ORIGINAL ARTICLE

Medians for second-trimester maternal serum markers: geographical differences and variation caused by median multiples-of-median equations

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Objectives: To establish gestational age-specific mid-trimester normal medians for the prenatal serum markers α fetoprotein (AFP), human chorionic gonadotropin (HCG) and unconjugated oestriol (uE3) for a Belgian population by using the Beckman Coulter Access chemiluminiscent immunoassays; to compare these data with data obtained from other geographical regions; to propose regression coefficients for regressed medians and analyse variation induced by different regression equations; to evaluate the effect of formulas used for gestation correction on estimating risk in Down's syndrome.

Design: Data derived from 862 fresh serum samples from women being screened for Down's syndrome pregnancy, composed of selected pregnancies deemed to be normal, were examined in a retrospective study. Regressed medians were calculated by using a first-degree logarithmic-linear fit of the raw data. Multiples-of-median (MoM) values estimated by using a simple logarithmic-linear equation were compared with those calculated with higher-degree polynomials chosen with a goodness-of-fit analysis. Model-specific variation was estimated and the effect on risk for Down's syndrome was evaluated.

Results: Regressed medians (Y) for Access serum markers AFP (IU/ml), HCG (IU/ml) and uE3 (nmol/l) for a Belgian population can be estimated with the equation $Y = 10^{(A+BX)}$ with X = decimal weeks. The best fit was obtained with a third-degree and a second-degree polynomial for AFP and uE3, respectively. Differences between the medians and among the slopes of the geographical populations were found to be significant (analysis of covariance, $p < 0.001$).

Conclusions: Belgian marker medians versus gestational time are found to show a pattern that is similar to that in the literature. The log-linear equation is observed to give a good fit and can be suggested as a tool for calculating median MoM values for Belgian laboratories that use Access biochemical prenatal markers.

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Risk estimation in second-trimester prenatal screening for chromosomal anomalies, such as Down's syndrome (trisomy 21) or Edwards's syndrome (trisomy 18), is often based on the triple test.¹ With this test, three maternal serum markers—namely, α fetoprotein (AFP), human chorionic gonadotropin (HCG) and unconjugated oestriol (uE3)—are measured and combined with maternal age to estimate risks at term.² Second-trimester screening is mostly carried out between gestation weeks 14 and 20. In that period, medians of AFP and uE3 rise, with lower values being observed in trisomy 21.³ Medians of HCG decrease between weeks 14 and 20, and higher values are observed in trisomy 21.³ As the levels of the three marker values change with gestational age, the results of these three markers are expressed as concentrations of multiples of median (MoM).⁴ These MoM values are compared with parametric population statistics⁵ and combined with a prior risk based on maternal age to calculate the likelihood of trisomy 21.⁶

Multivariate mathematics used in risk calculation is very complex and final outcomes depend on variables used in each intermediate step of the algorithm. The quality of the results obtained in such likelihood calculation depends therefore, among others, on the following variables: analytical performance of the immunoassays used to determine the biochemical markers,^{7–8} knowledge of the exact dating of pregnancy,⁹ medians used to calculate the MoM must be representative for the population studied, reliability of the regression coefficients describing the relationship between biochemical markers and gestation, and choice of the correct population parameters.¹⁰ Furthermore, outcomes vary considerably between the software packages available.¹¹

In this study, we wish to establish representative medians for the population studied for the Beckman Coulter Access (Beckman Coulter Inc, Fullerton, California, USA) chemiluminiscent immunoassays for AFP, HCG and uE3. Further, we will compare our medians with data from other regions obtained with the same immunoassays. Regressed medians will be calculated and the robustness of the regression coefficients proposed to estimate the medians will be studied. Variation in MoM values estimated with first-degree and higher-degree equations will be analysed and its effect on risk estimates will be discussed.

