

2.3. Precision study

According to the CLSI EP15-A3, the precision of the Pylon AMH assays was verified with serum samples of a high concentration and a low concentration. The samples were aliquoted and frozen at - 80 °C. The sample of each concentration was tested 5 times a day for 5 consecutive days. The repeatability CVs and within-laboratory imprecision CVs were calculated. The acceptance criteria of precision: repeatability CV $\leq$ 10 % and within-laboratory imprecision CV $\leq$ 15 %.

2.4. Sensitivity study

According to the CLSI EP 17-A2, the manufacturer-claimed limit of blank (LOB, 0.05 ng/mL) was verified by measuring two blank samples four times a day for three consecutive days. The limit of detection (LOD) was verified by measuring two AMH samples with the concentrations nearby the manufacturer's declared LoD (0.1 ng/mL) four times a day for three consecutive days. The limit of quantitation (LOQ) was verified by measuring two AMH samples of the concentration nearby the manufacturer's declared LoQ (0.3 ng/mL at 20 % CV) four times a day for three consecutive days.

2.5. Linearity study

A low-concentration pool was prepared using patients' sera with the AMH level close to LoD. A high-concentration pool was prepared by patients' sera of the AMH level close to the upper limit of detection range (24 ng/mL). According to CLSI EP6-A, the samples of six different concentrations were prepared by directly mixing the low-concentration and high-concentration sample pools in the ratios of 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5 respectively. Each sample was measured 3 times.

2.6. Reportable range

Two samples of high AMH concentrations within the detection range (24 ng/mL) were diluted by 5x, 10x and 20x respectively with serum whose AMH was below the LoD. All the samples and the dilutions were measured three times. The recovery rate (%) of each dilution was calculated by multiplying the mean concentration of the diluted sample with the corresponding dilution factor divided by the mean concentration of the undiluted sample. The predefined criterion for acceptable dilution was a recovery rate between 90 and 110 %.

2.7. Interference evaluation

Endogenous interfering substances such as hemoglobin, triglyceride, bilirubin, cholesterol and biotin were dissolved to the concentrations needed. Three serum pools of AMH were deteted after addition of interfering substances, AMH samples without interfering substances were used as controls. All the samples with interfering substances and the controls were measured in duplicates. As a default criterion, the percent recoveries calculated by dividing the measured concentrations in the samples with interferents by those of the controls should not exceed 90–110 %.

2.8. Comparison study

65 serum samples were measured by both the Pylon AMH assays and the Elecsys AMH assays. The correlation efficiency (R) between the results of the two methods was calculated by the Spearman rank correlation, the analysis and evaluation of consistency between two methods was obtained by Passing-Bablok regression analysis.

2.9. Establishment of pediatric reference intervals

Serum samples collected from 237 females and 346 males between 0 and 18 years old were measured using the Pylon AMH assays. The study cohort included 237 females and 346 males with clear apparent gender between 0 and 18 years old from Xinhua Hospital Affiliated to Shanghai Jiao Tong university School of Medicine. 583 children were divided into 8 groups. For children under 1 year old, the age grouping of this study referred to previous researches, which had found the peak time of AMH appeared at 0–1month [6], 3 months [11], 6 months [12],or 4 months-1 year old [13,14], so this study divided males into 3 groups: 0–30 d; $>$ 1month - 6 months; 7 months-1 year old, in order to observe the time when the peak concentration of AMH occurred in Chinese boys. Females under 1 years old used the same age grouping for direct comparison with males. For children over 1 year old were divided into 5 groups: 2 years old $-$4 years old; 5 years old $-$8 years old; 9 years old $-$11 years old; 12 years old $-$14 years old; 15 years old $-$18 years old. The age grouping was based on the Tanner stages of sexual development [15,16] and H. Jopling's research [7], the reasons were as followed: the prepubertal children younger than 9 years old might belonged to Tanner stage 1 and were subdivided into younger than 4 years old and 5–8 years old. the children at 9–11 years old might belonged to Tanner stage 2, puberty set on from 9 years old or 10 years old, hormonal changes might precede physical changes; the children at 12–14 years old might belonged to Tanner stage 3 or Tanner stage 4, it was also a stage of sexual maturity, when girls began to experience menarche and the formation of mammary glands, while boys experienced first nocturnal emission and voice mutation; the children at 15–18 years old might belonged to Tanner stage 5.

