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URINE FREE BETA hCG AND BETA CORE IN PREGNANCIES AFFECTED BY DOWN'S SYNDROME

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SUMMARY

Urine beta core was shown in recent studies to be markedly elevated in pregnancies affected by Down's syndrome in the late second trimester. Free beta human chorionic gonadotropin (hCG) has also been shown to be the most discriminatory maternal serum marker of Down's syndrome. Since free beta hCG is rapidly cleared from the maternal circulation, we have carried out a study to evaluate whether free beta hCG is elevated in the urine of pregnancies affected by Down's syndrome and to investigate whether urine beta core or urine free beta hCG may be used as possible screening markers. Urine samples from 29 cases of Down's syndrome, three cases of trisomy 18, and 400 control pregnancies were analysed for the two prospective markers. Results were corrected for urine concentration by expressing marker concentrations at a fixed creatinine concentration and then expressing the results as multiples of the median for unaffected pregnancies of the same gestation. The median value of beta core in the Down's syndrome pregnancies was 2·35 compared with 2·47 for free beta hCG. Free beta hCG distributions were closely similar to those in maternal serum. Using free beta hCG, we predict Down's syndrome detection rates of 58 per cent at a 5 per cent false-positive rate. Using beta core, however, this rate fell to 41 per cent. Measurement of free beta hCG in urine may present a feasible route for screening pregnant populations, particularly where community-based obstetric care is the norm and/or if early first-trimester screening becomes a reality.

KEY WORDS: free beta hCG; Down's syndrome; beta core; prenatal screening; urinalysis

INTRODUCTION

The measurement of free beta human chorionic gonadotropin (hCG) in maternal serum has been shown to be the single most effective marker of Down's syndrome in both the first and the second trimester of pregnancy (Spencer *et al.*, 1992, 1994). In the second trimester, in combination with maternal serum alpha-fetoprotein (AFP) and maternal age, screening programmes for Down's syndrome have been developed. In routine prospective screening practice, this simple two-analyte approach has been shown to achieve detection rates of 75 per cent at a 5 per cent false-positive rate when screening women of all ages (Spencer, 1994) or when screening only women under 35 years (Macri *et al.*, 1994, 1996; Hsu *et al.*, 1995). Mathematical models have predicted that free beta hCG combined with pregnancy-associated plasma protein A (PAPP-A) and maternal age in the first trimester of pregnancy could also achieve detection rates of 75 per cent (Cuckle, 1994) at a 5 per cent false-positive rate. In practice, rates of 63 per cent have been achieved in one study (Krantz *et al.*, 1996).

Elevated serum levels of free beta hCG are also reflected in amniotic fluid, with levels in Down's syndrome approaching 2·4 multiples of the median (MOM) (Spencer *et al.*, 1993b). In urine, the major metabolic excretion product of intact hCG involves the renal parenchymal cell breakdown of the beta subunit to a small molecular weight

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component referred to as beta core (10 kD) and this represents over 90 per cent of the immunoreactive hCG in urine from mid-pregnancy (Kato and Braunstein, 1983). Free beta hCG, on the other hand, because of its small molecular weight (23 kD) has a high renal clearance rate and is cleared from the circulation and excreted some 10–24 times more rapidly than those subunits combined as intact hCG (Cole, 1988; Wehmann and Nisula, 1979).

Cuckle *et al.* (1994) showed in a preliminary study that urinary beta core levels in seven cases of Down's syndrome ranged from 2·39 MOM to 11·91 MOM with a median of 6·24 MOM and in one case of trisomy 18, the value was very low (0·02 MOM). Hayashi and Kozu (1995), using a different assay, shortly after reported low levels in one case of trisomy 18 (0·18 MOM) but found levels in five cases of Down's syndrome ranging from 0·55 MOM to 2·99 MOM with a median of 1·33 MOM, significantly lower than found by Cuckle *et al.* (1994).

