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Performance Characteristics of the UniCel DxI 800 Immunoassay for the Maternal Serum Quadruple Test, Including Median Values for Each Week of Gestation, in Korean Women

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Background : Maternal serum prenatal quadruple screening includes testing for alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE3), and dimeric inhibin A (DIA). We evaluated quadruple screening using an automated platform and looked for any ethnic differences in the median values of each marker.

Methods : We measured the concentrations of each quadruple test analyte using the UniCel DxI 800 system (Beckman Coulter, USA) in 788 Korean mid-trimester maternal serum samples and calculated their median values using Benetech software (Benetech, Canada). We also compared the results with those obtained using the Immulite 2000 assay (Siemens Healthcare Diagnostics, USA) or ELISA (DSL, USA) in 442 samples.

Results : We obtained mid-trimester median values for each marker. The following are the comparative results for each test using the Immulite 2000 assay or ELISA (x) and the UniCel DxI 800 immunoassay (y): AFP, y=1.10x+0.01, r=0.925; uE3, y=0.28x+0.24, r=0.885; hCG, y=1.22x-3047.8, r=0.944; and DIA, y=0.86x+15.31, r=0.833. Assay results for each of the four markers showed good correlations. However, significant biases necessitated new median calculations of prenatal risk estimates in all four tests.

Conclusions : We established gestational age-specific second-trimester median values for four markers in Korean samples using the UniCel DxI 800 immunoassay system. Despite significant bias, there were good correlations between the results obtained using the UniCel DxI 800 immunoassay and those obtained using the Immulite 2000 assay. *(Korean J Lab Med 2010;30:126-32)*

Key Words : *Prenatal screening, Down syndrome, Immunoassay, Quadruple test*

INTRODUCTION

Maternal serum prenatal quadruple screening includes tests for alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE3), and dimeric

inhibin A (DIA). Measurements of maternal serum AFP, uE3, and hCG, as well as maternal age, are widely used in the second trimester for the identification of pregnancies at high risk of Down syndrome [1, 2]. The maternal serum mid-trimester triple marker test is also a widely accepted protocol in Korea [3-5]. The triple test (AFP, uE3, hCG) is known to have a 71.3% sensitivity at a 5% false-positive rate. However, when the gestational age is established by ultrasound, the quadruple test (AFP, uE3, hCG, DIA) has been demonstrated to have a better sensitivity of 77.5% at a 5% false-positive rate [6-8]. Many platforms are com-

mercially available for triple tests; however, DIA currently has only two platforms—either ELISA (Diagnostic Systems Laboratories, Webster, TX, USA) or the Beckman Coulter UniCel DxI 800 system or Access 2 (Beckman Coulter, Fullerton, CA, USA).

To date, DIA has been assayed using an ELISA method; however, this method is time consuming and requires considerable labor, thereby resulting in a long turnaround time. However, Beckman Coulter has launched a DIA product that is used in conjunction with an automated chemiluminescence analyzer, and Lambert-Messerlian et al. [9] recently published median values for DIA using the Beckman Coulter Access immunoassay system in 570 women of European descent. We established the gestational age-specific second-trimester median values for each marker of the quadruple test in Korean second-trimester pregnant women using the Beckman Coulter UniCel DxI 800 immunoassay system in order to make it applicable for use in the Korean population. In addition, we compared the results of the test for each marker using the UniCel DxI 800 system with those obtained using either the Immulite 2000 assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA) or ELISA.

MATERIALS AND METHODS

We recruited 788 pregnant Korean women at 15 to 20 weeks gestation who visited the clinics of Yonsei University Health System, or the clinics that refer the quadruple test to Seoul Clinical Laboratories for prenatal checkups. We stored serum samples from each patient in a deep freezer at −70℃ after completion of the requested quadruple marker tests using the Immulite 2000 assay or ELISA, and subsequently used these samples for the measurement of beta-hCG, AFP, uE3, and DIA using the UniCel DxI 800 system.

Test imprecision was evaluated by following NCCLS EP 5-A guidelines and using commercially available quality control materials (Lyphocheck Maternal Serum Control Levels 1, 2, and 3, Bio-Rad, Irvine, CA, USA) for 5 days.