The exclusion criteria were as follows: (1) diseases that affect the development of gonads, such as chromosomal abnormalities, cryptorchidism, inguinal hernia, and hermaphroditism; (2) using drugs that may affect gonadal function, such as various chemotherapy drugs; (3) chronic wasting diseases such as tumors and tuberculosis; (4) endocrine diseases such as diabetes, hyperthyroidism and obesity; (5) severe liver and kidney dysfunction.

To obtain the exact concentrations, the samples whose initial results were higher than the upper limit of the Pylon AMH assays were measured again after 5-20x manual dilutions using serum with AMH levels below the LoD (the reason using serum with AMH levels below the LoD as the diluent was due to matrix effects on detection).

2.10. Statistical analysis

MedCalc (version 20.0009) and Graphpad Prism 8.0 were used to analyze the data. Kruskal-Wallis analysis was used to compare the AMH levels between age groups. The correlation between AMH levels and ages was analyzed by Spearman rank correlation. $P < 0.05$ was considered as statistically significant. For males and females in each predefined age group, the outliers were detected by the Tukey method. The robust method with the Box-Cox transformation was used to calculate the reference ranges as the 2.5th percentiles and the 97.5th percentiles together with their 90 % confidence intervals (CI). Quantile regressions of AMH levels with age were fit with cubic smoothing splines using the R package QGam with qu of 0.05, 0.5 and 0.95. 95 % CI for the robust method were derived using a

bootstrap sampling method with 1000 iterations.

3. Results

3.1. Precision study

As showen at Table 1, the Pylon AMH assay had a repeatability CV of 4.6 % and a within-laboratory imprecision CV of 6.4 % at the mean concentration of 2.25 ng/mL and a repeatability CV of 3.7 % and a within-laboratory imprecision CV of 6.4 % at the mean concentration of 15.49 ng/mL.

3.2. Sensitivity study

All the twenty-four results of blank samples were below 0.05 ng/mL and the manufacturer-claimed LoB (0.05 ng/mL) was verified. For LoD (0.1 ng/mL), all the twenty-four results of the two low concentration samples (mean concentration: 0.140 ng/mL, 0.117 ng/mL) were greater than the LoB. For LoQ (0.3 ng/mL at CV = 20 %), two samples with mean concentration of 0.295 ng/mL and 0.304 ng/mL were measured and all the results were within their expected ranges (0.236–0.355 ng/mL and 0.243–0.364 ng/mL).

3.3. Linearity study and reportable range

The mean AMH levels of the low-concentration pool and the high-concentration pool were 0.1 ng/ml and 19.55 ng/mL, respectively. The average AMH concentration of each dilution was plotted as the y-axis with the theoretical value as the x-axis (Fig. 1). The linear regression equation was calculated as $y = 1.0005x - 0.2512$, with the correlation coefficient r of 0.9993. Thus, the method was linear within 0.1–19.55 ng/mL.

Two serum samples (18.23 ng/mL and 19.53 ng/mL) were diluted by 5x, 10x and 20x with negative samples (<0.1 ng/mL). The recovery rates of the dilutions were between 90.1 % and 109.0 % (Table 2). Thus, for the samples above the upper limit of detection range (24 ng/mL), manual dilution up to 1:20 could be performed prior to measurements to obtain the exact sample concentrations. The Pylon AMH assay could report up to a concentration of 391 ng/mL.

3.4. Interference evaluation

With hemoglobin, triglyceride, bilirubin, cholesterol and biotin in serum, the recoveries were within 90%–110 % in the different levels of AMH (Table 3). The results showed hemoglobin (up to 500 mg/dL), triglyceride (up to 500 mg/dL), bilirubin (up to 10 mg/mL), cholesterol (up to 800 mg/dL) and biotin (up to 3000 ng/mL) had no interference on Pylon AMH assays.

