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FIRST-TRIMESTER URINE FREE BETA hCG, BETA CORE, AND TOTAL OESTRIOL IN PREGNANCIES AFFECTED BY DOWN'S SYNDROME: IMPLICATIONS FOR FIRST-TRIMESTER SCREENING WITH NUCHAL TRANSLUCENCY AND SERUM FREE BETA hCG

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SUMMARY

We have examined maternal urine concentrations of beta core, free beta human chorionic gonadotrophin (hCG), and total oestriol in 373 control pregnancies and 43 pregnancies affected by aneuploidy (including 22 cases of Down's syndrome) in an attempt to see if any of the analytes have a value in Down's syndrome screening between the tenth and 14th week of pregnancy. We have compared the performance of these analytes against nuchal translucency measurement combined with maternal serum free beta hCG at the same period of pregnancy. Our results show that levels of urine free beta hCG and beta core are increased in Down's syndrome with average multiple of the median levels of 1·81 and 2·91, respectively. Urine total oestriol was reduced (0·83) whilst maternal serum free beta hCG was increased (1·72). In trisomy 18 the levels of all analytes were reduced, although serum free beta hCG was the most discriminating. The spread of results in the control and the Down's group for urine beta core was more than three times than that for serum free beta hCG and with urine free beta hCG it was two times wider. In combination with maternal age, urine total oestriol had a 32 per cent detection rate at a fixed 5 per cent false-positive rate; urine beta core 34 per cent, urine free beta hCG 36 per cent, maternal serum free beta hCG 44 per cent, and nuchal translucency 82 per cent. In combination with nuchal translucency, urine total oestriol added an extra 1 per cent detection, urine beta core an extra 2 per cent, urine free beta hCG an extra 3 per cent, and serum free beta hCG an extra 5 per cent. It is unlikely that any of the urine markers will be of value in first-trimester screening. Optimal first-trimester screening programmes will rely for the foreseeable future on nuchal translucency, serum free beta hCG, and possibly pregnancy-associated plasma protein A.

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INTRODUCTION

Free beta human chorionic gonadotrophin (hCG) in maternal serum is probably the single most effective biochemical marker of Down's syndrome in the first and second trimesters of pregnancy (Spencer *et al.*, 1992b, 1994). Combinations of free beta hCG with other biochemical markers have led to second-trimester prospective screening programmes with detection rates of 75 per cent at a 5 per cent false-positive rate (Spencer, 1994a)

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and preliminary first-trimester screening data in combination with pregnancy-associated plasma protein-A (PAPP-A) show detection rates of the order of 63 per cent at a 5 per cent false-positive rate (Krantz *et al.*, 1996; Wald *et al.*, 1996). Biophysical measurements such as nuchal translucency thickness (NT) in the tenth to 14th week of pregnancy have been shown to be increased in pregnancies affected by chromosomal anomalies (Nicolaides *et al.*, 1992). First-trimester screening programmes based on this modality, amongst women already at increased risk of Down's syndrome as a result of advanced maternal age, have shown detection rates of 84 per cent at a 5 per cent false-positive rate (Nicolaides *et al.*, 1994). Despite criticism of its effectiveness and doubts about the transferability to non-academic units (Bewley *et al.*, 1995; Roberts *et al.*, 1995), coordinated studies of unselected populations by suitably trained staff in non-academic environments have confirmed detection rates of 80 per cent at a 5 per cent false-positive rate (Nicolaides *et al.*, 1996).

Recently, detection rates as high as 80 per cent (at a 5 per cent false-positive rate) have been reported in the second trimester for the urine marker beta core (Cuckle *et al.*, 1994, 1995). Whilst one other study has confirmed that elevated levels of beta core are strongly associated with Down's syndrome (Canick *et al.*, 1995), other studies have shown either elevated but significantly lower values than the original studies (Spencer *et al.*, 1996) leading to detection rates of only 41 per cent (when combined with maternal age), or even median multiple of the median (MOM) values closer to 1·3 (Hayashi and Kozu, 1995). When urine free beta hCG levels were compared with urine beta core levels in the same second-trimester urine samples, a detection rate close to 60 per cent was achieved using urine free beta hCG and maternal age alone (Spencer *et al.*, 1996).

Third-trimester 24 h total oestrogen levels have been shown to be reduced in Down's syndrome pregnancies (Jorgensen and Trolle, 1972), and in the second trimester, maternal serum levels of unconjugated oestriol are, on average, also reduced (Canick *et al.*, 1988; Spencer, 1994b). As a result, Cuckle *et al.* (1995) studied levels of total oestrogens in random urine collected during the second trimester and also found reduced levels. On this basis, they further proposed a multimarker approach in urine and demonstrated that if total oestrogens was used in addition to beta core, then detection rates would increase by 2·7 per cent.

In order to extend our studies of urine beta core and free beta hCG, we have assessed the usefulness of these two urine markers along with total oestriol in the first trimester of pregnancy and have compared their effectiveness as screening markers against nuchal translucency and serum free beta hCG as established first-trimester markers of aneuploidy.

MATERIALS AND METHODS

The study population was derived from those women referred to the tertiary referral centre at King's College as a result of increased nuchal translucency measured in one of the participating demonstration project centres (Nicolaides *et al.*, 1996), or who presented for routine nuchal translucency scanning as a result of advanced maternal age or previous family history. Gestational age was determined by measurement of fetal crown–rump length (CRL) and nuchal translucency was measured as previously described (Nicolaides *et al.*, 1994). Blood and urine samples were collected from women in sterile containers at the time of nuchal translucency measurement. Urine samples were stored as aliquots at -20°C prior to blinded retrospective analysis. Maternal serum free beta hCG was measured prospectively on the next working day after storage at 4°C. Women with an increased risk of fetal aneuploidy as a result of increased nuchal translucency thickness (Pandya *et al.*, 1995) were offered fetal karyotyping by chorionic villus sampling (CVS) and pregnancy outcome was ascertained on all women.

In total, 43 pregnancies affected by aneuploidy were available for further study. This group consisted of eight cases of Turner's syndrome; 22 cases of Down's syndrome, of which three were included in a previous serum free beta hCG study (Noble *et al.*, 1995); seven cases of trisomy 18; five cases of trisomy 13; and one case of triploidy. None of the cases had been part of any previous urine study. A control group from those pregnancies resulting in the birth of an unaffected baby was established by selecting 373 cases collected within the same time scale as the affected group. Table I summarizes the descriptions of the two populations.