The median values for each of the four markers were calculated using Benetech PRA (Benetech Clinical Software

Solutions, Toronto, Canada), using serum samples from women with singleton pregnancies. The quadruple marker serum screening was conducted at Gangnam Severance Hospital (formerly Yongdong Severance Hospital, renamed in March 2009), Yonsei University (Seoul, Korea), and Seoul Clinical Laboratories, an independent medical laboratory. We used the completed week for gestational age estimation. Exclusion criteria for the subjects were as follows: 1) age less than 18 yr, 2) those achieving pregnancy by assisted reproductive technologies, 3) gestational age estimated only by non-ultrasound methods (e.g., LMP), and 4) ethnic origin other than Korean. This study was approved by the institutional review board of the Gangnam Severance Hospital, where a triple marker test was initiated in 1995 and a quadruple marker test has been available since 2007. The clinical laboratory of Gangnam Severance hospital covers three hospitals (Severance Hospital, Gangnam Severance Hospital, and Yongin Severance Hospital) of the Yonsei University Health System.

Quadruple tests (AFP, uE3, hCG, DIA) were conducted using the UniCel DxI 800 system (Beckman Coulter), and the AFP, uE3, and hCG values were compared with the values determined using the Immulite 2000 assay. In addition, DIA results were compared with those obtained by ELISA using 442 stored samples.

Passing-Bablok linear regressions, Spearman correlations,

Table 1. Precision of the Beckman Coulter DxI for the quadruple assay

| Sample	Level	Mean	CV (%)	
			Within-run	Total
AFP (ng/mL)	Low	9.94	3.36	4.45
	Middle	30.14	4.25	4.14
	High	76.21	3.84	3.84
uE3 (ng/mL)	Low	0.46	5.85	5.77
	Middle	1.45	3.02	2.79
	High	4.61	4.05	4.50
Beta-hCG (IU/mL)	Low	7,955	2.30	2.12
	Middle	24,300	5.39	5.07
	High	51,426	3.73	4.81
DIA (pg/mL)	Low	150.4	3.91	3.88
	Middle	390.4	5.23	5.83
	High	802.9	5.27	5.36

Abbreviations: AFP, alpha-fetoprotein; uE3, unconjugated estriol; hCG, human chorionic gonadotrophin; DIA, dimeric inhibin A.

and Bland−Altman plots were generated using Analyse−It software version 1.71 (Leeds, UK).

RESULTS

Total coefficients of variation (CV) were estimated according to CLSI Guideline EP5−A2 [10] and the results ranged from 2.1% to 5.8% for low, from 2.8% to 5.8% for middle, and from 3.8% to 5.4% for high variation (Table 1).