3.5. Method comparison

The results of 65 serum samples measured by the Pylon AMH assay were compared to those by the Elecsys AMH assay. Pylon AMH and Elecsys AMH assays displayed a good correlation coefficient (with the Spearman's rho 0.980, $P < 0.0001$). Pylon AMH deviated from the linearity with the value of 1.019 slope (95%CI: 0.9494–1.0863) and had a constant bias with an intercept value of −0.135 (Fig. 2).

3.6. Pediatric reference intervals

The serum samples of 238 females and 346 males aged between 0 and 18 years old were included in the study. The median level and the reference interval (the 2.5th and 97.5th percentiles) for each predefined age group of the males and the females were shown in Table 4. The AMH levels of different age groups were significantly different in both females ($P<0.001$) and males ($P<0.001$) (Fig. 3). The quantile regression showed that the changes of AMH levels with ages in these children (Fig. 4). In the females, the AMH levels were very low at birth (0–1 month, median: 0.20 ng/mL) and rose gradually afterwards. During the puberty, female AMH levels increased to a mild peak between 9 and 11 years old (median: 3.92 ng/mL) before a small fall around 12–14 years old, and they increased again in the late adolescence in our data. In comparison, the male AMH levels showed much greater variability. The males had high AMH levels at birth (0–1 month, median: 95.10 ng/mL) and increased quickly to a peak between 7 months and one year old (median: 158.80 ng/

Table 1
The results of the precision study at two levels.

	Level 1	Level 2
Mean(ng/mL)	2.25	15.49
Repeatability CV (%)	4.6	3.7
Repeatability SD(ng/mL)	0.1	0.58
Within-laboratory imprecision CV (%)	6.4	6.4
Within-laboratory imprecision SD(ng/mL)	0.14	0.99

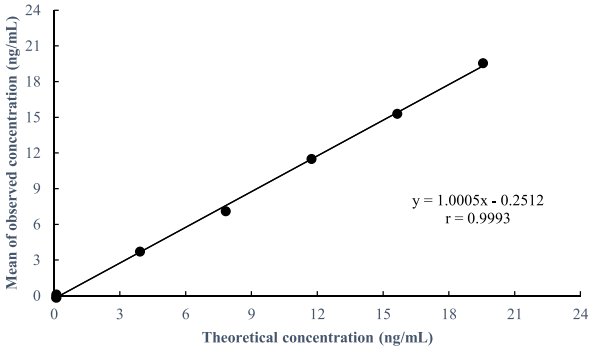

Fig. 1. Linearity study of the Pylon AMH assays.

Table 2
Reportable range of the Pylon AMH assays.

	Dilution factors	Average diluted concentrations (ng/mL)	Undiluted concentrations (ng/mL)	Recovery rate (%)
Sample 1	5x	3.61	18.23	99.1
	10x	1.85		101.6
	20x	0.99		109.0
Sample 2	5x	3.52	19.53	90.1
	10x	1.88		96.2
	20x	1.03		105.3

Table 3
Interference evaluation of the Pylon AMH assays.

Interferent	Pool 1		Pool 2		Pool 3	
	AMH, ng/mL	Recovery	AMH, ng/mL	Recovery	AMH, ng/mL	Recovery
Control	0.109	–	2.127	–	9.823	–
Hemoglobin, 500 mg/dL	0.099	99.9 %	2.14	100.6 %	9.757	99.3 %
Triglyceride, 500 mg/dL	0.096	99.9 %	2.047	96.2 %	9.993	101.7 %
Bilirubin, 10 mg/dL	0.102	99.9 %	2.06	96.9 %	9.967	101.5 %
Cholesterol, 800 mg/dL	0.102	99.9 %	2.113	99.3 %	9.783	99.6 %
Control	0.092	–	1.41	–	3.1	–
Biotin, 3000 ng/mL	0.094	100 %	1.36	96.5 %	3.13	99.1 %

The measured AMH concentrations and percentage recoveries were presented as mean.

