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Urinary Free Beta hCG, Beta Core Fragment and Total Oestriol as Markers of Down Syndrome in the Second Trimester of Pregnancy

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In a study of 69 random urine samples from cases of Down syndrome and 405 samples from unaffected pregnancies, we have assessed the value of various candidate markers that have been proposed as tools for screening for Down syndrome. We found that the marker urine free beta hCG in Down syndrome had a median MoM of 3·53 (95 per cent confidence interval 2·48–4·68) and at a 5 per cent cut-off would have identified 49 per cent (34/69) of cases. Urine beta core had a median MoM of 4·95 (3·87–8·62) and at a 5 per cent cut-off would have identified 39 per cent (27/69) of cases. Total oestriol had a median MoM of 0·65 (0·55–0·80) and at a 5 per cent cut-off would have identified 35 per cent (24/69) of cases. In conjunction with maternal age, the modelled detection rate increased to 55·8 per cent for free beta hCG, 49·8 per cent for beta core and 48·8 per cent for total oestriol. In combination free beta hCG, total oestriol and maternal age would have detected 68 per cent of cases for a 5 per cent false-positive rate. Using analyte ratios to obviate the need to correct for urine dilution in our study (rather than correcting to a fixed creatinine concentration) was not shown to be as effective as correcting using urine creatinine. Urine markers on the whole are unlikely to be of practical screening value considering the 85 per cent to 90 per cent detection rates achievable in the first trimester using a combiantion of ultrasound and maternal serum biochemistry.

KEY WORDS: Down syndrome screening; urine free beta hCG; urine beta core; urine total oestriol

INTRODUCTION

Over the last decade, screening for Down syndrome in the second trimester has become an established part of obstetric practice in many developed countries, primarily through the measurement of maternal serum biochemical markers. Of the biochemical markers that have been investigated in the second trimester free beta hCG in combination with AFP and maternal age can detect over 70 per cent of Down syndrome pregnancies at a 5 per cent false-positive rate (Spencer, 1994) and this compares favourably with triple-marker protocols (Macri and Spencer, 1996; Spencer, 1997a). Recent work has shown that screening is possible in the first trimester using a combination of ultrasound measurements of nuchal translucency thickness and the maternal serum markers free beta hCG and pregnancy associated plasma protein-A (PAPP-A) and that detection rates of 85–90 per cent may be achievable (Spencer *et al.*, 1992; Macri *et al.*, 1994; Spencer, 1997b; Brambati *et al.*, 1991; Spencer *et al.*, 1994; Wald *et al.*, 1996; Krantz *et al.*, 1996; Berry *et al.*, 1997; Nicolaides *et al.*, 1997; Grudzinskas and Ward, 1997; Cuckle, 1997; Spencer *et al.*, 1999). Matching this level of sensitivity in the second trimester depends on the recognition of better markers. One such avenue of investigation has been urine markers.

Cuckle *et al.* (1994) first reported that levels of the beta core fragment of hCG were markedly (6·24 MoM) elevated in the maternal urine of pregnancies affected by Down syndrome. Expanding on the initial 7-case study with an additional 17 cases, Cuckle *et al.* (1995) confirmed the initial observation with a median MoM of 6·02. Canick *et al.* (1995, 1996) in two separate studies further confirmed elevations close to 5 MoM. Studies by Hayashi and Kozu (1995) and Spencer *et al.* (1996) could not confirm such elevations leading to speculation over assay suitability and specificity (Cole, 1995). However, more recent reports from Isozaki *et al.* (1997), Hallahan *et al.* (1998) and Lam *et al.* (1997) using specific assays have also shown the median MoM to be much lower (2·4 to 4·1 MoM) than in the earlier studies. Similarly, four studies in the first trimester of pregnancy have also shown only a modest elevation of 1·1 to 2·9 MoM (Cuckle *et al.*, 1996; Macintosh *et al.*, 1997; Kornman *et al.*, 1997; Spencer *et al.*, 1997a).

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Table 1—Measured (regressed) median values of urine free beta hCG, urine beta core and urine total oestriol in the unaffected population

Gestational week	Number	Free beta hCG IU/mmol creatinine	Beta core ng/mmol creatinine	Total oestriol nmol/mmol creatinine
14	20	4·89 (4·61)	279·5 (243·8)	475·2 (554·1)
15	40	4·58 (4·20)	218·3 (209·4)	843·9 (815·7)
16	60	3·65 (3·83)	181·4 (179·9)	1010·5 (1077·4)
17	60	3·31 (3·49)	154·0 (154·5)	1419·9 (1339·0)
18	60	2·93 (3·18)	104·4 (132·7)	1700·5 (1600·7)
19	60	2·79 (2·90)	107·6 (114·0)	1896·6 (1862·3)
20	50	3·00 (2·64)	92·3 (97·9)	2118·0 (2124·0)
21	35	2·83 (2·40)	71·3 (84·1)	2325·9 (2385·6)
22	20	2·97 (2·19)	101·5 (72·3)	2615·6 (2647·3)
23	—	(2·00)	(62·1)	(2909·0)
24	—	(1·82)	(53·3)	(3171·0)
26	—	(1·51)	(39·4)	(3694·0)

In addition to beta core fragment (sometimes referred to as UGP or UGF) a small number of studies have also measured free beta hCG in urine. In these studies (Spencer *et al*., 1996; Cole *et al*., 1997a; Hayashi *et al*., 1996; Hallahan *et al*., 1998; Spencer *et al*., 1997a; Baviera, 1997; Cole *et al*., 1997c) levels in pregnancies affected by Down syndrome in either the first or second trimester are two to three times higher than normal and are similar to the levels observed in maternal serum (Macri *et al*., 1994) and amniotic fluid (Spencer *et al*., 1997b).