MATERIAL AND METHODS

A study was carried out at the following six Belgian laboratories: Centraal laboratorium Hasselt; Labo MCT; Centre Hospitalier Notre Dame—Reine Fabiola, Département Hormonologie—Site Fabiola; Centraal laboratorium St-Vincentius ziekenhuis Deinze; Laboratory Van Poucke; and C H Jolimont—Lobbes Site Jolimont Haine-Saint-Paul.

Data derived from 862 serum samples were examined in a retrospective study. The samples were from women being screened for Down's syndrome pregnancy, composed out of selected pregnancies. These pregnancies were deemed to be normal based on triple test results and genetic analysis of amniotic cells in doubtful cases. As is standard practice in Belgium, gestational age was estimated by ultrasound or by

Abbreviations: AFP, α fetoprotein; ANOVA, analysis of variance; HCG, human chorionic gonadotropin; uE3, unconjugated oestriol; MoM, multiples of median; VIF, variance inflation factor

the first day of the last menstrual period, if dates of scanning were unavailable. Fresh maternal serum from gestation weeks 14–20 was used to measure levels of mid-trimester AFP, HCG and uE3 with the Beckman Coulter Access reagents for AFP, HCG and uE3 (catalogue number 33210, 33500 and 33570 with their corresponding calibrators) on the Beckman Coulter Immunochemistry Analyzers, Access base, Access2 and DxI.

Second-trimester values of the three serum markers were measured and regressed medians were calculated for each completed gestational week with a log-linear least-squares regression by using the ungrouped data. Data for gestational week 20 were not included because the number of data points was insufficient to establish representative statistics. Belgian medians for gestation weeks 14–19 were compared with those from other geographical regions with analysis of covariance.

The regressed medians were calculated using a log-linear relationship of the first degree. The markers were transformed to $\log_{10}$ data and the weeks were on an arithmetic scale. To check whether this simple regression gives a good fit and produces robust estimates of the regression coefficients, a complete polynomial analysis up to the fourth degree was carried out for the three prenatal biochemical marker values. For each parameter a summary and an analysis of variance (ANOVA) table were constructed wherein significance tests were carried out for the incremental improvement given by each increase in the degree of the polynomial being fitted. For each degree partial regression coefficients, their standard errors, *t* values and variance inflation factors (VIFs) were calculated. VIF values gave an indication on the reliability of the regression coefficients estimated. VIF values higher than 100 indicated instability.^{12, 13}

Medians calculated with the polynomial regression giving the best fit were compared with those estimated with the simple (first-degree) log-linear relationship.

Data were analysed with MedCalc for Windows V.7.4.1.2; BIOMstat V.3.4 (Exeter Software) for Windows, a statistical software for biologists; Microsoft Excel 2002, a data analysis tool add-in; and RiskCalc V.1.3a, a Down's risk calculation package.

RESULTS

Medians

Measured AFP concentrations ranged from 7.54 to 180 IU/ml with a geometric mean of 28.6 IU/ml (*n* = 862). Measured HCG concentrations ranged between 2.7 and 118.2 IU/ml with a geometric mean of 26.4 IU/ml. Assayed uE3 concentrations ranged from 0.43 to 22.6 nmol/l with a geometric mean of 3.33 nmol/l. Geometric means of the three serum markers obtained in the participating laboratories were homogeneous (ANOVA; *p* > 0.05) in gestation weeks 14, 16, 17, 18 and 19 (HCG and AFP). In week 15, differences between the laboratory geometric means were significant (ANOVA; *p* ≤ 0.002; *df* = 3, *n* = 369). This can be explained, because in week 15 mean gestational age of the women in the participating laboratories was different (*p* < 0.001). Individual laboratory data for week 19 were too few to interpret the uE3 results.