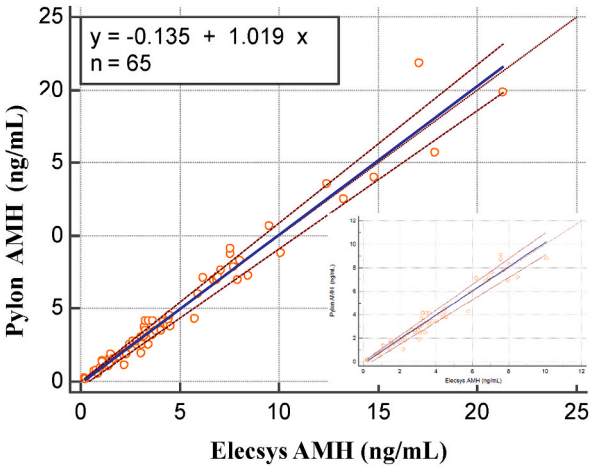

Fig. 2. Passing-Bablok regression of the results measured by the Pylon AMH assays and the Elecsys AMH assays.

Table 4
The AMH reference intervals of different gender and age groups.

Group	age	Female (ng/mL)				Male (ng/mL)			
		N	2.5th (90%CI)	Median	97.5th (90%CI)	N	2.5th (90%CI)	Median	97.5th (90%CI)
1	0–30 d	26	0 (0–0.001)[a]	0.20	7.24 (3.40–13.23)	43	25.88 (17.41–37.22)	95.10	213.23 (182.45–242.93)
2	1 m–6 m	35	0.05 (0–0.21)	1.71	7.65 (5.38–10.04)	48	46.62 (29.38–67.25)	158.68	330.89 (282.94–378.55)
3	7 m-1 y	34	0.25 (0.17–0.38)	1.13	4.31 (3.17–5.88)	34	45.83 (30.91–66.03)	158.80	387.49 (323.29–453.93)
4	2-4 y	31	0.39 (0.22–0.67)	2.06	6.59 (4.91–8.38)	43	32.51 (17.79–50.87)	124.67	234.76 (209.60–260.67)
5	5-8 y	36	0.05 (0–0.41)	2.67	7.96 (6.46–9.36)	46	16.48 (12.54–21.84)	61.33	199.57 (161.49–243.79)
6	9-11 y	27	0.14 (0–0.71)	3.92	11.79 (9.47–14.14)	45	1.40 (0.12–5.82)	51.68	163.74 (135.46–196.54)
7	12-14 y	23	0.68 (0.59–0.85)	1.52	8.23 (3.77–38.04)	52	2.52 (2.07–3.11)	9.15	58.07 (37.26–89.68)
8	15-18 y	26	0.63 (0.41–1.04)	3.03	12.16 (8.38–16.31)	35	1.52 (1.07–2.17)	6.31	33.33 (22.43–49.04)

[a] The calculated 2.5th percentile value and its CI of this group of females were lower than the LoD.

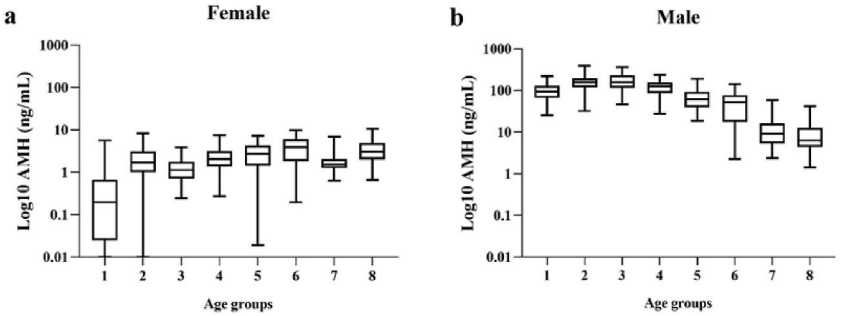

Fig. 3. AMH levels between different age groups in females (Kruskal-Wallis analysis $P<0.001$) and males (Kruskal-Wallis analysis $P<0.001$). The AMH concentrations were plotted on logarithmic scales for direct comparison between males and females. The sample size and ages for each group were indicated in Table 3.