Maternal serum free beta hCG was measured in duplicate with the CIS immunoradiometric assay [CIS (U.K.) Ltd, High Wycombe, Bucks, U.K.]. The analytical performance of this assay has been detailed elswhere (Spencer *et al.*, 1992b; Macri

Table I—Maternal age at delivery, gestational age, CRL, and storage time in the unaffected and affected study groups

	Affected group	Control group
Mean (median) age (years)	35·79 (36·2)	36·54 (37·5)
Range	24·7–46·6	18·3–46·8
Median gestation (days)	85	87
Range	77–98	74–98
Median CRL (mm)	58	61
Range	45–84	39–89
Median storage time (days)	174	292
Range	38–398	55–467
Number	43	373

et al., 1993). Maternal urine free beta hCG was measured by the same assay with samples diluted 1 in 5 with zero diluent prior to analysis.

Urine beta core was measured in duplicate using the Ciba Corning Diagnostics UGP enzyme immunoassay (Triton UGP EIA, Ciba Corning, Almeda, CA, U.S.A.). Urine samples were diluted to within the analytical range of the assay by appropriate dilution (between 1 in 5000 and 1 in 30 000) in the zero calibrant supplied with the kit. The analytical performance of this assay has been previously described (Spencer *et al.*, 1996; Canick *et al.*, 1995).

Total oestriol was measured in duplicate using the Johnson and Johnson Oestriol (total) II radioimmunoassay (Johnson and Johnson Clinical Diagnostics Ltd, Amersham, U.K.). Urine samples were either analysed neat or diluted 1 in 5 in normal male serum. The between-batch precision of the assay was 3·5 per cent at 50 nmol/l, 2·3 per cent at 200 nmol/l, and 3·5 per cent at 600 nmol/l over seven batches.

In order to take into account the varying degrees of urine-concentrating effects with individual patients, all urine analyte measurements were corrected to a standard urine creatinine concentration. Urine beta core was therefore expressed as nmol/mmol creatinine, urine free beta hCG as IU/mmol creatinine, and total oestriol as nmol/mmol creatinine. Urine creatinine was measured with a standard Jaffe reaction procedure (Spencer, 1986) on a Hitachi 717 after a 1 in 30 dilution in 0·9 per cent saline.

To take into account gestational age variation in analyte levels and to make a direct comparison with nuchal translucency measurements, all analyte values (corrected for urine creatinine if appropriate) were converted to multiples of the median based on the oberved analyte median in each crown–rump length band in decade intervals (40–49, 50–59, 60–69, 70–79, 80–89 mm). For nuchal translucency data, the difference between the nuchal translucency measurement and the appropriate normal median for crown–rump length (delta value) was calculated using previously established normal population medians (Pandya *et al.*, 1995).

Assessment of the performance of various marker combinations as potential screening procedures was examined using standard statistical modelling techniques (Royston and Thompson, 1992). We used the measured population parameters for the urine markers, published normal parameters for serum free beta hCG (Berry *et al.*, 1995) with data for Down's pregnancies ascertained from a meta analysis (Cuckle, 1996) of 340 cases from the world's literature (unpublished data, Spencer, 1996), and data on nuchal translucency from the combined King's College studies (unpublished data, Nicolaides *et al.*, 1996). Using these population parameters, a series of random MOM values or delta nuchal translucency values (Pandya *et al.*, 1995) were selected from within the distributions of the affected and unaffected populations for each analyte. These values were then used to calculate likelihood ratios for the various marker combinations and the expected Down's syndrome detection rate was then calculated at a given false-positive (5 per cent) assuming the maternal age distribution of England and Wales (Office of Population Censuses and Surveys, 1991–1994) and the age-related a priori risk of Down's syndrome in the first trimester (Snijders *et al.*, 1994). Repeated simulations through the model enabled confidence intervals to be established.

RESULTS

The observed median values of serum free beta hCG, urine free beta hCG, urine beta core, and urine total oestriol are shown in Table II and plotted as the median, fifth and 95th centiles in Figs 1 and 2.

For serum free beta hCG, the median values showed a gradual fall consistent with the levels observed by Berry *et al.* (1995) in a large series of over 8000 pregnancies and consistent with smaller published series (Spencer *et al.*, 1994; Brizot *et al.*,

Table II—Median analyte values in the first-trimester control group

CRL (mm)	CRL days	Number	Serum free beta hCG (IU/l)	Free beta hCG (IU/mmol creatinine)	Beta core (nmol/mmol creatinine)	Total oestriol (nmol/mmol creatinine)
40–49	74–80	54	44·0	8·4	12·0	91·9
50–59	80–85	107	34·0	8·9	7·3	119·9
60–69	86–91	89	33·0	8·2	9·6	184·9
70–79	91–95	82	22·5	8·3	14·7	236·0
80–89	96–100	28	23·5	6·7	8·0	298·8