The median MoM equations were established by least-squares regression of the logarithmic-transformed marker values against gestational age (in decimal weeks). AFP and HCG results were expressed in IU/ml and uE3 in nmol/l. The following equation was used for the three serum markers to calculate median serum marker values for a particular decimal gestation time:

$$\text{Median serum marker value} = 10^{(A+B \times \text{gestation age})}$$

where *A* and *B* are intercept and slope, and gestation age is in decimal weeks. The regression coefficients (SE) for AFP are *A* = 0.36800 (0.07197) and *B* = 0.06767 (0.00446); for HCG,

A = 2.88970 (0.09845) and *B* = −0.09133 (0.00611); and for uE3, *A* = −1.33070 (0.06154) and *B* = 0.11528 (0.00382). The three regressions are highly significant (*p* < 0.001; *n* = 862), with coefficients of determination *R*² = 0.2109 for AFP, *R*² = 0.2065 for HCG and *R*² = 0.5148 for uE3.

Comparison of Belgian medians with those in the literature

Table 1 compares regressed medians for the Belgian population with those from other geographical areas. The Belgian medians showed a similar trend for the three markers as those from the other regions. By using logarithmic (base 10)-transformed medians of all regions, individual regional slopes were calculated for each of the three markers. Individual regional slopes for AFP varied between *B* = 0.05305 for Canada and *B* = 0.06767 for Belgium; for HCG between *B* = −0.0691 for Canada and *B* = −0.09133 for Belgium; and for uE3 between *B* = 0.09711 for Canada and *B* = 0.11528 for Belgium. Differences between the median values of the different regions and individual regional slopes were compared using analysis of covariance. Table 2 shows results of this analysis. From these analyses of covariance, it can be concluded that for each of the three markers, major differences were observed between the adjusted means of the medians (see table 2 for formula) and differences among slopes for the different geographical populations studied. Only in AFP, differences among adjusted means of the medians from the UK and Belgium were not significant (statistics not shown).

Results of the polynomial regression analysis

HCG

For HCG, the simple log-linear relationship was the only one giving a significant *F* test, meaning that the other degrees produced a non-significant improvement in the fitting (table 3). As the first-degree regression was sufficient for HCG, no further analysis was carried out for this parameter and the coefficients of the first-degree equation were used to calculate the medians.

AFP

AFP values were $\log_{10}$ transformed. For AFP, the polynomial ANOVA (data not shown) indicated that a third-degree polynomial gave a better fit (*p* = 0.0092) than the simple linear regression. The VIF values for the different terms of the third-degree equation were high (higher than 100 000), which indicated very unstable estimates. The third-degree equation gave a significant fit of the data (*F*s = 79.4; *df* = 3, 858; *R*² = 0.21723; *p* < 0.001) as follows:

$$\log_{10} \text{AFP} = 21.5335097 - 3.7732995X + 0.2311950X^2 - 0.0046147X^3$$

where *X* = decimal weeks.

Table 4 compares median AFP values obtained with the first-degree and the third-degree polynomial equations. From this comparison, the two estimates were clearly very similar. Only the value with an asterisk fell outside the 95% CI range. So the first-degree equation gave a reasonably good fit of the data and was chosen for its simplicity.

uE3

From a polynomial regression and ANOVA on the logarithmic-transformed data (data not shown), only the second-degree polynomial explained a significantly greater variation of the sample (*F*s = 6.2091; *p* = 0.0129; *df* = 1, 857) when compared with the simple linear regression. The VIF values of the calculated second-degree coefficients were 462.446 for coefficients 1 and 2. The second-degree equation is

$$\log_{10} (\text{uE3}) = -3.0171824 + 0.3188427X - 0.0061006X^2$$

where *R*² = 0.51829; *F*s = 462.11; *df* = 2, 859.