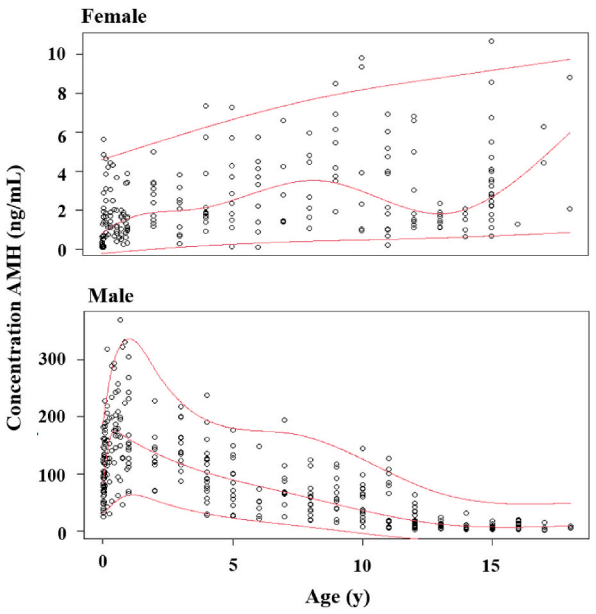

Fig. 4. Quantile regressions (5th, 50th and 95th percentiles) of AMH with age by smoothing splines in males and females.

mL) before they began to decline. A plateau of the male AMH levels was reached during the puberty and a slower decrease was shown in the late adolescence. Overall, there was a positive correlation between AMH levels and ages in females aged 0–18 years ($r = 0.424$, $P < 0.001$) while a negative correlation was observed between AMH levels and ages in males ($r = -0.735$, $P < 0.001$). The AMH range was wider in the males (minimum: 1.4 ng/mL, maximum: 409.6 ng/mL) than that in the females (minimum: 0 ng/mL, maximum: 13.4 ng/mL) under 18 years old. The AMH levels in females were much lower than those in males of the same ages, especially in the

neonates.

4. Discussions

In this study, the analytical performances of the Pylon AMH assay were evaluated, and its pediatric reference intervals were established. AMH is a biomarker of human gonadal function, which is useful in the diagnosis of DSD as well as the management of precocious or delayed puberty in children [17–20]. AMH is secreted by ovarian granulosa cells in females and the levels of AMH before adolescence are very low, while AMH in males is secreted by the Sertoli cells of testes and maintains very high during boyhoods [21]. Thus, the assays for AMH measurements in children require both high sensitivities and wide detection ranges. The imprecision study showed good analytical performance of the Pylon AMH assays with a total CV <7 %. Linearity was 0.1–19.55 ng/mL with pre-dilution by 20x, the Pylon assay allowed detection of AMH as high as 391 ng/mL. The Pylon AMH assays were not affected by the presence of hemoglobin, triglyceride, bilirubin, cholesterol and biotin which was common in clinical samples. The assay also had high sensitivities, which was achieved by the CEFIA method that utilized a novel fluorescent material, Cy5-streptavidin-polysaccharides to amplify fluorescent signals without increasing background noises [10]. The results of the Pylon AMH assays correlated with those of the Elecsys assays with similar value assignments. These demonstrated that the Pylon AMH assays are adequate for use in children. In addition, the Pylon assays are unitized tests and their test costs will not be affected by daily test volumes, different from bottled reagents which are only cost-effective for laboratories with large test volumes due to the limited shelf life after opened.

