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Comparison of the Automated Fluorescent Immunoassay System With Roche Elecsys and Beckman Coulter Access 2 Assays for Anti-Müllerian Hormone Measurement

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Background: Since 2017, automated assays have been used in most clinical laboratories for anti-Müllerian hormone (AMH) level measurement. We evaluated the analytical performance of the newly developed automated fluorescent immunoassay system (AFIAS) AMH assay (Boditech Med, Gangwon-do, Korea) in comparison with the Roche Elecsys and Beckman Coulter Access 2 AMH assays.

Methods: Analytical performance of the AFIAS AMH assay was assessed in terms of linearity, repeatability, and within-laboratory precision (CV%) using human recombinant AMH samples according to the Clinical and Laboratory Standards Institute (CLSI) guidelines EP05 and EP06. Using 293 serum samples collected from an infertility clinic, the AMH levels were compared across AFIAS, Elecsys, and Access 2 AMH assays according to the CLSI EP09 guidelines.

Results: The AFIAS AMH assay results were linear across the measurement range of 0.420–72.386 pmol/L AMH, with repeatability of 6.341%. CV% of the AFIAS AMH assay for three levels of control, 1.786, 7.143, and 56.857 pmol/L, were 5.801%, 5.714%, and 6.228%, respectively. The results of the three AMH assays showed strong correlation: AFIAS and Elecsys [slope, 1.055 (95% confidence interval (CI), 1.022–1.088) and Spearman's rho, 0.978 (95% CI, 0.973–0.983)], Elecsys and Access 2 [slope, 0.813 (95% CI, 0.791–0.834) and Spearman's rho, 0.986 (95% CI, 0.983–0.989)], and AFIAS and Access 2 [slope, 0.836 (95% CI, 0.821–0.853) and Spearman's rho, 0.984 (95% CI, 0.980–0.988)].

Conclusions: The AFIAS AMH assay may be an alternative to the Roche Elecsys and Beckman Coulter Access 2 AMH assays.

Key Words: Anti-Müllerian hormone, Automated fluorescent immunoassay system AMH assay, Performance, Comparison

INTRODUCTION

Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein linked by disulfide bonds with a molecular weight of 140 kDa and belongs to the transforming growth factor-β (TGF-β) superfamily. AMH production by embryonic Sertoli cells induces Müllerian ducts; thus, this hormone plays a vital role in male sex differentiation [1]. In women, granulosa cells of the small antral

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and pre-antral follicles produce AMH, and the AMH expression pattern is suggested to correlate with the number of early growing follicles [2]. Therefore, AMH in women may serve as an indicator of the ovarian reserve and a predictor of the ovarian response to hyperstimulation [2-8].

Since the development and market introduction of ELISAs by Diagnostic Systems Lab (DSL, Webster, TX, USA) and Immunotech (IOT, Marseilles, France) two decades ago, several AMH immunoassays, including automated assays, have been developed [9-12]. The Elecsys AMH assay (Roche Diagnostics, Basel, Switzerland) and the Access 2 AMH assay (Beckman Coulter, Brea, CA, USA) are widely used automated assays. Owing to their large assay capacity, they are suitable for clinical laboratories [12]. However, small *in vitro* fertilization (IVF) laboratories and clinics require automated assays combined with point-of-care (POC) instruments for performing small numbers of assays.

Recently, Boditech Med (Chuncheon, Korea) developed an automated fluorescent immunoassay system (AFIAS) AMH assay, which is an automated one-step immunoassay with time-resolved fluorescence detection using an all-in-one cartridge consisting of all factory-calibrated reagents. The AFIAS AMH assay uses only 100 µL of blood and takes only 3–15 minutes to deliver the result. We evaluated the analytical performance of the AFIAS AMH assay in comparison with that of the Elecsys and Access 2 AMH assays.

MATERIALS AND METHODS

Samples

Serum samples were collected prospectively at the infertility clinic of MizMedi Hospital, Seoul, Korea, between December 6, 2018 and July 20, 2019, with approval of the Ethics Committee on Human Subjects of MizMedi Hospital (MMIRB2018-10). In total, 332 participants (age range, 19–49 years) were enrolled in this study and provided informed consent. Thirty-nine participants were excluded from the analyses for the following reasons: nine participants withdrew consent, four were not Korean, and

26 had serum AMH levels below or above the measurement range of the AFIAS AMH assay. Collected serum samples from the remaining 293 women (mean age, 34.27 ± 5.13 years) were aliquoted into two tubes for standard laboratory AMH level measurement. For assay comparison, samples were stored in the freezer (–20°C) until AMH level measurement. Sample stability was assessed by analyzing the differences in AMH levels between fresh serum samples (day 0) and frozen/thawed samples using a reference instrument (Beckman Coulter Access 2), according to the European Medicines Agency (EMA) guidelines [13].

