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hCG AND THE FREE β-SUBUNIT AS SCREENING TESTS FOR DOWN SYNDROME

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SUMMARY

Published studies have reached varying conclusions as to the benefit of replacing human chorionic gonadotropin (hCG) measurements with the free β-subunit of hCG (the free β-subunit) for Down syndrome screening. One study reports 14 per cent higher detection for the free β-subunit, while another finds an actual loss in detection. To explore this issue further, we directly compared the screening performance of hCG and the free β-subunit, alone and in combination with other serum markers, using banked sera obtained prior to amniocentesis and karyotyping. Altogether, 52 Down syndrome and 5065 unaffected pregnancies were studied. Sera were thawed and assayed for hCG and the free β-subunit over 1 year. At a 5 per cent false-positive rate, the detection rate for hCG in combination with maternal age and alpha-fetoprotein was higher than when the free β-subunit was substituted (62 versus 57 per cent). Ultrasound dating and adding unconjugated oestriol both increased detection. The present findings, along with those from six case–control studies (our re-analysis), indicate that the screening performances of hCG and the free β-subunit are similar (median change in detection 0, range -8 to $+3$ per cent). Under optimal sample collection and transportation conditions, laboratories can expect to achieve similar screening performance using either hCG or the free β-subunit measurements.

KEY WORDS: Down syndrome; prenatal screening; human chorionic gonadotropin; free β-subunit

INTRODUCTION

Approximately two-thirds of the 4 million annual pregnancies in the United States are screened for Down syndrome in the second trimester (Palomaki *et al.*, 1997). Most of these pregnancies are screened using a combination of maternal age and alpha-fetoprotein (AFP), unconjugated oestriol (uE3), and human chorionic gonadotropin (hCG) measurements. This combination detects an estimated 60 per cent of Down syndrome pregnancies with an initial positive rate of about 5 per cent (Wald *et al.*, 1988; Haddow *et al.*, 1992). In 1990, it was reported that substituting the free β-subunit of hCG (the free β-subunit) for traditional hCG measurements could increase Down syndrome detection by 20 per cent (Macri *et al.*, 1990). In that study, however, hCG was not measured and a direct comparison could not be made. When direct comparisons were made in subsequent studies, varying conclusions were reached about the free β-subunit, ranging from a loss of 1 per cent in Down syndrome detection to a gain of 14 per cent (at comparable false-positive rates) (Spencer, 1991; Ryall *et al.*, 1992; Spencer *et al.*, 1992; Wald *et al.*, 1994; Nørgaard-Pedersen *et al.*, 1994; Aitken *et al.*, 1996). These case–control studies differed in their methods of identifying Down syndrome cases and controls, methods of gestational dating, and modelling techniques. These differences might explain some of the observed heterogeneity. The present study used serum specimens and clinical information from a

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cohort of women having amniocentesis for reasons other than positive serum screening results. Data from this study population allow an unbiased comparison to be made of the relative performance of hCG and the free *β*-subunit univariately and in combination with other serum markers.

MATERIALS AND METHODS

Study population

Study subjects were drawn from the original 5385 pregnant women enrolled in a collaborative study at 14 prenatal diagnostic centres in the United States between 1990 and 1992 (Haddow *et al.*, 1994). That study was sponsored by the Maternal and Child Health Bureau, Department of Health and Human Services (MCJ-237014-02-2), and the National Institute of Child Health and Human Development. Informed consent was obtained prior to amniocentesis, along with a blood sample and selected demographic and pregnancy-related information (race/ethnicity, gestational dating by both the first day of the last menstrual period (LMP) and ultrasound measurements, maternal weight, smoking status, number of fetuses, and other relevant data). The present study was restricted to pregnancies between 14 and 21 completed weeks' gestation, based on LMP dates. Karyotypes were obtained for all of the pregnancies; 52 Down syndrome and 5065 unaffected pregnancies were within the gestational age window and were included in the analysis. Other chromosome abnormalities were excluded from the study.