Method comparisons were performed using Passing−Bablok regression and Bland−Altman plots. Passing−Bablok regression produced variable slopes ranging from 0.28 to 1.22, and Spearman correlation coefficients ranged from 0.833 to 0.944 (Fig. 1). The Bland−Altman plot demonstrates with average bias for each test with the 95% confidence interval in parentheses were as follows: AFP, 10.7% (−13.7% to 35.2%); uE3, −91.1% (−125.1% to −57.0%); hCG, 10.8% (−19.6% to 41.2%); DIA, −8.0% (−53.7% to 37.8%) (Fig. 2).
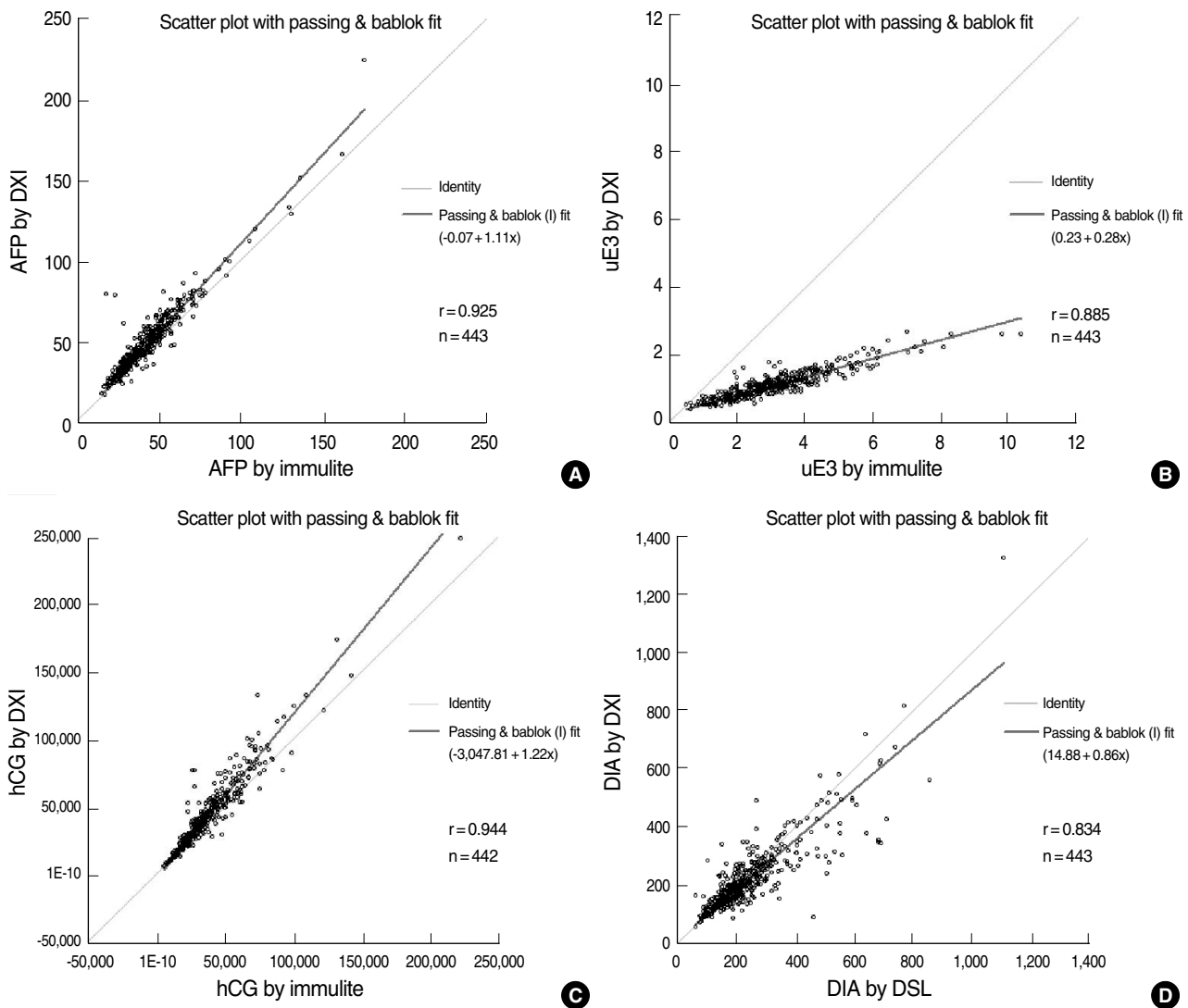