Measurement

We followed the CLSI EP06 guidelines to measure the linearity of the AFIAS AMH assay [14]. We prepared a series of human recombinant AMH samples with known AMH levels (Boditech Med), ranging from a low (near the lower limit of quantification) to a high level (close to the upper limit of quantification). We analyzed each sample in 10 replicates and generated linear and non-linear regression equations [14].

Repeatability and within-laboratory precision of the AFIAS AMH

Table 1. Summary of linear and non-linear regression analyses of the AFIAS AMH assay results

Model	Y-intercept	X	X^2	X^3	Standard error
Linear					
Coefficient	−0.004	1.013	NA	NA	0.425
P	0.971	<0.001	NA	NA	
Polynomial 2nd order					
Coefficient	−0.062	1.055	−0.004	NA	0.427
P	0.629	<0.001	0.461	NA	
Polynomial 3rd order					
Coefficient	−0.044	1.022	0.005	−0.001	0.431
P	0.752	<0.001	0.879	0.779	

Abbreviations: AFIAS, automated fluorescent immunoassay system; AMH, anti-Müllerian hormone; NA, not available.

Table 2. Repeatability and within-laboratory precision of the AFIAS AMH assay

Control AMH level	Control target (pmol/L)	Range of control (pmol/L)	Measured mean (pmol/L)	Repeatability		Within-laboratory precision	
				SD (pmol/L)	CV (%)	SD (pmol/L)	CV (%)
Low	1.786	1.434–2.151	1.793	0.101	5.642	0.104	5.801
Medium	7.143	5.704–8.556	7.130	0.403	5.659	0.407	5.714
High	56.857	45.513–68.269	56.891	3.190	5.607	3.543	6.228

Abbreviations: AFIAS, automated fluorescent immunoassay system; AMH, anti-Müllerian hormone; SD, standard deviation; CV, coefficient variation.

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assay were calculated using three control human recombinant AMH samples with known AMH levels according to a modified protocol presented in the CLSI EP05 guidelines: 20 days, two runs daily, and six replicates per run [15].

Statistical analysis

The results obtained using the AFIAS, Elecsys, and Access 2 AMH assays were compared using Passing–Bablok regression analysis and Bland–Altman methods, according to the CLSI EP09 guidelines [16]. Statistical analyses for Passing–Bablok regression, Bland–Altman plot, and Spearman's correlation were conducted using MedCalc software (version 19.2.0, MedCalc Software bvba, Ostend, Belgium) and R software (R Core Team 2014, Vienna, Austria). A P value <0.05 was considered statistically significant.

RESULTS

Linear results were obtained across the measurement range of 0.420–72.386 pmol/L, with repeatability of 6.341% for all samples (Table 1). The repeatability and within-laboratory precision of the AFIAS AMH assay were 5.607%–5.659% and 5.714%–6.228%, respectively, which were within the ranges reported by the manufacturer (Table 2).

Age-specific AMH levels in the 10th, 50th, and 90th percen-
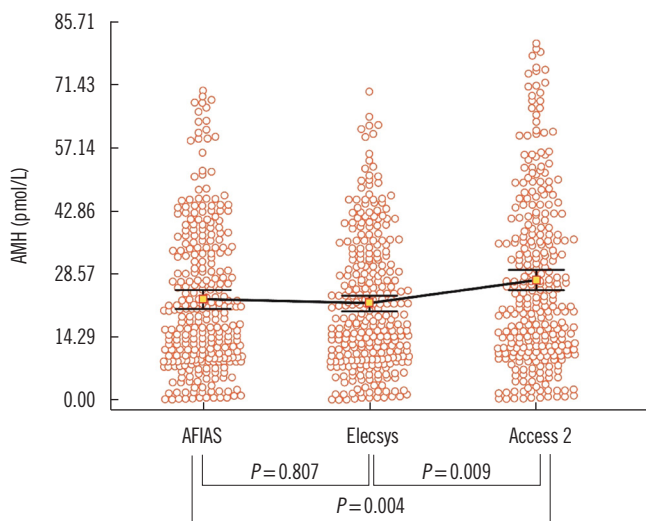
Fig. 1. Distribution of AMH levels measured using the AFIAS, Elecsys, and Access 2 AMH assays. Squares and bars represent the median of measured data and 95% CI.
Abbreviations: AMH, anti-Müllerian hormone; AFIAS automated fluorescent immunoassay system; CI, confidence interval.