As early as 1972 Jorgensen and Trolle (1972) observed low levels of total oestriol in the third trimester of urine from women with pregnancies affected by Down syndrome. Canick *et al*. (1988) some 16 years later extended this association by demonstrating low levels of unconjugated oestriol in the maternal serum of women with pregnancies affected by Down syndrome and some five years later (Cuckle *et al*. (1995) confirmed low levels of total oestrogens in second-trimester maternal urine of affected cases and Spencer *et al*. (1997b) confirmed these low levels in the first trimester of affected cases. Recently, it has been proposed by Cole *et al*. (1997c) and by Kellner *et al*. (1997), that a ratio of beta core:total oestriol may be of value in obviating the need to employ a creatinine correction for urine dilution, since by taking the ratio, dilution effects would be cancelled out. Both workers have reported detection rates close to 80 per cent at a 5 per cent false-positive rate using this procedure.

In view of the varied data published on urine beta core in the second trimester we have sought to investigate further the levels of beta core, free beta hCG and total oestriol in a new large set of Down syndrome cases. We have also sought in this present study to evaluate the use of analyte ratios with total oestriol as proposed by Cole *et al*. (1997c) and Kellner *et al*. (1997).

MATERIALS AND METHODS

Study population

Women with a pregnancy affected by Down syndrome confirmed by amniocentesis were asked to provide a random urine sample. Urine from a control group of 405 women attending the antenatal clinic with normal pregnancy outcomes were similarly collected. All samples were collected into sterile containers and stored at −20°C prior to being shipped frozen to the analysis laboratory (Harold Wood Hospital) except for samples collected in the locality of the testing laboratory which were stored at −20°C until analysis. In all, 69 samples were available from cases of Down syndrome. The reasons for prenatal diagnosis were advance maternal age in 27 cases, increased risk of Down syndrome based on second-trimester biochemical screening in 38 cases and ultrasound signs in 4 cases.

The median maternal age of the control group was 34·5 (range 23–43) and that of the Down syndrome group 36·0 (range 21–44). The median gestation of the control group was 18 (range 14–22) and that of the Down syndrome group 19 (range 14–26).

Analytical methods

Free beta hCG was measured with the CIS immunoradiometric assay (CIS (U.K.) Ltd., High Wycombe, Bucks, U.K.). The assay uses the capture antibody FBT11 which does not cross-react with beta core. The analytical performance of this assay has been described previously (Spencer *et al*., 1992; Macri *et al*., 1993). All urine samples were analysed diluted one in five with assay diluent or neat if more appropriate. The samples were analysed in five separate batches with the sample outcome blinded to the assayist.

Table 2—Population parameters in the unaffected and affected populations for the various analytes

Statistic	Control	Down syndrome
Urine free beta $\log_{10}$ mean	− 0·041	0·559
Urine free beta $\log_{10}$ SD	0·3920	0·4895
Urine beta core $\log_{10}$ mean	0·063	0·747
Urine beta core $\log_{10}$ SD	0·5040	0·5972
Total oestriol $\log_{10}$ mean	0·00	− 0·164
Total oestriol $\log_{10}$ SD	0·1940	0·3932
Correlation free beta versus beta core	0·1637	0·2569
Correlation free beta versus total oestriol	− 0·0346	0·1005
Correlation total oestriol versus beta core	0·0387	0·2766
Beta core: total oestriol $\log_{10}$ mean	0·023	0·875
Beta core: total oestriol $\log_{10}$ SD	0·5207	0·8616
Free beta: total oestriol $\log_{10}$ mean	0·024	0·895
Free beta: total oestriol $\log_{10}$ SD	0·5208	0·8488

Urine beta core was measured using the UGF-EIA Toa kit (Toagosei Co. Ltd., Minato-Ku, Tokyo 105-8419, Japan). The assay has been shown to be specific for the beta core fragment only (Cole, 1995) and shown to give the highest median MoM and the highest projected detection rate in a small comparison of 12 different assays for detecting hCG-related molecules in the urine of 14 Down syndrome cases (Cole *et al*., 1997c). The assay had a between-assay precision of 6·0 per cent at 156 pg/ml. All urine samples were analysed at an initial dilution of between 1 in 50 and 1 in 2500 in assay diluent. The samples were analysed in 10 separate batches with the sample outcome blinded to the assayist.

Urine total oestriol was measured using the Ortho-Clinical Diagnostics Oestriol (total) II radioimmunoassay kit (Ortho-Clinical Diagnostics, Amersham, U.K.). Urine samples were analysed at a dilution of 1 in 50 in normal male serum. The samples were analysed in five separate batches with the sample outcome blinded to the assayist. The between-assay precision was 4 per cent at 50 nmol/l, 2·5 per cent at 200 nmol/l and 3 per cent at 600 nmol/l.

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 expressed as ng/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 21 dilution on 0·9 per cent saline. Additionally, the urine beta core and free beta hCG concentrations were expressed as ratios against total oestriol concentration as described by Cole *et al*. (1997b) and Kellner *et al*. (1997).

Statistical analysis

To take into account gestational age variation in analyte levels, all corrected analyte values (either creatinine corrected or ratios) were converted to multiples of the median based on linear or $\log_{10}$ regressed weighted medians by completed week of pregnancy. In order to assess the performance of individual markers or their combination, standard statistical modelling techniques (Royston and Thompson, 1992) were used. Using the established population parameters, a series of random MoM values were generated from within the distributions of the affected and unaffected populations. These values were then used to calculate likelihood ratios using the univariate or bivariate procedure (Reynolds and Penney, 1990) and the expected Down syndrome detection rate was calculated at a given false-positive rate (5 per cent) using the maternal age distribution of England and Wales (Office of Population Censuses and Surveys, 1991–1994). Statistical analysis of data was performed using Astute, a statistical software add-in for Microsoft Excel 5 (DDU Software, University of Leeds, U.K.). Statistical parameters such as log SD were calculated using all of the MoM data for each analyte.