Serum samples

Blood samples were obtained using a separator clot tube, centrifuged, and stored in the refrigerator until shipment. The samples and clinical information were shipped bi-weekly by express mail to the Foundation for Blood Research in Scarborough, Maine. Upon receipt, a portion of the sample was assayed for AFP, uE3, and hCG, and the remaining serum was coded, aliquoted, and immediately frozen at -20°C. Nearly all samples were received within 2 days of shipment. The informed consent agreement included a provision that excess serum could be frozen and stored for future studies.

Assays

Sera were retrieved and thawed at the rate of approximately 100 per week over 1 year, in the order in which they were originally received and without knowledge of the karyotype. The free *β*-subunit and AFP were assayed using the Wallac DELFIA hAFP/Free-hCG*β* Dual Assay (Wallac Oy, Turku, Finland) (Nørgaard-Pedersen *et al.*, 1994). This assay has less than 0·03 per cent cross-reactivity with hCG and has a high correlation ($r = 0{\cdot}99$) with the CIS ELSA F-*β* hCG (CIS Bio International, Gif-Sur-Yvette Cedex, France). Measurements of hCG were repeated with the same kit (hCG MAIA Clone) used on the fresh samples [Biodata Diagnostics (formally Serono) distributed by Polymedco, Inc., Cortlandt Manor, New York, U.S.A.]. The AFP and hCG measurements were repeated to provide assurance that stored serum samples were labelled and retrieved accurately and to assess possible degradation. This design also ensured that the free *β*-subunit and hCG measurements were performed in the same sample and under the same conditions.

Calculation of observed screening performance

Gestational age-specific median values were established for the free *β*-subunit and hCG by weighted exponential regression analysis, using unaffected pregnancies with gestational age based on LMP dates. All values were then converted to multiples of the median (MOM). MOM levels were adjusted for maternal weight using a published methodology (Neveux *et al.*, 1996). Means and standard deviations for the analytes were estimated using data from the linear portion of the probability plots. Detection and false-positive rates were calculated at fixed MOM cut-off levels for comparisons of hCG and the free *β*-subunit, and at fixed Down syndrome risk cut-off levels (calculated using maternal age and the relevant MOM values) for the multivariate comparisons. Risks were assigned to each study subject using a published algorithm (Wald *et al.*, 1988) with subsequently described modifications (Wald *et al.*, 1994). The AFP and uE3 values were those obtained in the original study, while the free *β*-subunit and hCG values were those obtained on the thawed samples.

Calculation of modelled screening performance

Down syndrome screening performance was estimated using a published model (Royston and

Thompson, 1992) in which the distribution parameters of the current analyte measurements were combined with the maternal age distribution in the United States in 1993 (National Center for Health Statistics, 1997). The original publication describing multiple marker screening (Wald *et al.*, 1988) and our earlier analysis (Haddow *et al.*, 1994) were both based on LMP dates. Large published datasets indicate that any systematic differences between LMP and biparietal diameter (BPD) dating are the same for both Down syndrome and unaffected pregnancies (Wald *et al.*, 1993b). The separation of analyte measurements between Down syndrome and unaffected pregnancies should therefore be the same, regardless of the dating method. The current modelling uses only one estimate of the log mean and is based on menstrual dates, but the standard deviations and correlation coefficients in the model are derived separately from each of the dating methods. Truncation limits contained in the Appendix are used for modelling the current data. Truncation limits are not used when modelling the screening performance of the six other published studies, because most do not provide sufficient data for determining such limits. Several refinements are incorporated into the current analysis (e.g., improved maternal weight adjustment, updated population parameters, and new truncation limits) that will lead to improved screening performance estimates in comparison with an earlier publication (Haddow *et al.*, 1994).

Additional sera collected as part of routine screening

The free β-subunit concentrations can increase in maternal sera stored at room temperatures or higher (Stevenson *et al.*, 1993; Cuckle and Jones, 1995; Sancken and Bahner, 1995; Beaman *et al.*, 1996). To evaluate whether such increases might influence our findings, we obtained 1000 sera from women undergoing routine prenatal screening. Sera were stored in the refrigerator prior to transport by courier (on kool packs or at ambient temperature) on the evening of the day of collection. Most sera were processed the next day and spent no more than 8 h at room temperature. Samples were assayed for free β-subunit at the rate of approximately 20 per week over the same time period as the thawed samples were being assayed. Distributions of the free β-subunit measurements in fresh and stored samples were then compared.