In a follow-up study including 24 Down's syndrome cases, Cuckle *et al.* (1995) found beta core values in Down's syndrome ranging from 1·01 MOM to 15·70 MOM with a median of 6·02 MOM, thus confirming their initial study. Similarly, Canick *et al.* (1995), using a different assay yet again, showed levels of beta core ranging from 2·71 MOM to 12·57 MOM (median 5·34) in some 14 cases of Down's syndrome.

On the basis of the known superiority of free beta hCG in serum screening and the observed faster clearance rate of the free beta subunit and the comparable values obtained in amniotic fluid in affected cases, we postulate that in urine we may also find raised levels of free beta hCG in pregnancies affected by Down's syndrome. In this study, we therefore sought to establish if free beta hCG is elevated in the urine of pregnancies affected by Down's syndrome and to confirm if beta core is also elevated in such pregnancies. A preliminary report of part of this study has been presented as an abstract (Spencer *et al.*, 1995).

MATERIALS AND METHODS

Women with an aneuploid pregnancy confirmed by amniocentesis or chorionic villus sampling (CVS) were asked to provide a random urine sample. In all, 29 samples were available from cases of Down's syndrome and five samples from cases of trisomy 18. The reasons for prenatal diagnosis in these cases were advanced maternal age in 15 cases of Down's syndrome and three cases of trisomy 18, ultrasound-visible abnormalities in two cases of Down's syndrome, previous genetic history in one case of Down's syndrome and one case of trisomy 18, and abnormal maternal serum screening results in 11 cases of Down's syndrome and one case of trisomy 18. All samples were collected into sterile containers and stored at −20°C prior to refrigerated shipment to the analysis laboratory at Oldchurch Hospital, where the samples were again kept at −20°C prior to analysis. A group of 400 control samples from unaffected pregnancies were collected across the gestational range 9–22 weeks from one centre (Oldchurch Hospital) to establish a suitable reference range at each gestational week. Samples were collected as for the affected group and stored at −20°C prior to analysis. Table I shows the population descriptors for the affected and unaffected groups. In addition, maternal blood samples were collected from the control group at the time of urine collection. After clot retraction, the separated serum samples were kept at 4°C until analysis either on the day of collection or on the day following collection, as is our normal laboratory practice.

Table I—Maternal age at delivery and gestational age (GA) distribution in the unaffected and affected study groups

	Unaffected	Down's syndrome	Trisomy 18
Median age (years)	29	37	37
Range (years)	19–36	21–44	25–43
Median GA (weeks)	15	19	15
Range (weeks)	9–22	14–24	14–20

Free beta hCG (urine and serum) was measured in duplicate with the CIS immunoradiometric assay [CIS (U.K.) Ltd, High Wycombe, Bucks, U.K.]. The assay uses the capture antibody FBT11 and this antibody does not recognize beta core (L. Cole, personal communication to CIS). The analytical performance of this assay has been detailed elsewhere (Spencer *et al.*, 1992; Macri *et al.*, 1993). All samples were diluted 1 in 5 with zero diluent prior to analysis and analysed in five separate assays.

Urine beta core was measured in duplicate using the Ciba Corning Diagnostics UGP enzyme immunoassay (Triton UGP EIA, Ciba Corning, Alameda, CA, U.S.A.). All urine samples were diluted 1 in 5 000–30 000 in the zero calibrator diluent supplied with the kit. The urine samples were analysed in ten separate assays and the between-assay precision of the asssay was 9·7 per cent at 3 pmol/l and 4 per cent at 10 pmol/l. The calibrator range was 0–16·0 pmol/l and the observed detection limit (three times the standard deviation at zero dose) over ten assays was 0·15 pmol/l. The cross-reactivity of this assay with intact hCG, free beta hCG, and free alpha hCG has been recently shown to be less than 0·11 per cent (Canick *et al.*, 1995).