RESULTS

Table 1 shows the observed and best-fit weighted medians of urine beta core, urine free beta hCG and urine total oestriol for the unaffected population. Each of the three analytes investigated in this study showed varying degrees of fit to $\log_{10}$ Gaussian distribution in both the unaffected and Down syndrome populations when tested using the Kolmogorov and Anderson Darling tests of linearity at the 5 per cent levels. There was also a reasonable fit to $\log_{10}$ Gaussian distributions for the ratios of the analytes. The parameters of the distributions for individual analytes and for the ratios are summarized in Table 2.

The distributions of analytes results (MoM) with gestation in the Down syndrome pregnancies are shown in Fig. 1 for free beta hCG, in Fig. 2 for beta core and in Fig. 3 for total oestriol. The corresponding distributions for the ratios (in MoM) are shown in Fig. 4 for free beta hCG:total oestriol and in Fig. 5 for beta

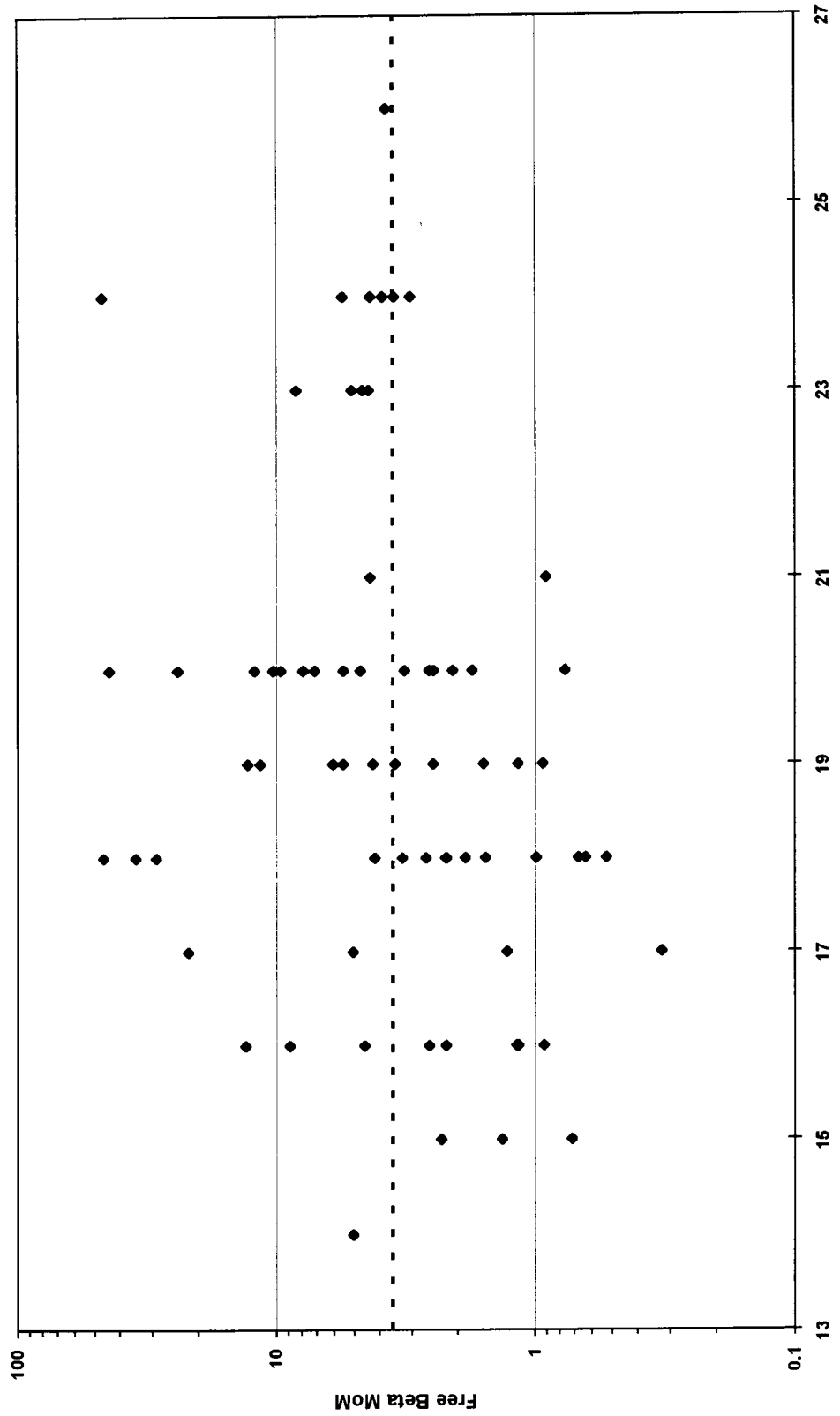

Fig. 1—Urine free beta hCG MoM in 69 T21 cases and the 95th centile of normality (– – –)