RESULTS

The AFP and hCG values originally obtained in the 5117 serum samples were highly correlated with those obtained after retrieval from freezer storage ($r = 0{\cdot}970$ and $r = 0{\cdot}979$, respectively). The high correlation for both analytes indicates that sample integrity was maintained and that samples were retrieved reliably. Figures 1a and 1b show the high correlation between hCG and the free β-subunit measurements in stored sera from the 5065 unaffected and 52 Down syndrome pregnancies.

Table I shows the observed detection and false-positive rates for hCG and the free β-subunit at selected MOM cut-off levels. At 2·0 MOM, the hCG measurements are associated with a 9·2 per cent false-positive rate and a 50 per cent detection rate; the free β-subunit measurements are associated with a 15 per cent false-positive rate and a 52 per cent detection rate. The higher false-positive rate for the free β-subunit reflects the greater variability in the distribution of measurements. When the MOM cut-off levels are selected to provide false-positive rates of 3, 5, and 7 per cent, the hCG measurements detect 27, 35, and 42 per cent of the Down syndrome pregnancies. The corresponding detection rates for the free β-subunit are 27, 33, and 40 per cent. At all three false-positive rates, the observed detection rates for hCG are the same or slightly higher.

Figures 2a and 2b show probability plots for hCG and the free β-subunit measurements with regression lines fitted to the data between the fifth and 95th centiles. Both analytes fit a log Gaussian distribution reasonably well, but the free β-subunit measurements are more variable, as evidenced by higher standard deviations (steeper slopes) for both the unaffected and the Down syndrome pregnancies. The population parameters for these two analytes are given in the Appendix, along with those for AFP and uE3. Those population parameter are used with each woman's analyte levels and age to assign Down syndrome risk for each pregnancy. Based on these risks, Table II shows the observed detection rates for the 52 Down syndrome pregnancies at selected false-positive rates. At a 5 per cent false-positive rate, hCG (in combination with maternal age) yields 7 per cent more detection than the free β-subunit, and the combination of AFP and hCG yields about 8 per cent more detection than the combination of AFP and the free β-subunit. The combination of AFP,