Table 1 Regressed median values for the Beckman Coulter Access reagents compared with median values from other subpopulations

AFP (IU/ml)							
Gestational age (weeks/days)	n	Belgium*	95% CI	Canada	Germany	UK	USA
14/3	93	22.1	21.2 to 23.1	–	21.8	23.6	–
15/3	373	25.8	25.1 to 26.6	27.5	25.3	27.0	25.7
16/3	207	30.2	29.4 to 31.0	31.1	29.2	30.8	29.7
17/3	94	35.3	33.9 to 36.7	35.1	32.5	35.2	34.4
18/3	61	41.2	39.0 to 43.6	39.7	37.5	40.2	39.7
19/3	32	48.2	44.6 to 52.0	44.9	46.3	46.0	46.0
20/3	–	–	–	50.6	–	52.5	53.2

HCG (IU/ml)						
Gestational age (weeks/days)	n	Belgium	95% CI	Canada	Germany	UK
14/3	93	37.3	35.1 to 39.7	–	34.9	40.0
15/3	373	30.2	29.0 to 31.5	42.4	26.5	33.2
16/3	207	24.5	23.6 to 25.5	34.1	21.4	27.6
17/3	94	19.9	18.8 to 21.0	28.3	18.9	22.9
18/3	61	16.1	14.9 to 17.4	24.1	17.3	19.1
19/3	32	13.0	11.7 to 14.5	21.2	14.8	15.9
20/3	–	–	–	19.1	–	13.2

uE3 (nmol/l)						
Gestational age (weeks/days)	n	Belgium	95% CI	Canada	Germany	UK
14/3	93	2.15	2.06 to 2.24	–	2.22	2.26
15/3	373	2.8	2.73 to 2.89	2.53	3.02	2.84
16/3	207	3.66	3.56 to 3.75	3.19	3.92	3.58
17/3	94	4.77	4.59 to 4.95	3.99	5.00	4.51
18/3	61	6.22	5.89 to 6.57	4.96	6.21	5.67
19/3	32	8.11	7.54 to 8.72	6.21	7.95	7.14
20/3	–	–	–	7.77	–	8.99

AFP, α fetoprotein; HCG, human chorionic gonadotropin; uE3 unconjugated oestriol.

AFP results expressed in ng/ml are multiplied by 0.826 to convert to IU/ml. uE3 results expressed in ng/ml are multiplied by 3.47 to convert to nmol/l.

*Measurements were carried out with the Beckman Coulter Access reagents on the Accessbase, Access2 and DxI analyzers.

Table 2 Summary table for analysis of covariance

Serum marker	Differences among adjusted mean of the medians	Differences among slopes	Common slope
α Fetoprotein	0.0069 (4, 25)	8.844×10^{-7} (4, 21)	0.06057
Human chorionic gonadotropin	6.032×10^{-11} (3, 20)	0.0041 (3, 17)	–0.07812
Unconjugated oestriol	1.019×10^{-8} (3, 20)	2.733×10^{-7} (3, 17)	0.10464

p Values are shown, testing heterogeneity among prenatal markers obtained for different geographical populations. Adjusted (adj) means of the medians are calculated with the common regression slope with the formula $\text{median}_{i(\text{adj})} = \text{median}_i - \text{common slope} (GA_i - GA)$, where GA_i is mean gestational age for region i and GA is the grand median of all regions.

Individual slopes are calculated for each prenatal marker and each region. Differences among slopes test whether the differences among separate regional slopes are significant.

The populations studied are from Belgium, the UK, Germany, Canada and the USA (only α fetoprotein). Degrees of freedom are shown in parentheses. Common slopes shown have no parametric value, as the differences between the individual regional slopes are highly different.

Table 3 Summary of analysis of variance table of a polynomial regression analysis for human chorionic gonadotropin

Source	df	SS	MS	Fs	p Value
Explained	4	11.15350	2.78838	55.7534	0.120×10^{-42}
Degree 1	1	11.15178	11.15178	222.9792	0.492×10^{-45}
Degree 2	1	0.00098	0.00098	0.0197	0.8885
Degree 3	1	0.00002	0.00002	0.0004	0.9844
Degree 4	1	0.000072	0.00072	0.0144	0.9044
Unexplained	857	42.86084	0.05001		

df, degrees of freedom; Fs, sample statistic of the F distribution; MS, mean square; SS, sums of squares. Degree 1, simple log-linear relationship; degree 2, second-degree polynomial; degree 3, third-degree polynomial; degree 4, fourth-degree polynomial.