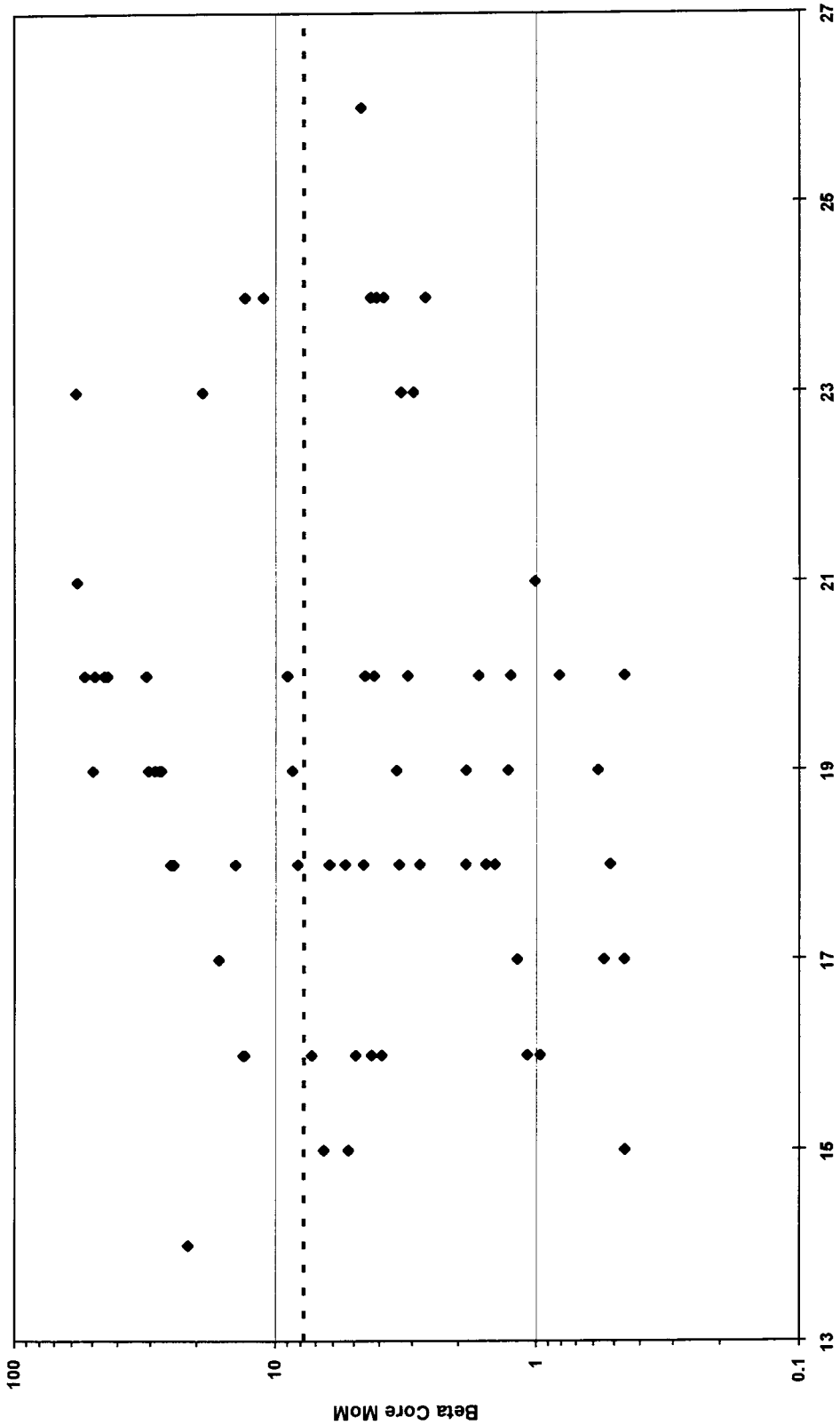

Fig. 2—Urine beta core MoM in 69 T21 cases and the 95th centile of normality (– – –)