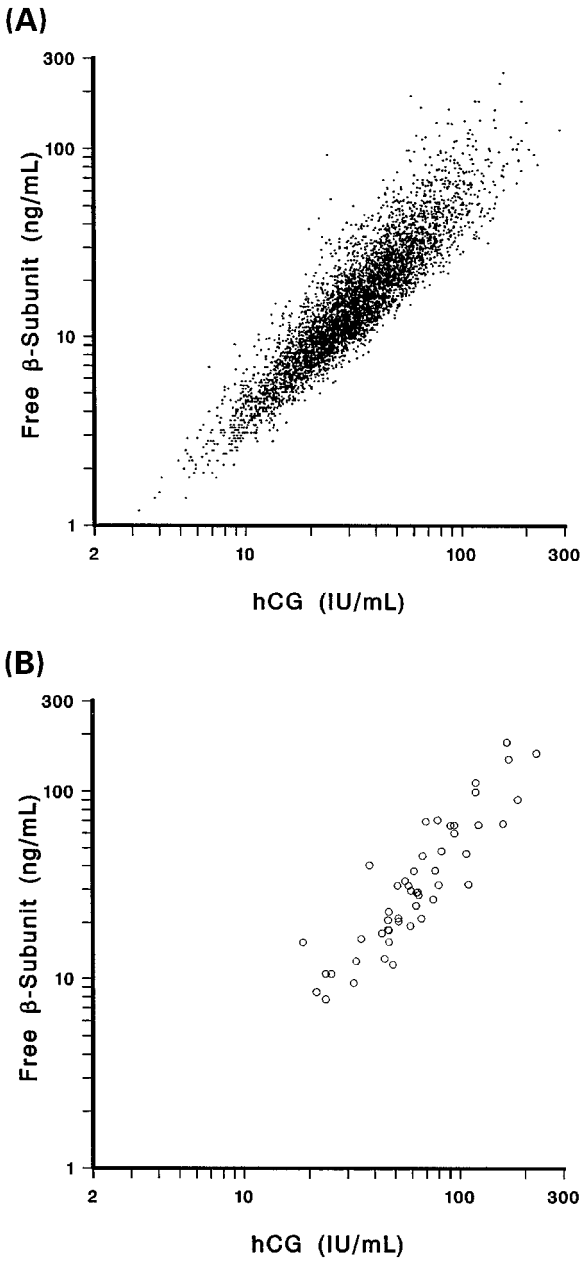

Fig. 1—Relationship between the measurements of human chorionic gonadotropin (hCG) and the free *β*-subunit in unaffected (a) and Down syndrome (b) pregnancies. Measurements of both analytes were those obtained on the sera after storage. The correlation coefficients for the 5065 unaffected and 52 Down syndrome pregnancies are 0·889 and 0·876, respectively

uE3, and hCG detects 6 per cent more Down syndrome pregnancies than AFP, uE3, and the free *β*-subunit.

Table I—Observed Down syndrome detection rates (DR) and false-positive rates (FPR) at various multiple of the median (MOM) cut-off levels for human chorionic gonadotropin (hCG) and the free *β*-subunit

MOM cut-off level	hCG		Free *β*-subunit	
	FPR (%)	DR (%)	FPR (%)	DR (%)
1·0	50	87	50	85
1·2	35	85	38	81
1·4	25	77	30	73
1·6	18	75	24	65
1·8	13	64	18	56
2·0	9·2	50	15	52
2·2	6·6	37	12	46
2·4	4·7	35	9·6	42
2·6	3·4	29	7·7	40
2·8	2·8	23	6·3	37
3·0	2·0	23	5·2	33
3·2	1·4	19	4·4	27
3·4	1·1	14	3·8	27
3·6	0·9	12	3·4	27
3·8	0·6	12	2·6	23
4·0	0·5	9·6	2·4	23

Modelling can provide a more accurate estimate of screening performance than direct observation, by smoothing statistical irregularities. Tables III, IV, and V utilize such modelling to show Down syndrome screening performance for various combinations of maternal age and serum markers. Screening performance is displayed at selected false-positive rates (Table III), detection rates (Table IV), and risk cut-off levels (Table V). In each table, separate estimates are provided, according to whether dating is based on LMP or BPD measurements. Screening performance is consistently better with hCG than with the free *β*-subunit and improves when BPD measurements are used. Adding uE3 measurements to either of the double-marker combinations yields about a 5 per cent increase in detection when dating is by LMP and a 7 per cent increase when dating is by BPD.

Modelled detection rates (Table III) are at least 5 per cent higher than observed detection rates (Table II) for all combinations of markers. This is primarily due to the difference in the underlying maternal age distributions. The observed screening performance is derived from a population of women who are nearly all age 35 years or older,