Table 4 Regressed medians obtained with a simple log-linear relationship and with a log-higher-degree-polynomial compared, for α fetoprotein and unconjugated oestriol

Week	Regressed median log-linear	L1: lower 95%CI limit	L2: upper 95%CI limit	Regressed median log-higher-degree linear equation
α Fetoprotein (IU/ml)				
14	22.10	21.15	23.10	22.89
15	25.83	25.07	26.61	25.28
16	30.18	29.36	31.03	30.27
17	35.28	33.92	36.69	36.88*
18	41.23	38.95	43.64	42.88
19	48.18	44.64	52.00	44.66
Unconjugated oestriol (nmol/l)				
14	2.15	2.06	2.24	2.06
15	2.8	2.73	2.89	2.82
16	3.66	3.56	3.75	3.75
17	4.77	4.59	4.95	4.86
18	6.22	5.89	6.57	6.12
19	8.11	7.54	8.72	7.50*

Table 4 compares uE3 medians obtained with the first-degree and the second-degree polynomial equations. Except for the value with an asterisk, the two estimates were very similar and not different from each other at the 5% level. So the first-degree equation gave a reasonably good fit of the data and was chosen for its simplicity.

DISCUSSION

Simple log-linear equations of the first degree proposed in this study can be used to estimate gestational age-specific Access medians for the three Access serum markers of the triple test. A comparison of the Belgian regressed medians calculated in this study showed that the AFP medians compared very well with those from other countries¹⁴⁻¹⁶ and overall (SD) differences were -0.9% (4.2%), 0.5% (5.3%), 2.7% (1.7%) and 5.0% (3.4%) higher than those of the UK, Canada, the USA and Germany, respectively. Overall differences between our data and those of other countries¹⁴⁻¹⁶ varied between -31.7% (4.4%) and 3.5% (10.9%) for HCG and between -3.3% (3.7%) and 20.2% (8%) for uE3. Typical imprecisions (coefficient of variance in %) for Beckman Coulter's Access chemiluminiscent AFP, HCG and uE3 are $<7\%$, $<5\%$ and $<8\%$,⁷ respectively (data from MAS Real Time QC, for AFP and HCG). Hence for uE3, regional differences can be explained by analytical imprecision, but for HCG this is not the case. Similar differences between geographical medians, as high as 25%, have been reported for gestational age-specific medians for dimeric inhibin-A.¹⁷ For AFP and HCG, noticeable differences between races have been reported.¹⁸⁻¹⁹ For uE3, the situation is more complex. Differences between black and white women are not notable, whereas differences between white women and Hispanic and South-Asian women are marked.¹⁸⁻¹⁹ Despite the regional

differences, our data show a trend similar to that of published data (table 1).

For AFP and uE3, a third-degree polynomial and a second-degree polynomial equation gave a better fit of the data. Table 1 shows regressed medians obtained with the log-linear approach and the best fit equations were relatively similar. MoM values were determined by dividing biochemical marker concentrations by the gestational age-specific median value. Hence variation in MoM values was determined by the method of estimating regressed medians. Figure 1 shows variation between MoM values calculated with the simple first-degree log-linear equation and with the best-fit polynomial. Clearly, from this simulation, maximum differences between the two approaches occurred in gestational week 19. Maximum difference for uE3 was -10.8% and increased to -16.0% for AFP and a gestational age of 19 weeks+6 days. To estimate the effect of the variation induced by the median MoM equation on risk for Down's syndrome, the following exercise was carried out. For three simulated patients aged 35 years, risks were derived for each day of gestational week 19 and for gestational dates (weeks+days): 14+3, 15+3, 16+3, 17+3 and 18+3. Cuckle's⁵ population parameters but no correction factors were used. Risks for three different simulated patients A, B and C were estimated with the likelihood ratio method.²⁰ Serum marker values of the three patients are as in table 5 and were chosen to generate a negative risk (R), a positive risk and an intermediate risk sample with regard to the consensus risk cut-off value of 1 in 250.²¹ Variation in week 19, calculated as $(\text{largest R/smallest R}-1) \times 100\%$, ranged between 15% and 113% for the intermediate sample, between 13% and 114% for the positive sample and between 4% and 58% for the negative sample. Furthermore, the simple log-linear MoM