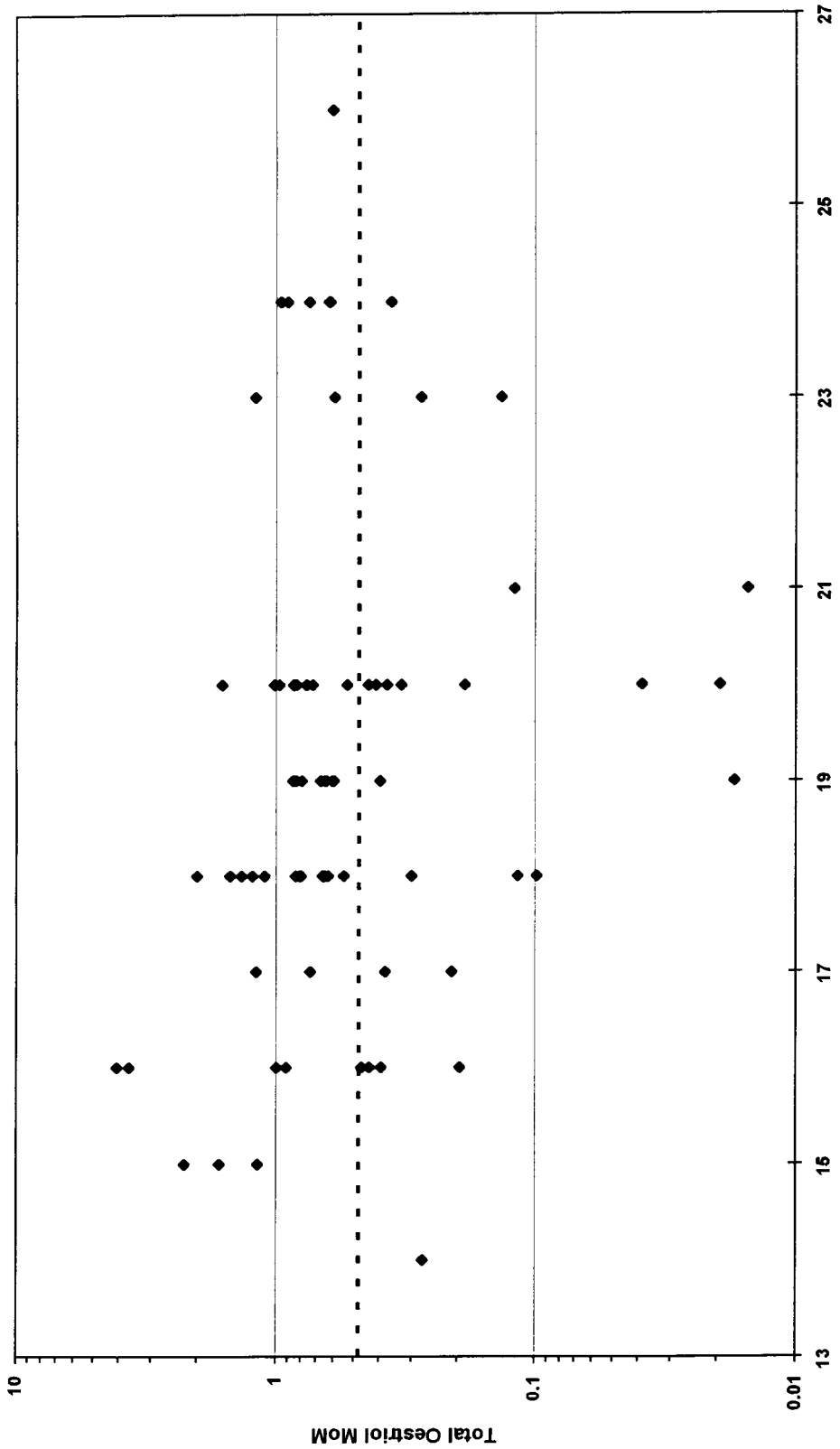

Fig. 3—Urine total oestriol MoM in 69 T21 cases and the 5th centile of normality (– – –)

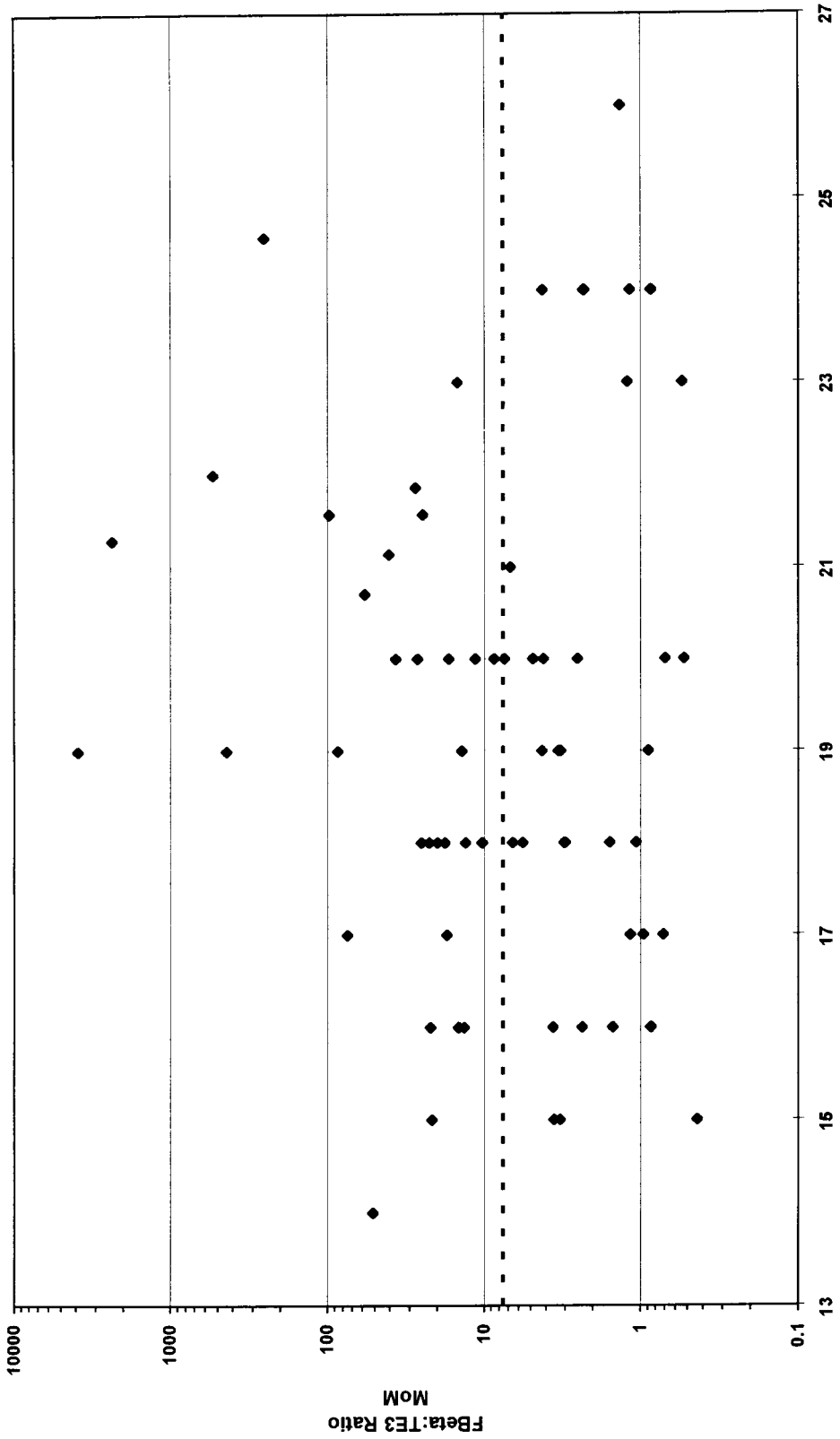

Fig. 4—Urine free beta hCG: total oestriol ratio (MoM) in 69 T21 cases and the 95th centile of normality (– – –)