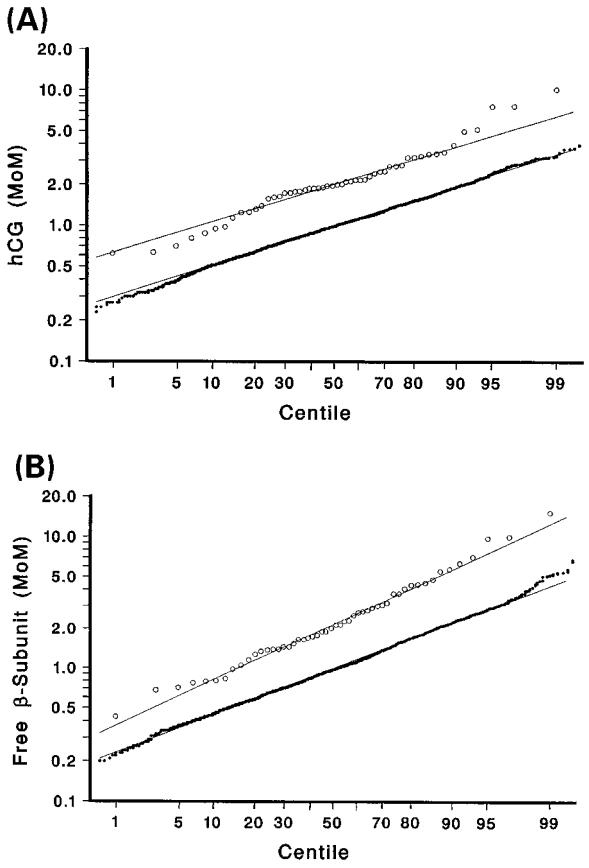

Fig. 2—Probability plots for human chorionic gonadotropin (hCG) (a) and the free β-subunit (b) for both unaffected (●) and Down syndrome (○) pregnancies. The multiple of the median (MOM) values for individual pregnancies are plotted on the vertical logarithmic scale. The horizontal scale indicates the Gaussian centile, based on the rank of the observation within its category. If the points plotted for each category follow an approximate straight line, the distribution is log Gaussian

Table II—Observed Down syndrome detection rates at three false-positive rates for combinations of maternal age and alpha-fetoprotein (AFP), unconjugated oestriol (uE3), human chorionic gonadotropin (hCG), and the free β-subunit (free β)

False-positive rate (%)	Maternal age and serum markers	Down syndrome detection rate (%)
3	hCG	37
	free β	31
	AFP and hCG	48
	AFP and free β	46
	AFP, uE3, and hCG	52
	AFP, uE3, and free β	46
5	hCG	44
	free β	37
	AFP and hCG	58
	AFP and free β	50
	AFP, uE3, and hCG	58
	AFP, uE3, and free β	52
7	hCG	54
	free β	50
	AFP and hCG	64
	AFP and free β	58
	AFP, uE3, and hCG	64
	AFP, uE3, and free β	58

while the modelled screening performance is based on the maternal age distribution of a general pregnancy population (where only about 10 per cent are age 35 years or older). Maternal age is a more effective marker when applied to the general population where age-associated risks vary widely, compared with a population of older women where the age-associated risks, although higher, are less varied.

Table VI summarizes the observed and modelled differences in Down syndrome detection rates for protocols combining hCG or the free β-subunit with maternal age and AFP measurements as reported in six published studies (and the present study). The change in detection resulting from substituting the free β-subunit for hCG measurements ranges from a gain of 9 per cent to a loss of 5 per cent, according to the authors' estimates. All of these studies provided population parameters (means, standard deviations, and correlation coefficients) which allowed a re-analysis by modelling, whether or not modelling was actually done in the original study. This re-analysis has the advantage of using the same underlying maternal age distribution and truncation limits, and holds other modelling-related features constant. At a 5 per cent false-positive rate, the change in detection resulting from substituting the free β-subunit for hCG measurements in the bivariate model ranges from a loss of 8 per cent to a gain of 3 per cent. The present study shows a loss in detection of 5 per cent. The last two columns show the individual modelled detection rates for all of the studies. For example, the detection rate using the free β-subunit measurements is estimated to be 60 per cent for the 1991 study by Spencer, when hCG measurements are used instead, the detection rate is 61 per cent. The modelled detection rates for

Table III—Modelled Down syndrome detection rates at three false-positive rates for combinations of maternal age and alpha-fetoprotein (AFP), unconjugated oestriol (uE3), human chorionic gonadotropin (hCG), and the free β-subunit (free β)

False-positive rate (%)	Maternal age and serum markers	Detection rate (%)	
		LMP	BPD
3	hCG	42	42
	free β	39	36
	AFP and hCG	55	57
	AFP and free β	50	51
	AFP, uE3, and hCG	60	64
	AFP, uE3, and free β	55	59
5	hCG	50	51
	free β	48	47
	AFP and hCG	62	65
	AFP and free β	57	59
	AFP, uE3, and hCG	67	71
	AFP, uE3, and free β	62	67
7	hCG	57	57
	free β	54	53
	AFP and hCG	68	70
	AFP and free β	62	64
	AFP, uE3, and hCG	73	76
	AFP, uE3, and free β	67	71

LMP = last menstrual period; BPD = biparietal diameter.