Fig. 1. Comparison study of each of the four tests of the quadruple marker by DxI vs Immulite or DSL ELISA. (A) AFP, (B) uE3, (C) hCG, (D) DIA.
Abbreviations: DXI, UniCel DxI 800 (Beckman Coulter, Fullerton, CA, USA); Immulite, Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, IL, USA); DSL ELISA (DSL, Webster, TX, USA); AFP, alpha-fetoprotein; uE3, unconjugated estriol; hCG, human chorionic gonadotrophin; DIA, dimeric inhibin A (DIA).
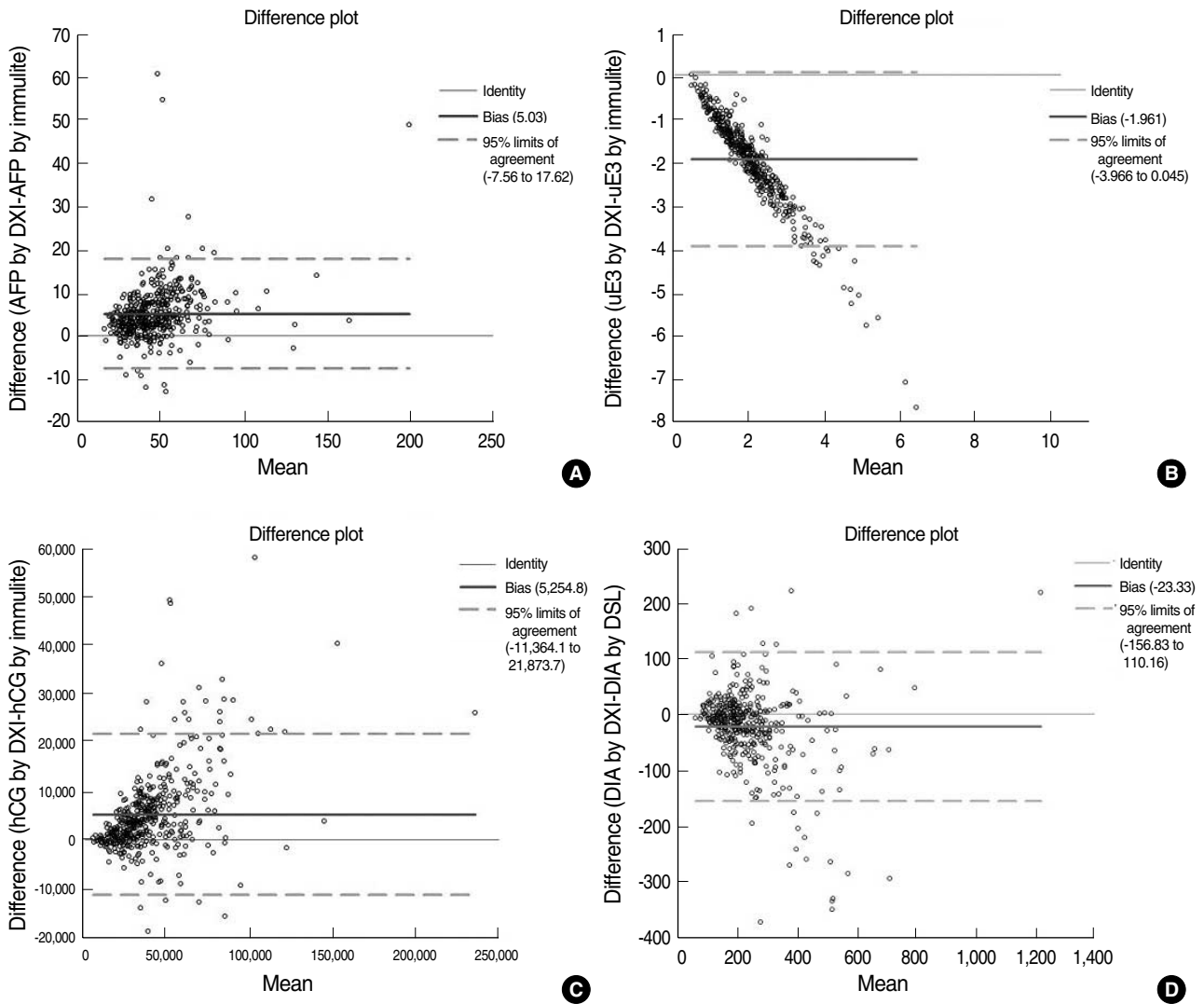
Fig. 2. Bland-Altman plots for each of the four markers in the quadruple test using Unicel DxI 800 vs Immulite 2000 or DSL ELISA. (A) AFP, (B) uE3, (C) hCG, (D) DIA.
Abbreviations: See Fig. 1.