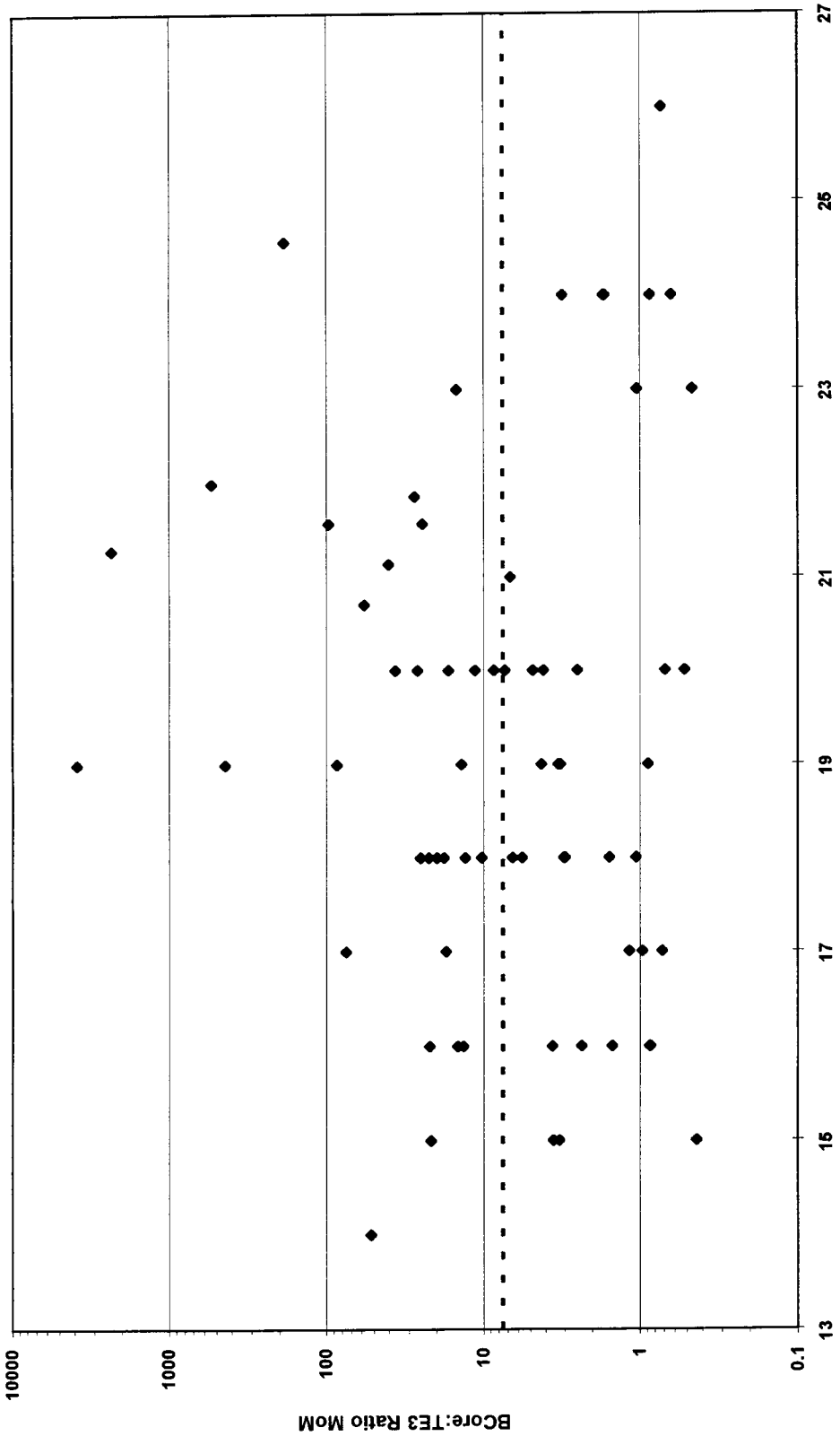

Fig. 5—Urine beta core: total oestriol ratio (MoM) in 69 T21 cases and the 95th centile of normality (– – –)

Table 3—Meta analysis of published data for urine beta core in cases of Down syndrome

Study	Number of cases	Median MoM	Comment
First trimester			
Cuckle *et al.* (1996)	9	1·06	
Macintosh *et al.* (1997)	9	1·16	
Kornman *et al.* (1997)	5	1·30	
Spencer *et al.* (1997a)	22	2·91	
Hallahan *et al.* (1998)	5	2·89	
Total	50	2·10	
Second trimester			
Cuckle *et al.* (1994)	7	6·24	Exclude, data included in Cuckle *et al.*, 1995 study
Cuckle *et al.* (1995)	24	6·02	
Canick *et al.* (1995)	14	5·34	Exclude, data included in Kellner *et al.*, 1997
Canick *et al.* (1996)	18	5·02	
Hayashi and Kozu (1995)	5	1·33	
Spencer *et al.* (1996)	29	2·35	
Isozaki *et al.* (1997)	13	4·10	
Hallahan *et al.* (1998)	8	2·18	
Lam *et al.* (1997)	29	3·44	
Kellner *et al.* (1997)	32	5·42	
Cole *et al.* (1997b)	12	4·50	Exclude, data included in Isozaki *et al.*, 1997 study
Total	158	4·14	

core:total oestriol. The correlation coefficients between pairs of markers in the Down syndrome and unaffected pregnancies, respectively, were: for free beta hCG versus beta core, 0·2569 and 0·1637; for free beta hCG versus total oestriol, 0·1005 and − 0·0346; and for total oestriol versus beta core, 0·2766 and 0·0387.

The detection rates achieved by different analytes and their combination when the population parameters in Table 2 were used to simulate population detection rates using the England and Wales population model at a fixed 5 per cent false-positive rate, revealed rates varying from 48·8 per cent to 68·4 per cent for the following combinations. Total oestriol—48·8 per cent; beta core—49·8 per cent; free beta hCG—55·8 per cent; beta core:total oestriol ratio—58·1 per cent; free beta hCG:total oestriol ratio—63·8 per cent; free beta hCG and beta core—66·5 per cent; beta core and total oestriol—67·7 per cent; free beta hCG and total oestriol—68·4 per cent.