Table IV—Modelled false-positive rates at three Down syndrome detection rates for combinations of maternal age and alpha-fetoprotein (AFP), unconjugated oestriol (uE3), human chorionic gonadotropin (hCG), and the free β-subunit (free β)

Detection rate (%)	Maternal age and serum markers	False-positive rate (%)	
		LMP	BPD
50	hCG	4·9	4·8
	free β	5·6	5·9
	AFP and hCG	2·2	1·8
	AFP and free β	3·1	2·8
	AFP, uE3, and hCG	1·6	1·1
	AFP, uE3, and free β	2·1	1·5
60	hCG	8·1	7·9
	free β	9·9	10·2
	AFP and hCG	4·3	3·6
	AFP and free β	6·0	5·4
	AFP, uE3, and hCG	3·1	2·3
	AFP, uE3, and free β	4·3	3·2
70	hCG	13·2	12·8
	free β	17·3	17·3
	AFP and hCG	8·1	6·9
	AFP and free β	11·3	10·2
	AFP, uE3, and hCG	5·9	4·6
	AFP, uE3, and free β	8·3	6·4

LMP = last menstrual period; BPD = biparietal diameter.

each of the combination of markers are consistent between the various studies, ranging from 54 to 62 per cent for the free β-subunit and from 55 to 62 per cent when hCG measurements are used.

In order to determine the possible influence of shipping and freezer storage on our modelling estimates, free β-subunit measurements were performed on 1000 fresh second-trimester sera. These measurements were then examined on a probability plot to evaluate how closely they fitted the line describing the distribution of measurements found in the stored samples (Fig. 3). The observations fitted the line well over most of the range. This indicates that the performance estimated from the stored samples is likely to represent that found in fresh samples. At the extremes, however, levels in the fresh sera were slightly higher than predicted. The small deviation at the upper end would yield a higher false-positive rate in actual practice than would be predicted by our modelling, because our modelling is based on the stored samples.

DISCUSSION

The present study took advantage of a bank of frozen sera obtained from an earlier cohort study (Haddow *et al.*, 1994). This well-characterized set avoided selection bias, because decisions for amniocentesis were based on maternal age and not on serum screening. Bias due to selective fetal loss was also avoided, because sera were obtained prior to amniocentesis, and karyotypes were available for all of the pregnancies. The complete ascertainment of Down syndrome cases in this study population, in combination with the availability of relevant pregnancy information, offered a unique opportunity to develop an unbiased estimate of the free β-subunit performance, both alone and in combination with other biochemical measurements. The samples were assayed over the course of 1 year to duplicate assay variability that occurs in actual practice.

Substituting the free β-subunit for hCG measurements in this study population yields a

Table V—Modelled Down syndrome detection and false-positive rates at three risk cut-off levels for combinations of maternal age and alpha-fetoprotein (AFP), unconjugated oestriol (uE3), human chorionic gonadotropin (hCG), and the free β-subunit (free β)

Down syndrome risk term (second trimester) Risk cut-off (1:*n*)	Maternal age and serum markers	Detection rate (%)		False-positive rate (%)		OAPR (1:*n*)	
		LMP	BPD	LMP	BPD	LMP	BPD
250 (190)	hCG	55	56	6·2	6·7	82	87
	free β	49	49	5·2	5·4	77	80
	AFP and hCG	63	65	5·1	5·1	59	57
	AFP and free β	56	58	4·7	4·7	61	59
	AFP, uE3, and hCG	60	67	3·1	3·7	38	40
	AFP, uE3, and free β	59	64	3·9	4·3	48	49
300 (230)	hCG	59	60	7·8	8·0	96	97
	free β	53	53	6·8	6·9	93	95
	AFP and hCG	66	68	6·2	6·1	68	65
	AFP and free β	60	61	5·8	5·8	70	69
	AFP, uE3, and hCG	63	70	3·8	4·5	44	47
	AFP, uE3, and free β	62	67	4·8	5·2	56	56
350 (270)	hCG	63	64	9·3	9·6	107	109
	free β	56	56	8·1	8·2	105	106
	AFP and hCG	68	71	7·3	7·2	78	74
	AFP and free β	62	64	7·0	7·0	82	80
	AFP, uE3, and hCG	66	72	4·5	5·3	59	54
	AFP, uE3, and free β	64	69	5·7	6·1	65	64

OAPR = odds of being affected given a positive result; LMP = last menstrual period dates; BDP = biparietal diameter.