CLSI C28-A3 proposes to establish reference intervals with at least 120 samples, but the guideline also suggests that because this number of samples may be an difficult task to achieve in the paediatric population, the robust method can be used for the establishment of reference intervals where there is no specific minimum number of required observations [22]. Although this study was unable to recruit large sample sizes in clinical practice, the reference intervals were determined using robust method, which met the requirements of the guideline. The pediatric reference intervals determined using the Pylon AMH assays showed sexual dimorphic patterns. In the males, this study found high concentrations of AMH at birth and AMH levels peaked between 7 months and 1 year old, before they declined significantly with the age. This trend consisted with the findings of other studies [7,11,12,23]. The increase of AMH during infancy in males is related to mini-puberty, which is a transient period of activity surge in hypothalamic-pituitary-gonadal axis activity and an important period of sexual development [24,25]. The secretion of gonadotropin such as luteinizing hormone and FSH increases a week after birth till around 6 months in males, which stimulates the proliferation and differentiation of testicular tissues and up-regulates the expression of AMH by the Sertoli cells, and AMH production is not regulated by the negative feedback of androgen during this period. These resulted in extremely high concentrations of AMH. Differences existed in when the peak of AMH appeared in males in the first year of life among studies [6,11–14], and the peak time in this study was 7 months–1 year after birth. The variation in the peak time may result from study designs, sample sizes and individual variations. The median of AMH levels between 7 months and 1 year old in this study was very similar to below 6 months, because the median and mean of AMH levels at 6 months were the second highest (except for 10 months), which increased the median of AMH levels at 1–6 months age group, but the upper limit value of 7 months–1 year was higher. It was reasonable to speculate that the proliferation of Sertoli cells began active from 6 months, which promoted AMH secretion. Furthermore, this study was consistent with Wang's speculation that AMH levels peak at 1 year of age and then begin to sharply decline until adolescence [23]. In contrast, the change of AMH concentrations in females from 0 to 18 years old was relatively small. This study showed that the levels of AMH in female was very low at birth and then rose slowly with a mild peak around the age of 9 further increased between 15 and 18 years. A slight increase of AMH concentration at aged 9–11 years old might indicate hormonal changes at the onset of puberty, Yates et al. [6] observed similar results in females aged 8–12 years old, and the studies including adults reported highest levels of AMH around the age of 25 when women have the strongest fertility [21,26]. The sample size of the elderly female groups were less than 40 individuals and a larger sample size would be better and lower the CI for each group, but this trend consisted with the findings of the other studies [6,7,21,26].

This is the first study to establish reference intervals for AMH in Chinese boys and girls from 0 to 18 years old. Comparison of the derived pediatric AMH reference intervals in boys from other countries, Median of AMH peak value in Chinese boys tend to be significantly higher than those in boys from British [6,7,27], but which is very close to another China research [23]. Comparison of the AMH reference intervals in girls from other countries, Chinese girls tend to have higher AMH levels from 0 to 18 years old than British girls [6,7]. It is reported Chinese women tend to have significantly greater AMH levels prior to age 25 than Caucasian women [28], and this study may provide a piece of evidence. This study results can provide important guides to establish reference intervals for other regions in China.

This study still had limitations. Firstly, the derived pediatric AMH reference intervals were established based on the results of a single-center study, children's serum samples collected only came from southern China (mainly in Shanghai). In the future, the study need to collect more AMH samples from different regions in China to expand the applicability of pediatric AMH reference intervals. Secondly, serum samples of this study were only tested by the Pylon immunoassay system analyzer, further research is needed to determine whether the reference intervals can be applied to Beckman immunoassay system analyzer.

In conclusion, this study showed that the Pylon AMH assays based on the CEFIA method had good analytical performances suitable for measurements in children and the assay-specific reference intervals in Chinese children was established.

CRediT authorship contribution statement

Li Li: Writing – original draft, Investigation, Data curation, Conceptualization. **Mingyi Li:** Writing – original draft, Investigation, Data curation, Conceptualization. **Wenqian Zhu:** Investigation. **Lisong Shen:** Investigation. **Limin Jiang:** Writing – review & editing,

Supervision, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Data availability

Data will be made available on request.

Acknowledgements

Thanks to the laboratory members for their contributions to this study.

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