In order to take account of varying degrees of urine concentration, all urine beta core and free beta hCG concentrations were corrected to a standard urine creatinine concentration. Beta core was therefore expressed in nmol/mmol creatinine and free beta hCG as IU/mmol creatinine. Urine creatinine was measured using standard Jaffe reaction procedures (Spencer, 1986) on a Hitachi 717 after a 1 in 30 urine dilution in 0·9 per cent saline.

In order to assess the performance of either marker, standard statistical modelling techniques (Royston and Thompson, 1992) were used to examine the screening potential of each marker. Using the established population parameters, a series of random MOM values was selected from within the distributions of the affected and unaffected distributions. These values were then used to calculate likelihood ratios and the expected Down's syndrome detection rate was then calculated at a given false-positive rate (5 per cent) assuming the maternal age distribution of England and Wales (Office of Population Censuses and Surveys, 1991–1994). Repeated simulations through the model enabled confidence intervals to be established.

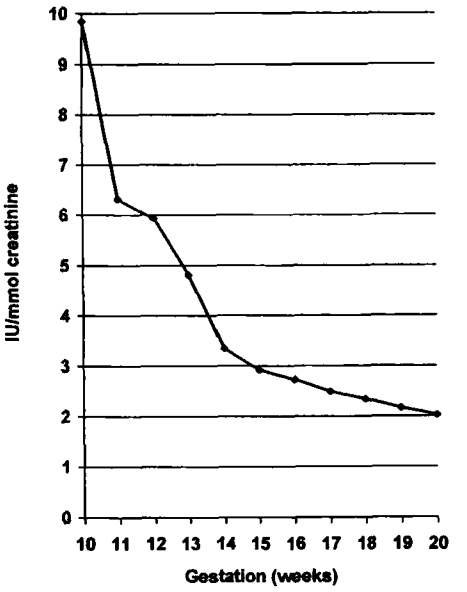

Fig. 1—Variation of median urine free beta hCG:creatinine ratios with gestational age

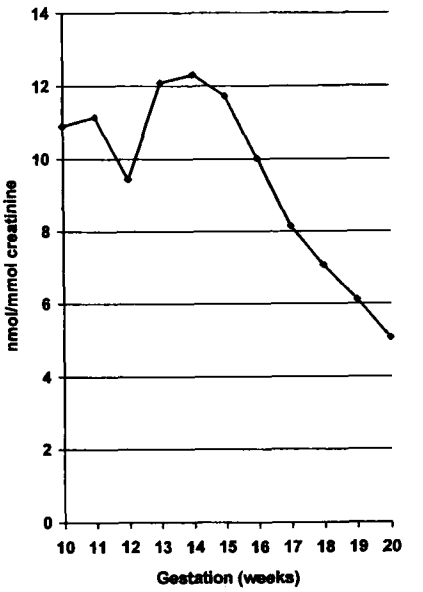

Fig. 2—Variation of median urine beta core:creatinine ratios with gestational age

RESULTS

Figures 1 and 2 show the median values of urine free beta hCG and beta core measured in the unaffected pregnancies. For free beta hCG, there is a progressive exponential decrease with gestational age which fits a log–log regression well. For beta core, there is a less pronounced variation with gestational age, reaching an apparent peak at 13 weeks and then declining. Table II shows the best-fit regressed weighted medians for the unaffected data set at gestational ages greater than 13 weeks. The unaffected control population (for pregnancies in the 13th–22nd week) showed a $\log_{10}$ Gaussian distribution of MOM for both beta core and urine free beta hCG (Figs 3 and 4). For urine

Table II—Best-fit medians for urine free beta hCG and beta core in the unaffected population

Median (beta core)=28·93 − 1·193 × GA week
$\log_{10}$ median (free beta hCG)=2·9121 − 2·0314 × $\log_{10}$ GA week