Table 3. Nomogram of AMH levels (pmol/L) by age

Age (yr)	Sample N	AFIAS AMH			Elecsys AMH			Access 2 AMH		
		10th percentile 95% CI	50th percentile 95% CI	90th percentile 95% CI	10th percentile 95% CI	50th percentile 95% CI	90th percentile 95% CI	10th percentile 95% CI	50th percentile 95% CI	90th percentile 95% CI
18–29	44	9.072 / 2.786-15.715	33.143 / 22.929-37.001	59.215 / 43.858-67.644	10.357 / 3.001-14.643	29.929 / 23.929-35.286	45.643 / 44.501-63.358	9.857 / 2.858-16.643	36.857 / 30.929-42.215	61.786 / 52.572-80.287
30–34	112	9.429 / 7.715-12.001	26.429 / 21.286-29.429	46.286 / 43.644-60.929	8.929 / 7.001-12.215	24.572 / 22.001-27.144	44.929 / 41.286-53.144	10.857 / 9.286-13.929	30.572 / 26.572-35.786	60.715 / 54.501-69.787
35–39	98	4.143 / 1.715-6.429	14.214 / 11.429-16.501	39.286 / 29.286-46.644	4.357 / 1.643-6.358	14.072 / 12.072-16.858	39.072 / 29.929-48.215	4.500 / 2.001-7.358	17.072 / 13.786-21.144	46.429 / 36.715-63.287
≥40	39	0.714 / 0.215-1.858	8.786 / 5.001-10.001	31.929 / 15.643-34.429	0.714 / 0.215-1.643	9.714 / 4.715-11.143	29.786 / 16.072-41.429	0.929 / 0.358-2.072	10.072 / 5.572-12.929	35.929 / 19.143-45.358

Abbreviations: AMH, anti-Müllerian hormone; AFIAS, automated fluorescent immunoassay system; CI, confidence interval.

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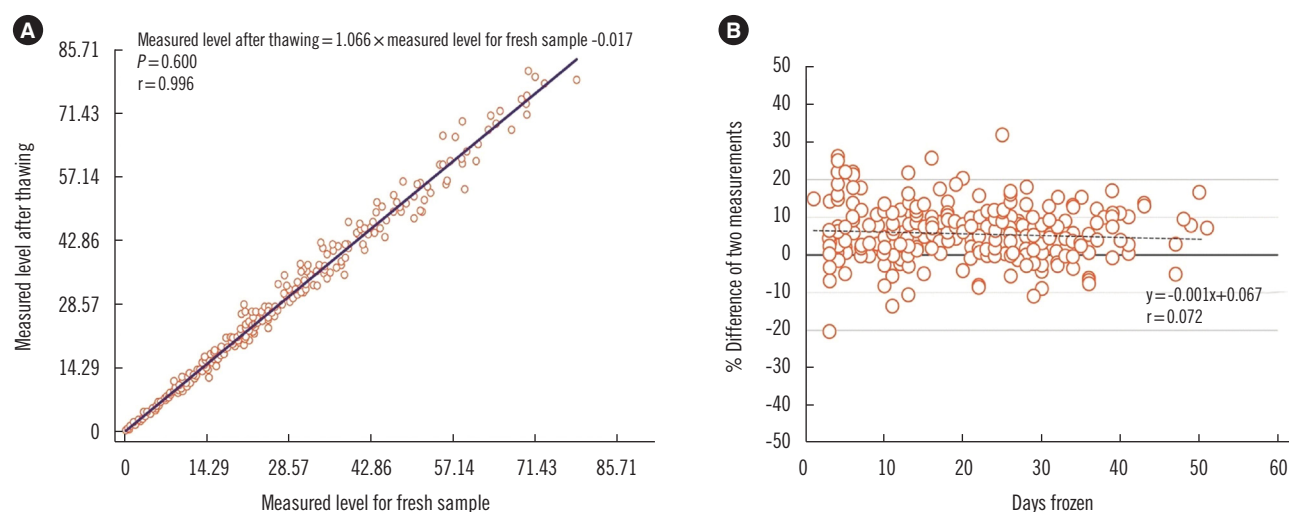
Fig. 2. Correlation between two measurements of fresh and frozen-thawed samples. (A) Scatter plots with regression line of two measurements. (B) Percent difference between two measurements. Thick and thin solid lines represent 0%, ±10%, and ±20% differences, respectively. Dotted line represents the regression line of the mean difference between the two measurements.

tiles, with 95% confidence intervals (CIs) calculated from the measurements obtained with each assay are shown in Table 3. The AMH levels obtained using the AFIAS and Elecsys AMH assays were similar ($P=0.807$), whereas the Access 2 AMH assay yielded higher values ($P=0.009$) (Fig. 1).