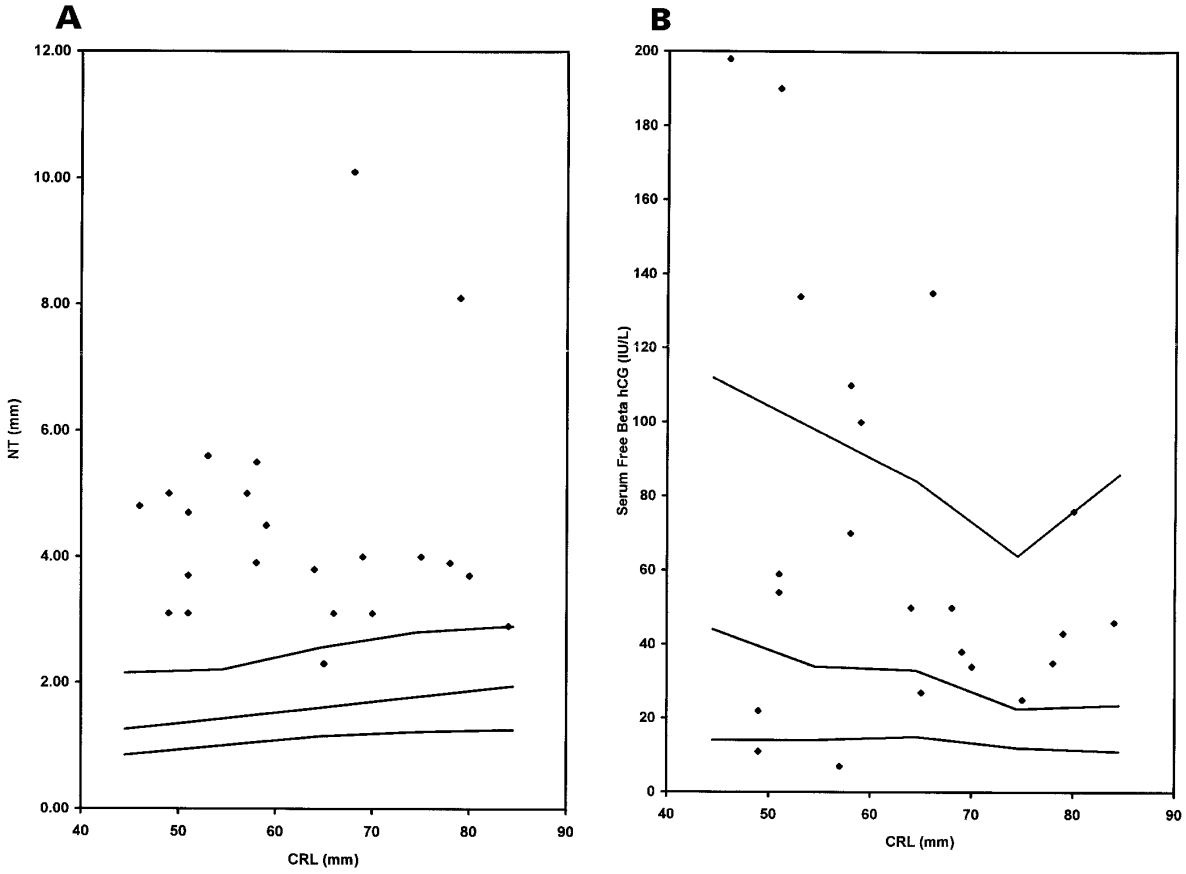

Fig. 1—(a and b)

1995; Krantz *et al.*, 1996; Wald *et al.*, 1996). The fall in serum free beta hCG was on average 1 IU/l (1 ng/ml) per day, as found by Berry *et al.* (1995).

For urine free beta hCG, the median values showed only a small change with gestational age which was less marked than observed in a previous smaller date set at this time (Spencer *et al.*, 1996).

For urine beta core, the median values also showed only a small change with gestational age which increased to a peak at around 13 weeks of gestation in a manner similar to that described previously (Spencer *et al.*, 1996). Absolute median values were similar to those obtained in this previous smaller series.

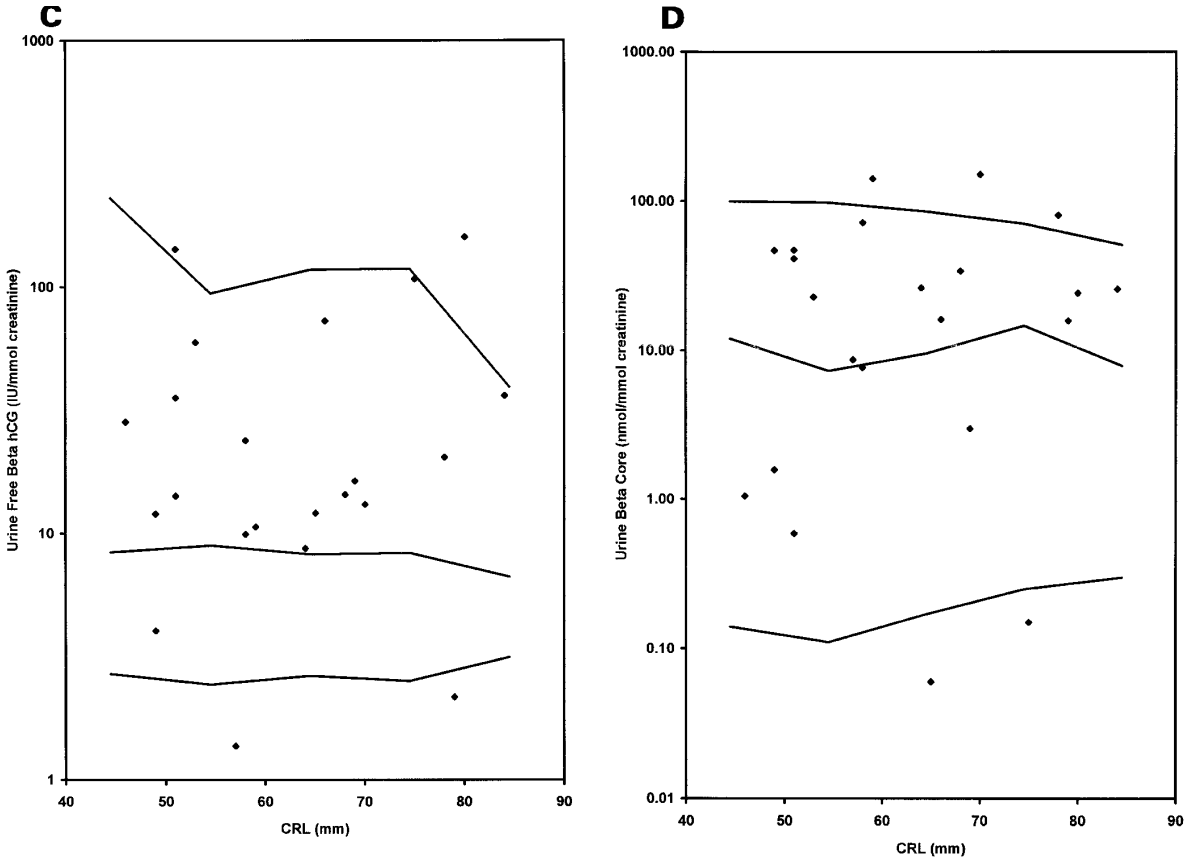

Fig. 1—(c and d)

For urine total oestriol, the median values showed an almost logarithmic increase with increasing gestation, approximating to a 6 nmol/mmol increase per day.