The median values for each of the four markers at 15–20 weeks of gestations are presented in Table 2.

DISCUSSION

All quadruple test assays conducted using the UniCel DxI 800 immunoassay system yielded results comparable with those obtained using the Immulite 2000 assay or ELISA, but with significant bias, thus necessitating the calculation of median values for prenatal risk estimation. The number of 19th week subjects was slightly less than the recommended number of 100 and there were only 17 subjects in the 20th week. However, the number of subjects in the 15th to the 18th gestational weeks was sufficient for regression analysis of the results for each marker. The median values of DIA in these Korean samples appeared to be higher than those in Western samples [9] (Table 3).

Rawlins et al. [11] reported that DIA Access and ELISA Passing–Bablok analysis yielded a slope of 0.88 (95% confidence interval [CI], 0.85 to 0.91), an intercept of −3.67 (95% CI, −16.26 to 6.09), an r value of 0.980, and a Bland–Altman analysis mean percentage difference of −14.6%. We

Table 2. Median values of each of the four quadruple markers determined using the Beckman Coulter DxI

Gestational week*	N. of samples	AFP (ng/mL)		uE3 (ng/mL)		hCG (IU/mL)		DIA (pg/mL)	
		Obs	Reg[†]	Obs	Reg	Obs	Reg	Obs	Reg
15th	130	40.9	39.7	0.8	0.9	46.2	46.7	222.4	222.3
16th	219	44.1	45.4	1.1	1.1	37.4	36.3	188.0	190.0
17th	160	53.4	52.0	1.4	1.4	28.7	30.2	185.3	178.1
18th	165	58.8	59.6	1.7	1.7	25.9	26.6	176.8	183.0
19th	97	67.0	68.2	2.0	2.1	26.6	24.6	209.2	206.3
20th	17	89.1	78.1	2.8	2.7	21.1	23.4	258.9	254.9
Total	788								

*Completed gestational week as determined by ultrasound; [†]Regression equations used were derived using Benetech software as follows: Median AFP=10$^{(0.05874×wk+0.69234)}$; Median uE3=10$^{(0.09983×wk-1.61100)}$; Median hCG=21.66705+54.07473×e$^{-0.53946×(wk-14)}$; and Median DIA=10$^{(0.02002×wk^2-0.70594×wk+8.47311)}$.
Abbreviations: AFP, alpha-fetoprotein; uE3, unconjugated estriol; hCG, human chorionic gonadotrophin; DIA, dimeric inhibin A; Obs, observed median values; Reg, regressed median values.

Table 3. Comparison of the median values (pg/mL) of dimeric inhibin A

Gestational week*	Lambert-Messerlian et al.[†] [9]			Rawlins et al.[†] [11]		Present study			
	N	Observed	Regressed	N	Observed	N	Observed	Regressed[‡]	Maternal weight-corrected values[§]
15th	82	157.3	147.0	2,422	166	130	222.4	222.3	225.9
16th	126	126.0	138.6	3,897	149	219	188.0	190.0	195.4
17th	106	134.3	135.0	2,851	150	160	185.3	178.1	187.2
18th	103	142.1	136.5	1,079	151	165	176.8	183.0	178.8
19th	90	147.4	143.0	526	161	97	209.2	206.3	206.2
20th	58	144.4	155.4	279	174	17	258.9	254.9	277.7
Total	565			11,054		788			

Completed gestational week as determined by ultrasound; [†]Beckman Coulter Access; [‡]Log-linear regression; [§]Median MoM=10 to the power (slope weight+intercept), slope=-0.00175, intercept=0.22616.

Table 4. Comparison of dimeric inhibin A (DIA) between automated assay and ELISA

Study	N	Slope	Intercept	r	Mean % difference	Mean ratio of the two assays
Lambert-Messerlian et al.* [9]	565	1.00	-0.776	0.960	NE	0.768
Rawlins et al.* [11]	119	0.88	-3.67	0.980	-14.6%	NE
Present study	447	0.86	14.88	0.834	-8.0%	0.949

*Beckman Coulter Access.
Abbreviation: NE, not evaluated.

confirmed similar biases and correlations. Lambert-Messerlian et al. [9] reported a slope of 1.001 (95% CI, 0.998 to 1.004) and an intercept of −0.776 (95% CI, −3.112 to 1.560) (Table 4). The UniCel DxI 800 system incorporates an analyzer that is essentially the same as that used in the Access 2 system manufactured by the same company, except that the former is like the integration of four units of the lat-

ter. Although we do not know the exact reasons why our results differ slightly from the previous studies [9, 11], we suspect that a difference in instrument (UniCel DxI 800 instead of Access) or random error, particularly in ELISA, could be the reasons for the difference. Beckman Coulter claim that there have been no calibrator changes in recent years.

DIA precision (within-run CV, 3.9–5.3%; total CV, 3.9–5.4%) in our study was similar to that of Rawlins et al. [11] (within-run CV, 3.2–3.6%; total CV, 4.1–4.2%) for similar levels (Table 1). The precision seemed to be better than that reported by Erickson et al. [12] (6.4–8.9%) using the ELISA method.