Figure 1 Variation (%) between serum marker multiples of median (MoM) values estimated with different methods for mid-trimester gestation. α Fetoprotein MoM values are calculated with a simple log-linear (II) equation and with a third-degree polynomial (best fit). Unconjugated oestrial MoM values are calculated with a simple log-linear equation and with a second-degree polynomial (best fit). Variation is estimated as $(\text{MoM (II)}/\text{MoM (best fit)} - 1) \times 100\%$.

Table 5 Down's syndrome risk estimated for three simulated patients (A–C) by using biochemical marker values

Serum marker levels	Patient		
	A	B	C
α Fetoprotein (IU/ml)	45	33.8	45
Human chorionic gonadotropin (IU/ml)	20	30.4	14
Unconjugated oestrial (nmol/l)	4.34	5.21	7.24

approach gave four positive results (from 19 weeks+3 days onwards) for sample A, whereas the best polynomial fit MoM equation gave only two positive results. This degree of variation did not occur in gestational weeks 15, 16 and 17 and 18 (fig 1, table 6) in which maximum variation was acceptable and less than 10%. Our study mainly investigated the effects of gestational age on the MoM values. Today most software packages available use both gestation and weight to

Take-home messages

- Mid-trimester normal medians for the Access AFP, HCG and uE3 biochemical prenatal markers for a Belgian population were established in a retrospective study.
- The marker medians versus gestational time show a pattern that is similar with that in the literature and the log-linear equation is proposed as a tool for calculating median MoM values for Belgian laboratories.
- Variation in Down's syndrome risk estimates, especially in gestational weeks 14 and 19, depends, among other factors, on the approach used for converting analytical results in MoM values.

correct the serum markers. Unfortunately, weight correction formulas for the Access parameters are at present not available in the literature. In a future study we will focus on the effect of weight as a variable in prenatal screening. Nevertheless, this simple simulation underlines the fact that risk calculation is very complex and that methods used to calculate intermediate results in the risk calculation algorithm need to be defined very strictly and synchronised between laboratories using the same methods and instrumentation to keep between-laboratory variations in risk outcome to acceptable limits. Our data prove that imprecision may depend, among other factors, on the approach used for converting analytical results into MoM values. Previously, it was shown that choices of the settings describing the parametric population models have a similar effect on risk outcomes.¹⁰

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REFERENCES

- 1 Canick JA. Prenatal screening for Down Syndrome. Current testing and future choices. *Clin Lab News* 2004 (Aug):12–5.
- 2 Palomaki GE, Haddow JE. Maternal serum alpha-fetoprotein, age, and Down syndrome risk. *Am J Obstet Gynecol* 1987;156:460–3.
- 3 Graves JC, Miller KE, Sellers AD. Maternal serum triple analyte screening in pregnancy. *Am Fam Physician* 2002;65:915–20.

Table 6 First-degree log-linear approach (method 1) and the best polynomial fit (method 2) for calculating the median multiples-of-median (MoM) results

Risk estimates (risk = 1/R)	Patient A, MoM calculus		Patient B, MoM calculus		Patient C, MoM calculus	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Gestational age (weeks+days)						
14+3	3577	3160	1356	1169	761	627
15+3	4508	4610	1685	1736	1854	1895
16+3	3802	3807	1402	1415	3019	3181
17+3	2145	1977	780	715	3244	3205
18+3	810	799	290	275	2400	2278
19+0	388	445	138	156	1674	1746
19+1	316	389	112	136	1498	1632
19+2	255	341	90	119	1330	1528
19+3	205	301	72	105	1172	1433
19+4	162	267	57	93	1023	1348
19+5	128	238	45	83	887	1272
19+6	100	213	35	75	762	1207

Patients aged 35 years at expected date of delivery.
 Population parameters used are from Cuckle⁵.