Figures 1a–1e and 2a–2e show the individual marker levels observed for each case of Down's syndrome and for each of the other aneuploid anomalies. Table III shows the median MOM for the various markers in question with the four major clinical groups trisomy 21 (Down's syndrome), trisomy 18, trisomy 13 and X0 (Turner's syndrome). Also shown in Table III are the results of Mann–Whitney *U*-tests of the affected populations compared with the unaffected populations for the biochemical markers. (This was not performed for the delta NT case since the control and affected groups were preselected on the basis of increased NT.) The data show that serum free beta hCG was significantly elevated in the trisomy 21 group and significantly lower in the trisomy 18 and trisomy 13 groups. Urine free beta hCG, however, was significantly increased only in the trisomy 21 group, whilst urine beta core was only just significantly increased in this group. Urine total oestriol was not significantly different from normal in the trisomy 21 group but was significantly lower in the trisomy 18 and Turner's groups.

Serum free beta hCG in the trisomy 21 group and the control population showed a Gaussian distribution after $\log_{10}$ transformation with Kolmogorov–Smirnov tests showing a probability of more than 0·2 for both groups. Table IV lists the statistical parameters associated with the distributions.

Urine free beta hCG, unlike in the second trimester (Spencer *et al.*, 1996), showed (Fig. 3) a significant deviation from linearity at higher levels even after $\log_{10}$ transformation (Kolmogorov–Smirnov probability of less than 0·01). The use of an upper boundary limit of 4·00 MOM resulted in exclusion of 20 per cent of the data but enabled Gaussian linearity to be established. In the trisomy

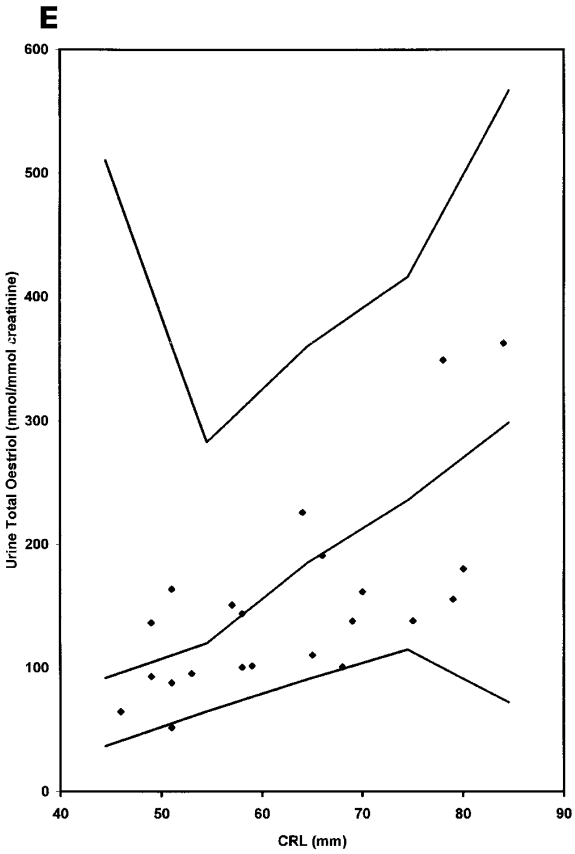

Fig. 1—(e)

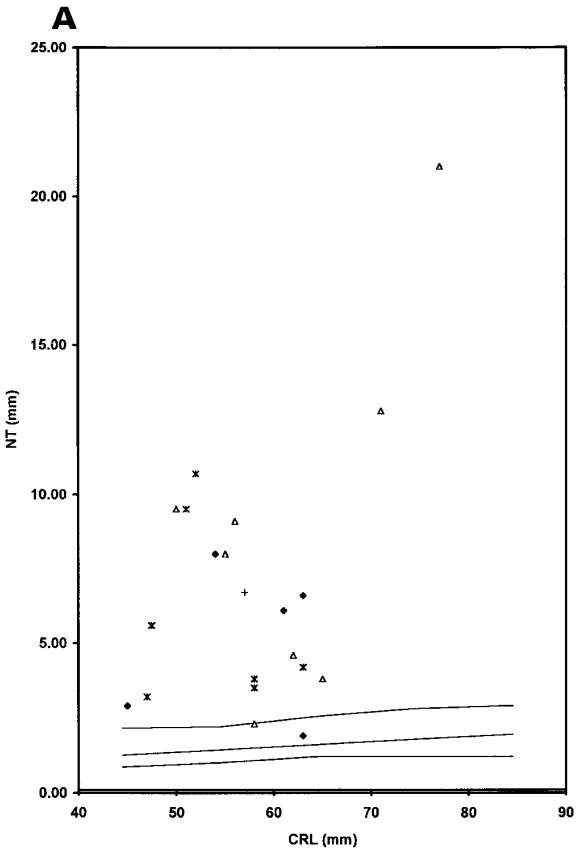

Fig. 2—(a)

Fig. 1—Individual results of the various marker levels for the 22 trisomy 21 cases plotted against gestational age measured by crown–rump length (mm) and alongside the fifth, 50th, and 95th centiles. (a) Nuchal translucency; (b) serum free beta hCG; (c) urine free beta hCG; (d) urine beta core; (e) urine total oestriol.

21 group, no deviation from a Gaussian distribution could be established after $\log_{10}$ transformation (P>0·10). Table IV shows the associated statistical parameters for the two groups.

Urine beta core also, unlike in the second trimester (Spencer *et al.*, 1996), showed (Fig. 4) a significant deviation from linearity at lower levels even after $\log_{10}$ transformation (Kolmogorov–Smirnov probability of less than 0·01). The use of a lower boundary limit of 0·10 MOM resulted in exclusion of 21 per cent of the data but enabled Gaussian linearity to be established. In the trisomy 21 group, no deviation from a Gaussian distribution could be established after $\log_{10}$ transformation (P>0·10). Table IV shows the associated statistical parameters for the two groups.

For both urine beta core and urine free beta hCG, the bimodal distributions represent a mixture of two Gaussian distributions and it was not possible to remove this biomodality by transformation of the data. As a best approximation, we have used the fitted mixture distributions (with truncation) to calculate risks.

Urine total oestriol showed a significant deviation from linearity (Fig. 5) at both extremes of the distribution even after $\log_{10}$ transformation (Kolmogorov–Smirnov probability of less than 0.01). The use of lower and upper boundary limits of 0·30 MOM and 3·0 MOM resulted in exclusion of only 3·5 per cent of the data but enabled Gaussian linearity to be established. In the trisomy 21 group, no deviation from a Gaussian distribution could be established after $\log_{10}$ transformation (P>0.10). Table IV shows the associated statistical parameters for the two groups.