We redetermined the AMH level in each sample using the Access 2 AMH assay to ensure sample stability under storage at –20°C. The measured levels for the thawed samples tended to be slightly higher (measured level after thawing = 1.066 × measured level for fresh sample - 0.017, Spearman's correlation coefficient r=0.996), but the differences were acceptable (96% of the samples showed a difference under 20%, while 76% samples showed a difference under 10%) (Fig. 2).

Strong correlations with no significant differences were found among the results of the three AMH assays (Fig. 3): The slope and Spearman's rho were 1.055 (95% CI, 1.022–1.088) and 0.978 (95% CI, 0.973–0.983) between AFIAS and Elecsys, 0.813 (95% CI, 0.791–0.834) and 0.986 (95% CI, 0.983–0.989) between Elecsys and Access 2, and 0.836 (95% CI, 0.821–0.853) and 0.984 (95% CI, 0.980–0.988) between AFIAS and Access 2, respectively. The Bland–Altman plot showed no significant systemic error across the AMH level measurement range among assays.

DISCUSSION

We found competent repeatability, within-laboratory precision, and adequate linearity of the recently developed AFIAS AMH assay with comparable measurement values to those of the Elecsys and Access 2 AMH automated assays.

AMH level measurement is a popular approach to assess the ovarian reserve and identify extreme responses before ovarian stimulation in IVF treatment, because the AMH level highly correlates with the antral follicle count [17, 18]. The usual starting point of ovarian stimulation for IVF is menstrual cycle day (MCD) 2 or 3. The starting dose of gonadotropin in ovarian stimulation is adjusted according to the ovarian reserve, and hormonal levels are checked in the early follicular phase (MCD 2–3). Determination of the levels of AMH and other hormones on the starting day of ovarian stimulation would greatly assist timely adjustment of gonadotropin dose. The impact of the proverbial biological clock, the so-called "ovarian age," on egg quality and quantity is also a concern in relatively older (>40 years) patients in IVF clinics, who do not want to delay ovarian stimulation. Hence, reducing the laboratory turn-around time of assays for AMH and other hormones is preferable.

In small- to medium-sized IVF clinics, blood samples are usually sent out to reference laboratories. Outsourcing of hormonal assays not only takes more time but may also affect the quality of the laboratory results. AMH level measurement with a short turn-around time would significantly contribute to patient satisfaction. To our knowledge, the AFIAS AMH assay is the only POC automated AMH assay currently available, offering an affordable on-site laboratory assay for small- to medium-sized IVF clinics with reduced laboratory assay capacity.