DISCUSSION

In the past few years a number of studies have investigated the potential value of markers in urine as a vehicle for screening for Down syndrome. Of the markers studied, beta core showed some initial promise in the second trimester but further studies have been unable to reproduce the median values in excess of 6 MoM showed by the early studies of Cuckle (Cuckle *et al.*, 1994, 1995). There may be many reasons for the wide variation in published data as shown in the meta analysis (Table 3). These reasons may include differences in assay specificity; errors due to the need to dilute samples for analysis in the order of 1 in 2000 to 1 in 30 000; matrix errors within the assay as a result of such high dilution; and variation in the sample collection and storage conditions. The question mark over assay specificity was raised by Cole (1995) and investigated further by Cole *et al.* (1997c). In this later study they showed that the Wako assay (with an equal detection for beta core fragment and free beta subunit) gave the lowest median MoM in a study of 14 Down syndrome cases and the assay used in our present study gave the highest median MoM. Of the reported studies only that of Hayashi and Kozu (1995) has used the non-specific Wako assay and this may have explained the low levels observed in their study. All other published data have used specific assays for beta core which have been targeted at tumour marker levels of beta core and are not designed for the massive amounts of beta core present in the first or second trimester of pregnancy. Most studies have required sample dilutions of the order of 5000 to 30 000 prior to analysis. Such high dilutions can obviously introduce analytical error in addition to possibly compromising the assay kinetics due to various matrix effects. None of the published studies have shown any data (such as recovery or dilution linearity) validating the assay performance at such extreme dilutions. In order to pursue further any possible value of beta core in screening for Down syndrome, specifically designed, validated and targeted first and second trimester pregnancy urine assays should be developed.

The data from our present urine study represent the largest single series of reported Down syndrome cases and has used an assay confirmed to be specific for beta core (Cole *et al.*, 1997c). Our study has confirmed that urine beta core is elevated in Down syndrome cases, with a median MoM of 4·95 being similar to the consensus (Table 3). The median MoM is higher than in our previous studies using the Chiron (Ciba

Corning/Triton) assay. If we exclude those samples beyond 22 weeks for which we had no control population and extrapolated medians, the median MoM and SD were not significantly different from the total data (5·3 MoM versus 5·0 MoM and 0·595 versus 0·597 for the SD). The median MoM from those cases identified by maternal serum screening was not significantly different from that obtained for those cases identified by advanced maternal age or ultrasound findings (5·25 MoM versus 4·82 MoM). The distribution of $\log_{10}$ beta core MoM in both the controls and Down syndrome populations fitted a Gaussian distribution well as has been shown in previous studies (Spencer *et al.*, 1996; Kellner *et al.*, 1997; Lam *et al.*, 1997). The width of the Down syndrome distribution in this present study is considerably wider than in our previous study (0·60 versus 0·26) but narrower than in our first-trimester series (0·94). Our estimate is also wider than the 0·31 to 0·37 observed by Lam *et al.* (1997), Kellner *et al.* (1997) and Cuckle *et al.* (1995) in the only other large-scale studies. As a consequence, our reported detection rate (at a 5 per cent false-positive rate) with beta core alone is 39 per cent. This is higher than our previous estimate of 21 per cent (Spencer *et al.*, 1996), consistent with the estimates of Hallahan *et al.* (1998) and Lam *et al.* (1997), but lower than the 58–68 per cent of Kellner *et al.* (1997), Isozaki *et al.* (1997) and Cuckle *et al.* (1995).

When combined with maternal age, the detection rate with beta core increased to 49·8 per cent compared with 41 per cent in our previous study (Spencer *et al.*, 1996). This rate is considerably lower than the 70–80 per cent observed by Kellner *et al.* (1997) and Cuckle *et al.* (1995), but is consistent with the 44 per cent obtained by Hallahan *et al.* (1998). We are unable now in two independent studies using two different methods of estimating beta core to confirm the early promise shown by this marker. In our hands as a single marker it performs no better than maternal serum free beta hCG and maternal age, and certainly poorer than the 70 per cent achieved by the maternal serum AFP/free beta hCG/maternal age combination (Macri and Spencer, 1996; Spencer, 1994, 1997a,b).

Considerably fewer studies have focused on the measurement of free beta hCG in urine, but compared with beta core there is a large body of agreement (Table 4) between the studies, despite claims of 'significantly greater stability problems' for free beta hCG in urine (Cole *et al.*, 1997a). This high level of agreement may reflect the fact that concentrations of free beta hCG in urine are similar to serum and therefore samples do not require dilution. Secondly, all the published studies use either the FBT11 antibody to free beta hCG or use a modified version of an assay shown to have excellent agreement with FBT11-based methods (Macri *et al.*, 1993).

The data from our present study confirm our earlier data (Spencer *et al.*, 1996). The median MoM in Down syndrome cases is 3·53, consistent with all other published series (Table 4) in the second trimester. If we exclude those samples beyond 22 weeks for which we had no control population and extrapolated medians, the median MoM was significantly lower than from the total data (2·62 MoM versus 3·53 MoM). The median MoM from those cases identified by maternal serum screening was surprisingly lower than that from cases identified by advanced maternal age or ultrasound findings (3·99 MoM versus 3·10 MoM). As in our previous study the log-transformed free beta MoMs showed a good fit to a Gaussian distribution in both populations. The measured standard deviations in both populations were slightly wider than in our previous study but still consistent with the range for controls of 0·27–0·39 from the studies of Spencer *et al.* (1996), Hallahan *et al.* (1998), Cole *et al.* (1997a,c) and the range 0·30–0·55 in Down syndrome cases from the same group of studies.