- 4 **Wald NJ**, Cuckle H, Brock JH, *et al.* Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet* 1977;**1**:1323–32.
- 5 **Cuckle H**. Improved parameters for risk estimation in Down's syndrome screening. *Prenat Diagn* 1995;**15**:1057–65.
- 6 **Reynolds TM**, Penney MD. The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy. *Ann Clin Biochem* 1990;**27**:452–8.
- 7 **MacRae AR**, Gardner HA, Allen LC, *et al.* Outcome validation of the Beckman Coulter access analyzer in a second-trimester Down syndrome serum screening application. *Clin Chem* 2003;**49**:69–76.
- 8 **Wald NJ**, Hackshaw AK, George LM. Assay precision of serum alpha fetoprotein in antenatal screening for neural tube defects and Down's syndrome. *J Med Screen* 2000;**7**:74–7.
- 9 **Bishop J**, Dunstan FD, Nix BJ, *et al.* The effects of gestation dating on the calculation of patient specific risks in Down's syndrome screening. *Ann Clin Biochem* 1995;**32**:464–77.
- 10 **Reynolds T**, Ellis A, Jones R. Down's syndrome risk estimates demonstrate considerable heterogeneity despite homogeneity of input. *Ann Clin Biochem* 2004;**41**:464–8.
- 11 **Muller F**, Aegerter P, Ngo S, *et al.* Software for prenatal down syndrome risk calculation: a comparative study of six software packages. *Clin Chem* 1999;**45**:1278–80.
- 12 **Sokal RR**, Rohlf FJ. Biometry: the principles and practice of statistics in biological research. 3rd edn. New York: W H Freeman, 1995:887.
- 13 **Rohlf FJ**, Slice DE. Biomstat for Windows. Statistical software for biologists. Version 3.3. User guide. New York: Applied Biostatistics, 1999:56.
- 14 **Mayer A**, Stibbe W. Evaluation des unkonjugierten Östriols im Rahmen der Triple-Diagnostik am Access®-Immunoassay-System. In: *Vitro.Das Beckman-Coulter-Magazin*. Beckman Coulter. 2000;**5**:37–9.
- 15 **Beckman Coulter**. Prenatal screening. *Bulletin* 3009, Beckman Coulter, 2000:1–2.
- 16 **Beckman Coulter**. AFP ONTD Physician's brochure. Information on alpha-fetoprotein testing. Bulletin 171305A. Beckman Coulter, 1999:1–4.
- 17 **Erickson JA**, Ashwood ER, Gin CA. Evaluation of a dimeric inhibin-A assay for assessing fetal Down syndrome: establishment, comparison, and monitoring of median concentrations for normal pregnancies. *Arch Pathol Lab Med* 2004;**128**:415–20.
- 18 **Wald NJ**, Kennard A, Hackshaw A, *et al.* Antenatal screening for Down's syndrome. Review. *Health Technol Assess* 1998;**2**:i–iv, 1–112.
- 19 **Benn PA**, Clive JM, Collins R. Medians for second-trimester maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol; differences between races or ethnic groups. *Clin Chem* 1997;**43**:333–7.
- 20 **Palomaki GE**, Haddow JE. Maternal serum alpha-fetoprotein, age, and Down syndrome risk. *Am J Obstet Gynecol* 1987;**156**:460–3.
- 21 **NHS**. Antenatal Screening-Working Standards. National Down's Syndrome Screening programme for England. Compiled March 2004. <http://www.screening.nhs.uk/downs/working-standards.pdf>.