Table VI—Differences in Down syndrome detection using maternal age and alpha-fetoprotein (AFP) in combination with either human chorionic gonadotropin (hCG) or the free β-subunit (free β)

Author (year)	Difference in detection (%)*			Our modelled detection rates (%)	
	Reported by author		Our re-analysis		
	Observed	Modelled	Modelled	With free β	With hCG
Spencer (1991)	+3	ND	−1	60	61
Ryall *et al.* (1992)	'Little'	ND	+1	58	57
Spencer *et al.* (1992)	+9	+9	+3	62	59
Wald *et al.* (1993a, 1994)	ND	−1	+3	58	55
Nørgaard-Pederson *et al.* (1994)	+7	ND	0	62	62
Aitken *et al.* (1996)	ND	−1	−8	54	62
Present study	−5	−5	NA	57	62

*Difference in Down syndrome detection is defined as the detection rate using maternal age in combination with AFP and the free β-subunit measurements minus the detection rate using maternal age in combination with AFP and hCG measurements. All modelled detection rates are at a 5 per cent false-positive rate; observed detection rates are as close to 5 per cent as possible. ND = not done; NA = not applicable.

slightly lower detection rate for Down syndrome, whether analysed univariately or in combination with other markers. The separation of the free β-subunit measurements between unaffected and affected pregnancies is greater than for hCG measurements and, on this basis, the free β-subunit

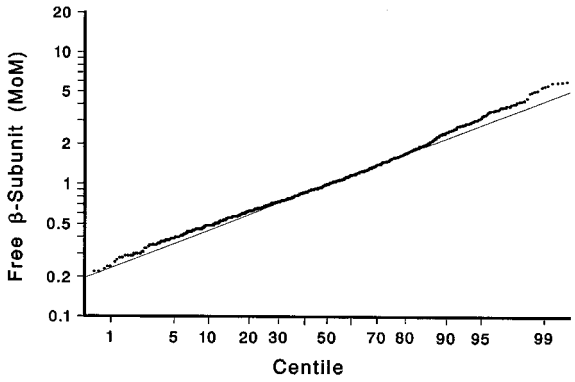

Fig. 3—Probability plot that compares distributions of the free *β*-subunit measurements in routinely collected fresh sera with those in stored sera from the unaffected pregnancies. The thin line represents the distribution of the free *β*-subunit measurements found in 5065 stored sera from unaffected pregnancies dated by the last menstrual period. The dots represent the free *β*-subunit measurements in 1000 fresh sera; 55 per cent of these were dated by ultrasound and the remainder by the last menstrual period

should be a better screening test. The spread of measurements (log standard deviation) from affected pregnancies is also greater for the free *β*-subunit, and this factor should also yield a higher detection rate. However, this is counterbalanced by the greater spread of the free *β*-subunit measurements in unaffected pregnancies; the variance is more than 40 per cent greater than for hCG measurements. This leads to a higher false-positive rate at any given detection rate. The net effect is that screening performance for the free *β*-subunit in the present study is slightly less good than for hCG.

Whole molecule hCG is present in 200 to 300-fold excess over the free *β*-subunit; even minimal dissociation due to transport delay or higher temperatures will raise the free *β*-subunit concentration (Knight and Cole, 1991). At room temperature, the free *β*-subunit measurements increased by 5–7 per cent per day in serum and by 10–14 per cent per day in whole blood (Cuckle and Jones, 1995). Much greater increases occur above 25°C (Sancken and Bahner, 1995). The management of samples used in the present study was designed to minimize *in vitro* increases in the free *β*-subunit. The success of this management was confirmed by the close agreement between the population variabilities (log standard deviations) in stored and fresh samples (Fig. 3). A contributing factor to the agreement may be that sterility was maintained because most sera were stored and shipped in serum separator tubes; increases in free *β*-subunit are minimized when antibiotics are added to stored samples (Kardana and Cole, 1997). The present study's performance estimates should be reliable for samples handled similarly, but they may not be appropriate for samples subjected to longer shipment times or higher temperatures (Beaman *et al.*, 1996; Stevenson *et al.*, 1993; Sancken and Bahner, 1995). The use of dried blood spots on filter paper has been suggested as a way to avoid this instability problem (Macri *et al.*, 1996). Certain disadvantages are inherent to this method, however, such as variability in haematocrit, sample application and elution, and recovery of the analytes from the blood spot (Hoffman *et al.*, 1996). In addition, a proportion of samples will be unacceptable and require resampling (Verloes *et al.*, 1995). No side-by-side study has yet been published comparing the Down syndrome risks obtained using serum versus blood spots on the same set of patients.