When individual marker levels (as MOM) were compared against each other and against delta NT,

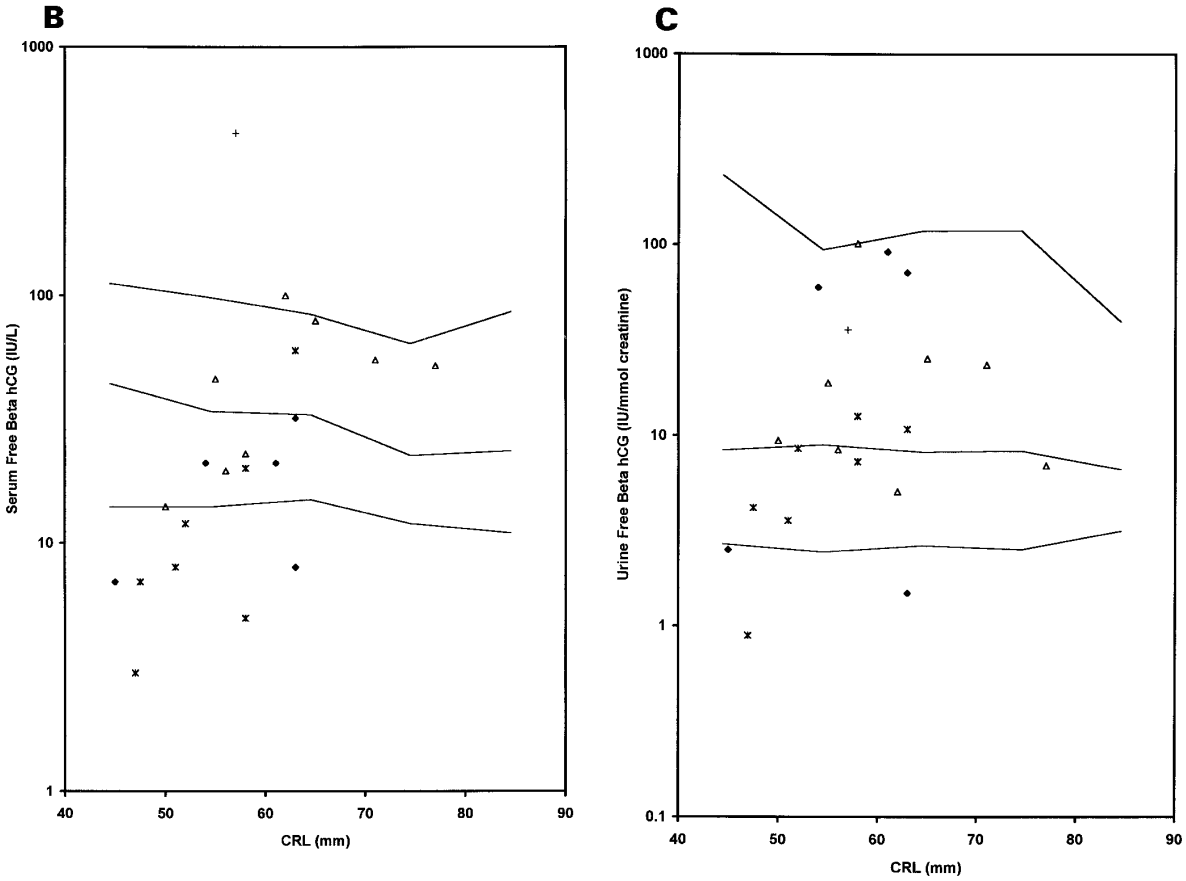

Fig. 2—(b and c)

the level of correlation shown in Table V was observed, with none of the correlations reaching statistical significance ($P>0{\cdot}05$ in all cases).

When the observed detection rates with individual analytes were examined at a fixed 5 per cent false-positive rate, serum free beta hCG identified 32 per cent (7/22) of cases of trisomy 21 and 71 per cent (5/7) of cases of trisomy 18 were less than the fifth centile. Urine free beta hCG identified only 9 per cent (2/22) of cases of trisomy 21 and 14 per cent (1/7) of cases of trisomy 18. Urine beta core identified only 14 per cent (3/22) of cases of trisomy 21 and 14 per cent (1/7) of cases of trisomy 18. Urine total oestriol identified only 5 per cent (1/22) of cases of trisomy 21 but 71 per cent (5/7) of cases of trisomy 18.

When Gaussian modelled detection rates with individual analytes were examined at the 5 per cent false-positive rate, serum free beta hCG would have identified 32 per cent of trisomy 21 cases, urine free beta hCG would have identified 18 per cent, and urine beta core and urine total oestriol would have identified no cases. This deviation from the observed detection rates emphasizes the inadequacy of the Gaussian model to deal with the distributions of urine analytes in the first trimester.

When the observed statistical parameters for urine free beta hCG, urine beta core, and urine total oestriol were used, along with those previously obtained for serum free beta hCG and delta NT, in the mathematical model of the pregnant population of England and Wales, detection rates using various marker combinations at a fixed 5 per cent false-positive rate rose from 32 per cent with urine total oestriol to 87 per cent when delta NT and serum free beta hCG were used (Table VI).