We compared triple marker data with the data from samples collected in other countries in a study that used the same Beckman Coulter analyzers—Access 2 or UniCel DxI 800 [13] (Table 5–7). The medians of AFP and hCG levels

Table 5. Regressed median values of alpha-fetoprotein (AFP) compared with those of samples from other countries

Gestational week*	AFP (IU/mL)						
	Vranken et al. [13]					Present study	
	Belgium	Canada	Germany	UK	USA	Korea[†]	Weight-corrected[‡]
14th	22.1	-	21.8	23.6	-	-	-
15th	25.8	27.5	25.3	27.0	25.7	32.8	31.9
16th	30.2	31.1	29.2	30.8	29.7	37.5	38.2
17th	35.3	35.1	32.5	35.2	34.4	43.0	43.8
18th	41.2	39.7	37.5	40.2	39.7	49.2	49.8
19th	48.2	44.9	46.3	46.0	46.0	56.3	55.8
20th	-	50.6	-	52.5	53.2	64.5	78.0

*Completed gestational week as determined by ultrasound; [†]Median values of this study were converted to IU/mL from ng/mL by multiplying by 0.826 for the comparison; [‡]Median values corrected for maternal weight using log-linear regression, Median MoM=10 to the power (slope*weight+intercept), slope=-0.00236, intercept=0.29407.

Table 6. Regressed median values of uE3 compared with those of samples from other countries

Gestational week*	uE3 (nmol/L)					
	Vranken et al. [13]				Present study[†]	
	Belgium	Canada	Germany	UK	Korea	Weight-corrected[‡]
14th	2.15	-	2.22	2.26	-	-
15th	2.80	2.53	3.02	2.84	3.12	2.93
16th	3.66	3.19	3.92	3.58	3.82	3.81
17th	4.77	3.99	5.00	4.51	4.86	4.87
18th	6.22	4.96	6.21	5.67	5.90	5.92
19th	8.11	6.21	7.95	7.14	7.29	6.99
20th	-	7.77	-	8.99	9.37	10.00

*Completed gestational week determined by ultrasound; [†]Median values of this study were converted to nmol/L from ng/mL by multiplying by 3.47 for the comparison; [‡]Median values corrected for maternal weight using log-linear regression, Median MoM=10 to the power (slope*weight+intercept), slope=-0.00061, intercept=0.09007.

Table 7. Regressed median values of human chorionic gonadotrophin (hCG) compared with those of samples from other countries

Gestational week*	hCG (IU/mL)					
	Vranken et al. [13]				Present study	
	Belgium	Canada	Germany	UK	Korea	Weight-corrected[†]
14th	37.3	-	34.9	40.0	-	-
15th	30.2	42.4	26.5	33.2	46.7	43.9
16th	24.5	34.1	21.4	27.6	36.3	35.6
17th	19.9	28.3	18.9	22.9	30.2	26.6
18th	16.1	24.1	17.3	19.1	26.6	23.4
19th	13.0	21.2	14.8	15.9	24.6	23.2
20th	-	19.1	-	13.2	23.4	19.6

*Completed gestational week determined by ultrasound; [†]Median values corrected for maternal weight using log-linear regression, Median MoM=10 to the power (slope*weight+intercept), slope=-0.00258, intercept=0.29094.

in Koreans appear to be somewhat higher, although the uE3 levels appear to be similar. Crandall et al. [14] noted somewhat higher AFP concentrations in Asian samples. Bogart et al. [15] noted possibly higher hCG concentrations in Asian samples. uE3 concentrations have been reported to be similar in samples of European and African descent, but have not been reported for Asians [16]. Our study showed similar median values for uE3. Benn et al. [17] postulated that the differences observed in the various studies may be attributable to the inconsistent use of maternal weight correction, heterogeneity in the gestational ages of the screened populations, maternal age differences, and regional differences in the subpopulations of women included in each group.

In addition, Benn et al. [18] and Serdar et al. [19] reported that analytical variations have a great impact on second trimester risk estimation procedures and some articles have reported borderline cases as being problematic [18, 20]. A survey report of external quality control samples for 9 clinical laboratories in Korea showed variable results for the borderline cases that necessitated standardization [21].

An automated platform for quadruple screening has several advantages over manual ELISA, namely, better precision (3.9–5.8% vs 6.1–7.8%), faster turnaround time (1 hr vs 5 hr), and random access function vs batch mode in terms of DIA.

In conclusion, quadruple test assays conducted using the UniCel DxI 800 system yielded results comparable to those obtained using the Immulite 2000 assay, but with significant bias, thus necessitating the calculation of median values. We established median values for each week of gestation (15 to 20 weeks) for each marker in Korean women.

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