Our re-analysis of published data on relative screening performance is in reasonable agreement with the original estimates in three of the studies (Spencer, 1991; Ryall *et al.*, 1992; Wald *et al.*, 1994), but agrees less closely with three others. In the first of these latter studies (Spencer *et al.*, 1992), attention has already been called to an error in modelling the hCG measurements that results in an underestimated detection rate (Wald *et al.*, 1993c). Our re-analysis supports the revised estimate. In the second (Nørgaard-Pedersen *et al.*, 1994), approximately half of the discrepancy is explained because AFP population parameters are selectively used in combination with the free *β*-subunit measurements that better discriminate between unaffected and Down syndrome pregnancies. In the third study (Aitken *et al.*, 1996), our re-analysis of the detection rate for the free *β*-subunit combination agrees, but our estimated detection rate for the hCG combination is higher. Because no obvious explanation can be identified for this discrepancy, we obtained verification of our calculations from a second research group. When Data from the published studies and ours are examined together, the free *β*-subunit and hCG measurements appear, overall, to perform similarly.

Intervention trials are considered by some to be the ultimate confirmation of screening performance. It would not be possible, however, to compare directly the performance of different marker combinations in such trials unless the free

β-subunit and hCG were to be measured together in the same women and then acted upon. An alternative approach might be to compare screening performances between intervention trials. This is complicated, however, by confounding factors such as differences in maternal age distributions, the method and reliability of gestational dating, and the risk cut-off level chosen. Detection rates can also be overestimated due to under-ascertainment of Down syndrome cases not detected by screening. The effects of confounding are a particularly serious problem when the difference in detection rates between marker combinations is small. An analysis of published intervention trials that takes these confounders into account reaches conclusions (Palomaki *et al.*, 1996) similar to those found for the case–control studies described above.

The results from this study, in combination with other published studies, indicate that substitution of the free β-subunit for hCG measurements in either a double- or a triple-marker combination provides approximately equivalent screening performance.

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APPENDIX

Table A1 contains population parameters for Down syndrome and unaffected pregnancies for each of the markers. Separate parameters are provided for gestational ages based on the last menstrual period (LMP) or the biparietal diameter (BPD). These parameters are used for modelling Down syndrome screening performance in the present dataset.

Table A1—Population parameters for four serum analytes in the second trimester of pregnancy

Population parameter	Analyte	Unaffected		Down syndrome	
		LMP	BPD	LMP	BPD
Log Mean	AFP	0·0000	0·0000	− 0·1467	− 0·1331
	uE3	0·0000	0·0000	− 0·1774	− 0·1763
	hCG	0·0000	0·0000	0·3049	0·2968
	free β	0·0000	0·0000	0·3347	0·3166
Log SD	AFP	0·1527	0·1471	0·1571	0·1505
	uE3	0·1351	0·1307	0·1666	0·1543
	hCG	0·2260	0·2235	0·2174	0·2093
	free β	0·2717	0·2723	0·3292	0·3266
Correlation	AFP/uE3	0·3140	0·2223	0·3668	0·2562
	AFP/hCG	0·0951	0·1560	− 0·3118	− 0·1893
	AFP/free β	0·0391	0·0946	− 0·2964	− 0·1986
	uE3/hCG	− 0·2147	− 0·1400	− 0·4156	− 0·2434
	uE3/free β	− 0·2257	− 0·1451	− 0·4689	− 0·2776
	hCG/free β	0·8892	0·8757	0·8758	0·9456
Truncation limits	AFP	0·4to2·0 MOM			
	uE3	0·4to2·0 MOM			
	hCG	0·5to4·0 MOM			
	free β	0·5to4·0 MOM			