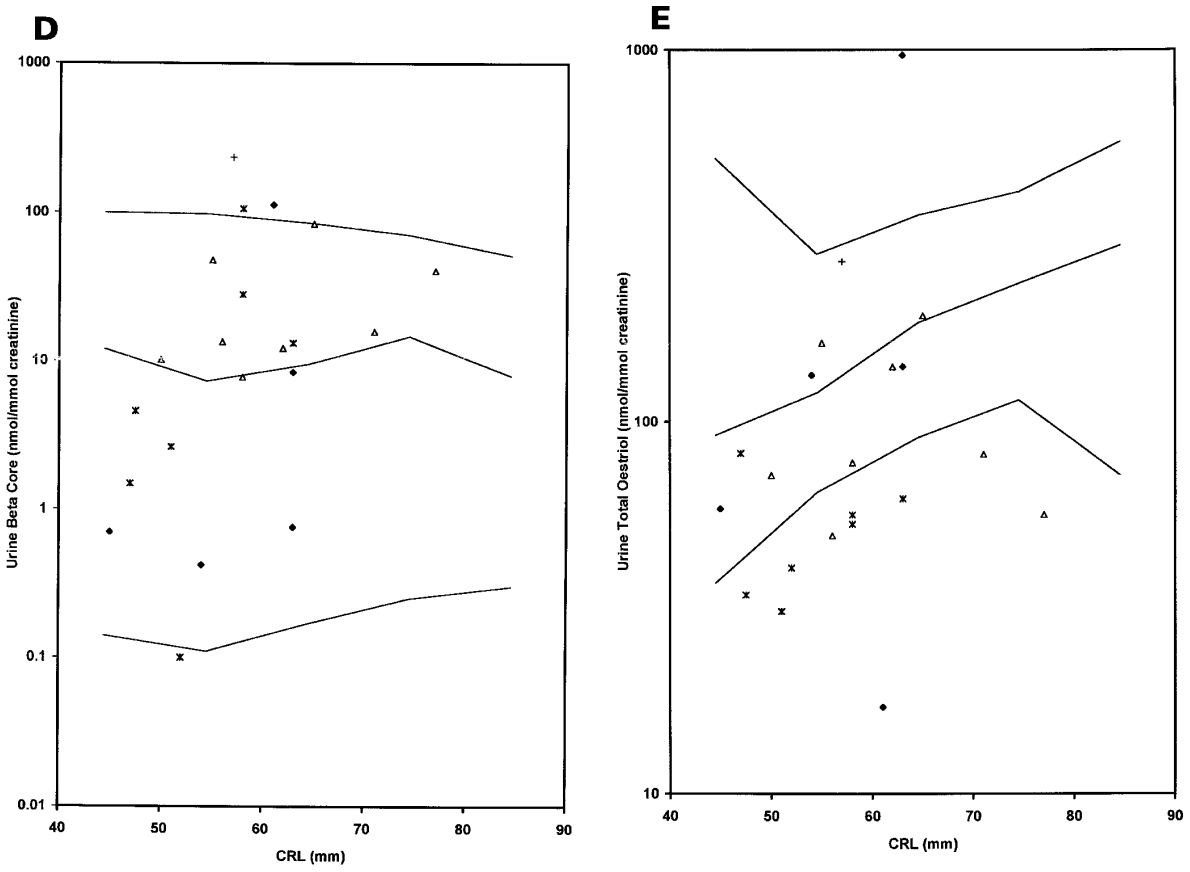

Fig. 2—(d and e)

Fig. 2—Individual results of the various marker levels for cases of trisomy 18 (*), trisomy 13 (◆), Turner's syndrome (△) and triploidy (+); plotted against gestational age measured by crown–rump length (mm) and alongside the fifth, 50th, and 95th centiles. (a) Nuchal translucency; (b) serum free beta hCG; (c) urine free beta hCG; (d) urine beta core; (e) urine total oestriol.

DISCUSSION

The serum free beta hCG concentration decreased with increasing gestational age and the median values obtained are consistent with previously published studies (Spencer *et al.*, 1994; Brizot *et al.*, 1995; Noble *et al.*, 1995; Krantz *et al.*, 1996; Wald *et al.*, 1996). The fall in serum free beta hCG was on average 1 IU/l (1 ng/ml) per day, as found by Berry *et al.* (1995). The observed standard deviation in the control group (0·2642) is very close to the 0·277 obtained in the large series by Berry *et al.* (1995) and is similar to that observed in smaller series (0·295, Spencer *et al.*, 1994; 0·2873, Wald *et al.*, 1996). In the Down's syndrome group, the measured standard deviation of 0·3732 is wider than that observed in a previous series (Spencer *et al.*, 1994) and wider than the 0·2870 in the series of Wald *et al.* (1996). The distribution of serum free beta hCG in both populations in the first trimester is very similar to that observed in the second trimester (Spencer *et al.*, 1992b). In this present study, serum free beta hCG alone identified 32 per cent of trisomy 21 cases at a 5 per cent false-positive rate; this is higher than the 18 per cent observed by Wald *et al.* (1996) but identical to that previously reported by Brizot *et al.* (1995). This compares with 44 per cent achieved in the second trimester (Spencer *et al.*, 1992b). In the trisomy 18 group, the median levels of serum free beta hCG were significantly lower than normal, confirming the initial observations of Spencer *et al.* (1992a) and Brizot *et al.* (1995). Furthermore, the levels appear on average to be even lower than

Table III—Median values and Mann–Whitney *U*-test results for the various markers in the major clinical groups

	Trisomy 21 (n=22)				Trisomy 13 (n=5)			
	Median	Range	Mann–Whitney z	Probability	Median	Range	Mann–Whitney z	Probability
Delta NT	2·33				4·56			
Serum free beta	1·72	0·21–5·76	3·44	0·0006	0·64	0·16–0·97	− 2·36	0·0181
Urine free beta	1·87	0·15–27·64	2·40	0·0162	6·71	0·18–11·19	0·47	0·6423
Urine beta core	2·91	0·01–20·33	1·96	0·0498	0·08	0·06–11·81	− 0·81	0·4187
Urine total oestriol	0·83	0·15–1·61	− 1·76	0·0790	0·76	0·09–5·23	− 0·75	0·4532
	Trisomy 18 (n=7)				Turner's syndrome (n=8)			
	Median	Range	Mann–Whitney z	Probability	Median	Range	Mann–Whitney z	Probability
Delta NT	2·63				7·10			
Serum free beta	0·24	0·07–1·82	− 3·15	0·0016	1·82	0·42–3·03	0·99	0·3199
Urine free beta	0·82	0·11–1·32	− 1·38	0·1674	1·58	0·62–11·43	0·95	0·3418
Urine beta core	0·38	0·01–13·57	− 0·27	0·7838	1·69	1·05–8·77	1·90	0·0568
Urine total oestriol	0·39	0·26–0·97	− 3·65	0·0003	0·63	0·24–1·37	− 2·55	0·0108

Table IV—Statistical parameters for the various analyte distributions in trisomy 21 and control pregnancies

	Serum free beta hCG	Urine free beta hCG	Urine beta core	Urine total oestriol
Log_{10} mean controls	0·0	0·0	0·0	0·0
Log_{10} SD controls	0·2642	0·5219	0·8235	0·2387
Log_{10} mean affected	0·212	0·230	0·254	− 0·067
Log_{10} SD affected	0·3732	0·5555	0·9379	0·1594
10th centile controls	0·48	0·37	0·03	0·56
50th centile controls	1·00	1·00	0·99	1·01
90th centile controls	2·30	7·54	6·16	1·78
10th centile affected	0·51	0·52	0·08	0·54
50th centile affected	1·72	1·87	2·91	0·83
90th centile affected	4·09	12·17	10·15	1·38