Table 4—Meta analysis of published data for urine free beta hCG in cases of Down syndrome

Study	Number of cases	Median MoM
First trimester		
Spencer *et al.* (1997a)	22	1·81
Baviera (1997)	6	2·12
Hallahan *et al.* (1998)	5	1·40
Total	36	1·71
Second trimester		
Spencer *et al.* (1996)	29	2·47
Hayashi *et al.* (1996)	3	3·52
Cole *et al.* (1997a)	13	3·90
Cole *et al.* (1997c)	14	2·80
Hallahan *et al.* (1998)	8	2·84
Total	67	2·86

The observed detection rate of 49 per cent for free beta hCG (at a 5 per cent false-positive rate) is in agreement with our 1996 figure of 41 per cent and the 54 per cent observed by Cole *et al.* (1997a). In combination with maternal age our figure of 56 per cent detection is higher than the 44 per cent observed by Hallahan *et al.* (1998). In this study and our previous study (Spencer *et al.*, 1996) free beta hCG in urine was a better discriminator of Down syndrome than urine beta core.

Few data have been published with respect to urine oestriol. Cuckle *et al.* (1995) confirmed the earlier findings of Jorgensen and Trolle (1972) of low levels of total oestrogens in the urine of pregnancies affected by Down syndrome and recently two other studies have shown low levels of total oestriol (Cole *et al.*, 1997b; Kellner *et al.*, 1997). Our present study confirms low levels of urine total oestriol in the second trimester of pregnancies affected by Down syndrome, with our median MoM of 0·65 being close to the consensus from the other studies (Table 5). If we exclude those samples beyond 22 weeks for which we had no control population and extrapolated medians, the median MoM was not significantly different from the total data (0·66 MoM versus 0·65). The median MoM from those cases identified by maternal serum screening was significantly higher than from cases identified by

Table 5—Meta analysis of published data for urine oestriol in cases of Down syndrome

Study	Number of cases	Median MoM
First trimester		
Spencer *et al.* (1997a)	22	0·83
Second trimester		
Cuckle *et al.* (1995)	24	0·74 (total oestrogens)
Cole *et al.* (1997b)	12	0·33
Kellner *et al.* (1997)	32	0·64
Total	68	0·62

advanced maternal age or ultrasound findings (0·91 MoM versus 0·59 MoM). The population distributions for controls shows a similar width to that found by Kellner *et al.* (1997) and Cuckle *et al.* (1996) but considerably tighter than that observed by Cole *et al.* (1997b). In the Down syndrome group the standard deviation was in agreement with that of Cole *et al.* (1997b), but higher than observed by Kellner *et al.* (1997) or Cuckle *et al.* (1995). Our detection rate using total oestriol alone (at a 5 per cent false-positive rate) is similar to the 42 per cent found by Cole *et al.* (1997b) but higher than the 22 per cent observed by Kellner *et al.* (1997). When combined with maternal age and detection rate was very similar to that achieved with beta core and maternal age.

When the analytes were combined together in a bivariate manner, adding urine total oestriol to either urine free beta hCG or urine beta core and maternal age, detection rates increased by some 12·6 per cent and 17·9 per cent respectively. This is considerably higher than the 6 per cent increase observed by Kellner *et al.* (1997) for the beta core–total oestriol combination, largely as a result of the poorer performance of total oestriol in the Kellner study. The studies of Kellner *et al.* (1997) and Cole *et al.* (1997b) showed combined beta core/total oestriol/maternal age detection rates of 80 per cent and 79 per cent, our present study can only show a detection rate of 68 per cent, somewhat poorer than suggested by these authors. The detection rate combining either beta core or free beta hCG with total oestriol was largely comparable.

Both Cole and Kellner have proposed using the raw beta core and total oestriol concentrations (uncorrected for creatinine) as a ratio and then using this ratio as if it were an individual analyte. They have suggested that this would obviate the need to measure creatinine, since the ratio would take into account the varying urine dilution. Our data show that the ratios approximate a Gaussian distribution, confirming the evidence of Kellner *et al.* (1997). The median MoM free beta:total oestriol ratio in Down syndrome was 6·55, which at a 5 per cent false-positive rate alone would have identified 45 per cent of cases, somewhat higher than with total oestriol alone but less than with free beta hCG alone. The median MoM beta core:total oestriol ratio in Down syndrome was 6·53, which would have identified 43 per cent of cases (as a 5 per cent false-positive rate). This compares with 9·32 MoM and a 62·5 per cent detection in the study of Kellner and 13 MoM and 75 per cent detection in the Cole study. When combined with maternal age in a univariate way, beta core:total oestriol and free beta hCG:total oestriol ratios showed 58 per cent and 64 per cent detection rates. This was less than predicted using the creatinine-corrected markers in a bivariate way (68 per cent). This observation is therefore contrary to the studies of Kellner *et al.* (1997) and Cole *et al.* (1997b) who found that the detection rates were comparable between the ratio and the creatinine-adjusted marker approaches.

In conclusion, therefore, this present study confirms that free beta hCG and beta core are elevated and total oestriol is lowered in the urine of pregnancies affected by Down syndrome in the second trimester. The wider standard deviation observed in our new study, however, results in detection rates being much lower than predicted from previous studies but consistent with our previous observations (Spencer *et al.*, 1996). The possible use of free beta hCG as a urine marker confirms our previous study (Spencer *et al.*, 1996). The addition of total oestriol to urine screening with free beta hCG could increase the detection rate to 68 per cent which is comparable with that achieved in second-trimester maternal serum screening, but we cannot confirm any benefits of using the ratio approach. Further evaluation of the use of urine marker screening using beta core is hampered by the lack of availability of suitably targeted and validated assays for second and first trimester pregnancy samples, although it is unlikely on the evidence so far that this would surpass the 85–90 per cent detection rate achievable in the first trimester using nuchal translucency, serum free beta hCG and serum PAPP-A.

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