This study is the first to compare three automated AMH as-
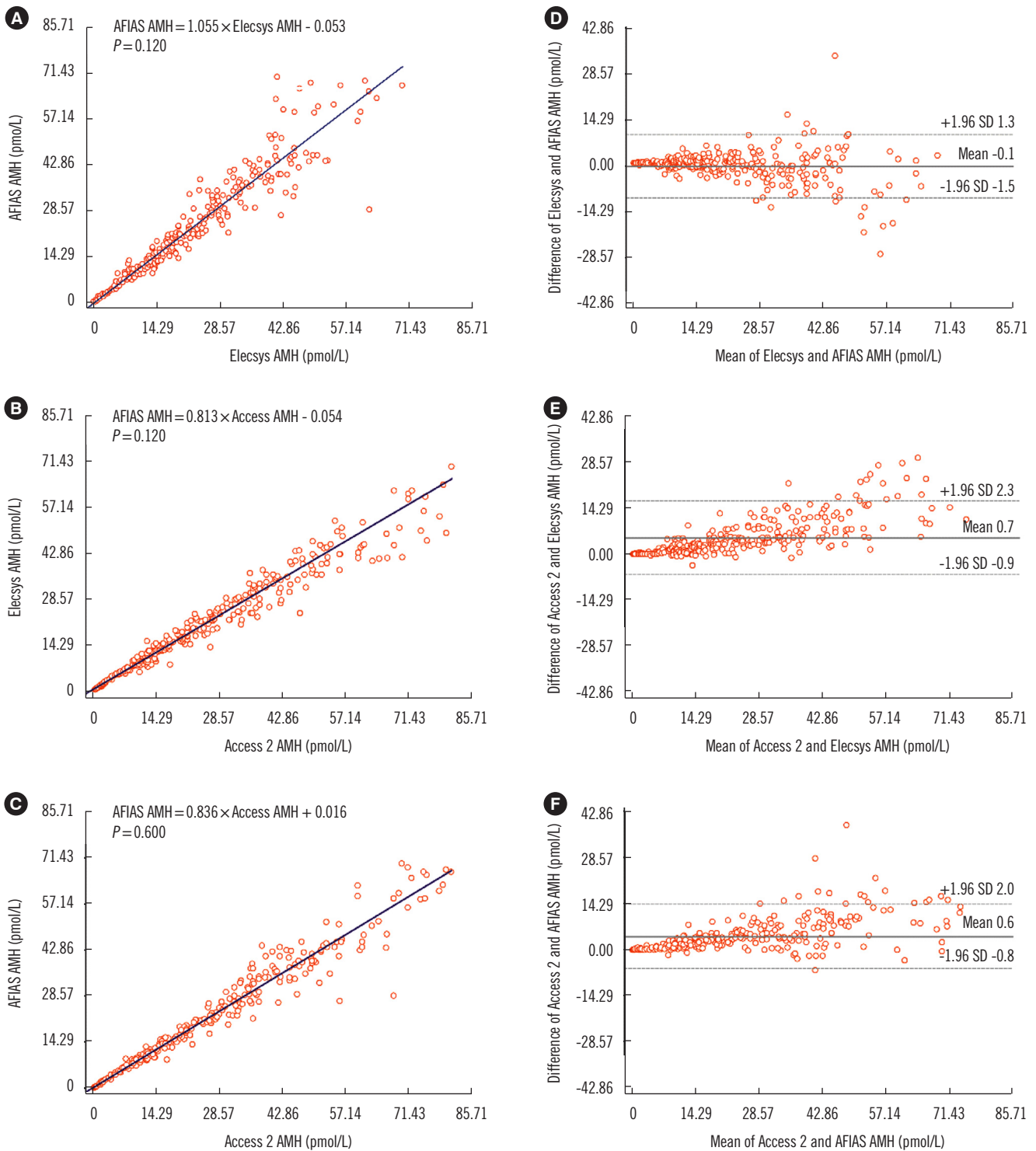
Fig. 3. Correlation analyses of the three AMH assays. (A), (B), and (C) show Passing–Bablok regression plots of the three AMH assays. Solid lines represent the regression of two measurements. (D), (E), and (F) show Bland–Altman plots of the appropriate comparison between the AMH assays.

Abbreviations: AMH, anti-Müllerian hormone; AFIAS, automated fluorescent immunoassay system.

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says (AFIAS, Elecsys, and Access 2) using serum samples from patients. As the AFIAS AMH assay results showed strong correlations with the results of the other two assays (Fig. 3), it could serve as a convenient alternative. The distributions of AMH levels measured using the AFIAS and Elecsys AMH assays were similar, whereas the Access 2 AMH assay showed a slightly wider distribution (Fig. 1). Similar distribution patterns among assays were found for age-specific AMH levels (Table 3). This indicates that assay-specific AMH levels need to be carefully interpreted. Since AMH level measurement has not yet been standardized [17, 18], it is preferable to use the same assay when testing AMH level serially for the same patient.

We only considered age and AMH level for each participant and did not analyze correlations of AMH levels with clinical profiles. Therefore, we cannot offer any practical suggestion regarding the most suitable AMH level for infertility treatment. Further, serum samples were obtained only from participants who had registered for infertility treatment. Therefore, our data do not indicate age-specific AMH levels in the general population of Korean women.

Despite these limitations, we showed the competent repeatability and within-laboratory precision and adequate linearity of AFIAS AMH assay, and its performance was satisfactory compared with the widely used Elecsys Access 2 AMH assays. Therefore, the AFIAS AMH assay can be an alternative to existing immunoassays.

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AUTHOR CONTRIBUTIONS

Han A designed the study, collected data, and wrote and revised the manuscript. Suh B supervised the laboratory work and proofread the manuscript. Lee G designed the study and collected the samples. Lee Y and Kim S collected samples. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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