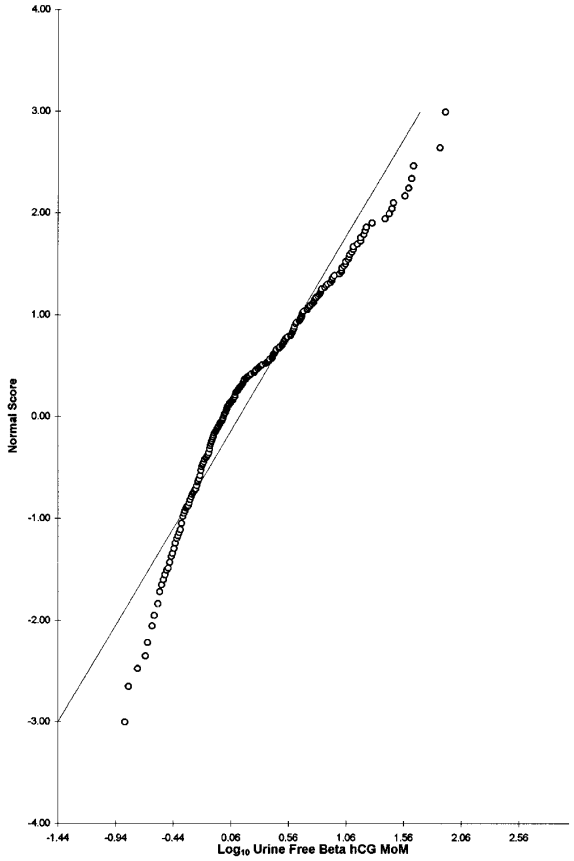

Fig. 3—Probability plot for urine free beta hCG in unaffected first-trimester pregnancies.

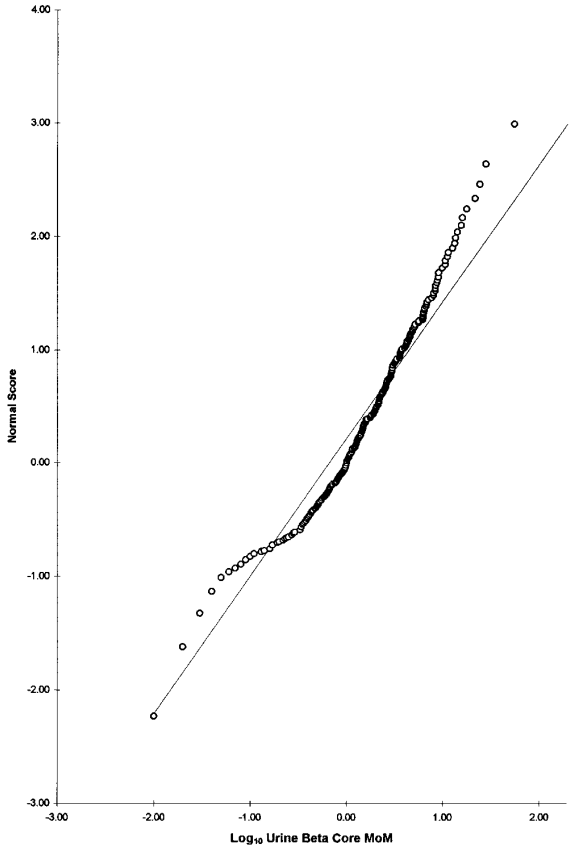

Fig. 4—Probability plot for urine beta core in unaffected first-trimester pregnancies.

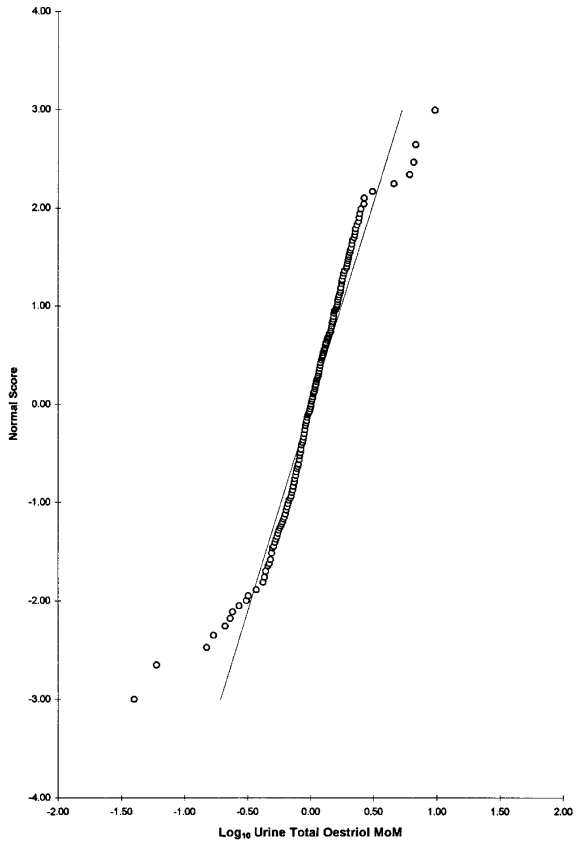

Fig. 5—Probability plot for urine total oestriol in unaffected first-trimester pregnancies

those observed in the second trimester (Spencer *et al.*, 1993). In the trisomy 13 group, the serum free beta hCG median levels were also significantly reduced, as observed previously by Brizot *et al.* (1995). In cases of Turner's syndrome, whilst the median values were increased this was not of statistical significance. In the second trimester, the serum free beta hCG values also tend to be increased (Laundon *et al.*, 1996). In the trisomy 21 group, the median MOM was significantly increased to 1·72; this is similar to the 1·85 MOM reported by one of us (Spencer *et al.*, 1992a) but less than the 2·20 reported by Macri *et al.* (1993) for an extended series of 39 cases and also lower than the 2·00 MOM in a series of 41 cases published by Brizot *et al.* (1995) and the extended series of an additional 41 cases (Noble *et al.*, 1995)

Table VI—Expected screening performance (detection rate) using various marker combinations in conjunction with maternal age at a fixed 5 per cent false-positive rate after ten simulations

	Detection rate (%)	95% confidence interval
Urine total oestriol	32	30–34
Urine beta core	34	31–37
Urine free beta hCG	36	33–39
Serum free beta hCG	44	42–46
Delta NT	82	80–84
Delta NT + urine total oestriol	83	81–85
Delta NT + urine beta core	84	81–87
Delta NT + urine free beta hCG	85	82–88
Delta NT + serum free beta hCG	87	85–89

Table V—Correlation coefficients between analytes and nuchal translucency

	Urine free beta hCG	Urine beta core	Urine total oestriol	Delta NT
Serum free beta hCG				
Controls	0·227	0·131	− 0·066	0·097
Affected	0·198	0·246	− 0·073	0·00
Urine free beta hCG				
Controls		0·009	− 0·354	− 0·258
Affected		0·101	− 0·062	− 0·014
Urine beta core				
Controls			0·060	0·020
Affected			0·066	− 0·136
Urine total oestriol				
Controls				0·044
Affected				0·103

in which the combined median was 2·00 MOM. The observed median of 1·72 is similar to that recently published by Wald *et al.* (1996) in a series of 77 cases. All together (excluding the present series), the world published series represents a total of 340 cases of trisomy 21 in the first trimester, for which the median MOM serum free beta hCG is 1·89. Correlation of serum free beta hCG with NT in both the trisomy 21 and the unaffected groups is very small, confirming the observations of previous studies (Brizot *et al.*, 1995; Noble *et al.*, 1995).

Levels of urine free beta hCG decline gradually across the first trimester but fall much less sharply than in serum. Levels of correlation between free beta hCG in serum and urine during the first trimester appear much smaller than the high (0·5682) correlation found in the second trimester. The observation of a low correlation in the first trimester is in agreement with the observations of Norman *et al.* (1987) at this time and the difference between the first and second trimester might suggest some change in the clearance rate of serum free beta hCG as gestation increases. The distribution of urine free beta hCG deviates significantly from a $\log_{10}$ Gaussian distribution at levels above 4 MOM, unlike in the second trimester, and the width of the distribution in both affected and unaffected cases is almost 1·7 times that found in the second trimester (Spencer *et al.*, 1996). The detection rate with urine free beta alone was much lower than in the second trimester and the median values for the trisomy 21 group were lower than the 2·47 observed in the second trimester whilst that for the trisomy 18 group was higher than the 0·22 observed in the second trimester (Spencer *et al.*, 1996).

Levels of urine beta core show only a small change with gestation which increases to a peak at around 13 weeks. Whilst our data are similar to those of our previous study (Spencer *et al.*, 1996), others have not observed this peak at 13 weeks (Cuckle *et al.*, 1994, 1995) and observed a steady decline from 11 weeks. Correlation with urine free beta hCG in the control group was much lower than the 0·3904 observed in the second trimester (Spencer *et al.*, 1996), but correlation with serum free beta hCG was as low as that observed in the second trimester. The distribution of urine beta core was non-Gaussian at low levels, which was not previously observed in the second trimester, although the width of the distribution in the first trimester was 2·1 times that observed in the second trimester. The median value in trisomy 21 cases was slightly higher than that observed in our previous second-trimester series (Spencer *et al.*, 1996) but still not as high as the value suggested by Cuckle *et al.* for the second trimester (Cuckle *et al.*, 1994, 1995). For trisomy 18, the median value was slightly higher than observed in a similar number of cases in the second trimester (Spencer *et al.*, 1996). The detection rate for urine beta core alone in the first trimester was very similar to the 21 per cent observed in our second-trimester study.

Urine total oestriol levels increased with increasing gestation, as similarly occurs for urine total oestrogens during the second trimester (Cuckle *et al.*, 1995). The level of correlation with other markers was not significant except for that with urine free beta hCG amongst the control group. As observed by Cuckle *et al.* (1995) for total oestrogens in the second trimester, first-trimester total oestriol levels deviate from Gaussian normality at the extremes of the distribution and require the use of similar truncation limits. The width of the distribution of total oestriol in the first trimester is similar to that for total oestrogens in the second trimester and shows a much tighter distribution than any of the other measured parameters in the first trimester. The median value of 0·83 amongst trisomy 21 cases is slightly higher than the 0·74 observed by Cuckle *et al.* (1995) for total oestrogens in the second trimester. Nevertheless, in the first trimester we confirm that, as stated by Cuckle *et al.* for the second trimester, urine total oestriol (oestrogen) is a less discriminating marker of trisomy 21 than either beta core or free beta hCG, with detection rates when combined with maternal age no better than 32–34 per cent. In comparison, detection rates for trisomy 18 are significantly better than with trisomy 21 and this adds support to the view that measurement of oestriol may be of greater value in screening for trisomy 18 (Palomaki *et al.*, 1995).

Of the markers available for use in screening for trisomy 21 in the first trimester, nuchal translucency measured under standardized conditions by trained personnel is clearly being demonstrated in a number of centres as the marker offering the greatest potential. Based on data from previous studies, we have shown that modelled detection rates of 82 per cent at a 5 per cent false-positive rate are consistent with observed detection rates of 75–80 per cent either in high-risk groups or in normal unselected populations. By combining biochemical markers with biophysical measurements,

further gains in the detection rate will be achievable. Serum free beta hCG, a marker with potential across a wide gestational window from 8 to 20 weeks, has been shown to add a further 5 per cent to the detection rate when used with maternal age and nuchal translucency, confirming the observation of a previous study (Noble *et al.*, 1995). The addition of the serum marker pregnancy-associated plasma protein-A to this combination may add a further 5 per cent to the detection rate but we still await the production of a viable commercial assay. Furthermore, any potential gain in detection will only be realized if blood samples can be collected prior to 11 weeks when the values of pregnancy-associated plasma protein-A in trisomy 21 cases are significantly lower than at the end of the first trimester (Bersinger *et al.*, 1994; Berry *et al.*, 1996; Wald *et al.*, 1996). Urine markers, however, whilst showing some disputed promise in the second trimester, appear to be of no extra value in the first trimester. Nuchal translucency, serum free beta hCG, and potentially pregnancy-associated plasma protein-A (Brizot *et al.*, 1994) remain the most likely direction of first-trimester screening in the next 5 years.

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