Gestational week	Number	Free beta hCG, IU/mmol creatinine		Beta core, nmol/mmol creatinine	
		Observed	Regressed	Observed	Regressed
13	30	4·62	4·45	12·09	13·42
14	43	3·85	3·84	12·31	12·23
15	92	3·35	3·33	11·73	11·04
16	44	2·92	2·92	10·01	9·84
17	45	2·50	2·59	8·15	8·65
18	23	2·35	2·30	7·16	7·46
19	21	2·17	2·06	6·12	6·27
20	25	2·03	1·86	5·10	5·07
21	24	1·72	1·68	4·09	3·88
22	25	1·65	1·53	2·89	2·68
23	—	—	1·40	—	1·49
24	—	—	1·28	—	0·30

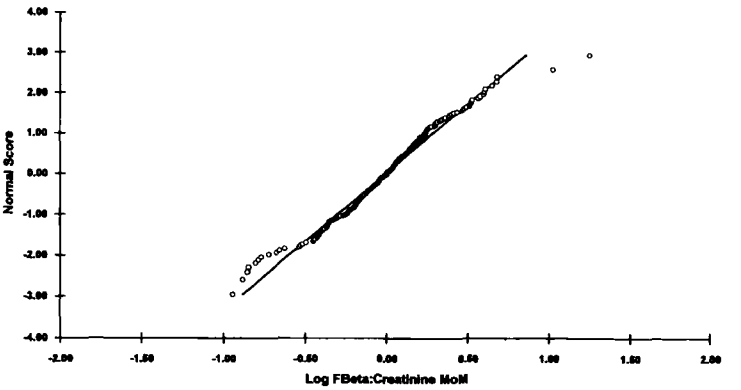

Fig. 3—Probability plot of urine free beta hCG:creatinine ratios as MOM for the unaffected population between 13 and 22 weeks' gestation. The solid line defines the best fit for the log Gaussian distributions

free beta hCG, the $\log_{10}$ mean was −0·0188 with a $\log_{10}$ SD of 0·2897 (very similar to that observed in serum) and the tenth to 90th centile was 0·44–2·19 MOM. In pregnancies greater than 15 weeks' gestation, the $\log_{10}$ SD was 0·2902. For beta core, the $\log_{10}$ mean was −0·02341 with a $\log_{10}$ SD of 0·414 and the tenth to 90th centile was 0·29–3·17 MOM. In pregnancies greater than 15 weeks' gestation, the $\log_{10}$ SD was 0·422.

As expected, there was a significant correlation between the concentration of free beta hCG in serum and urine taken at the same time in the unaffected pregnancy group, with a correlation coefficient of 0·5682. This was not the case for beta core, which showed no correlation with serum free beta hCG (r= −0·0485). In urine, there was a small but significant correlation between free beta hCG and beta core (r=0·3904).

Figures 5 and 6 show the distributions of urine free beta hCG and beta core in the Down's syndrome cases. For urine free beta hCG, the $\log_{10}$ mean was 0·4309 with a $\log_{10}$ SD of 0·356. The tenth, 50th, and 90th centiles were 1·02, 2·47, and 8·30, respectively. The 95 per cent confidence

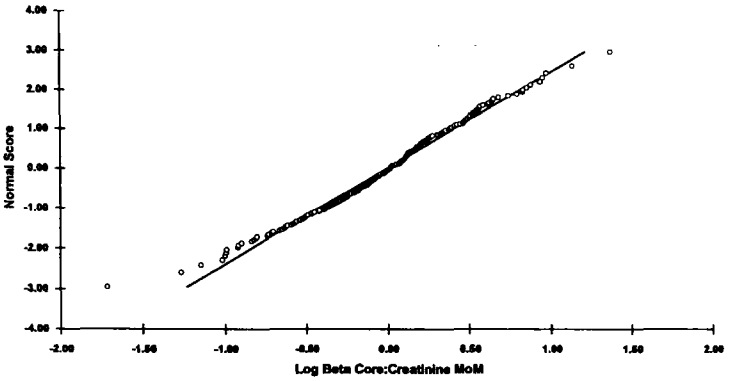

Fig. 4—Probability plot of urine beta core:creatinine ratios as MOM for the unaffected population between 13 and 22 weeks' gestation. The solid line defines the best fit for the log Gaussian distributions.

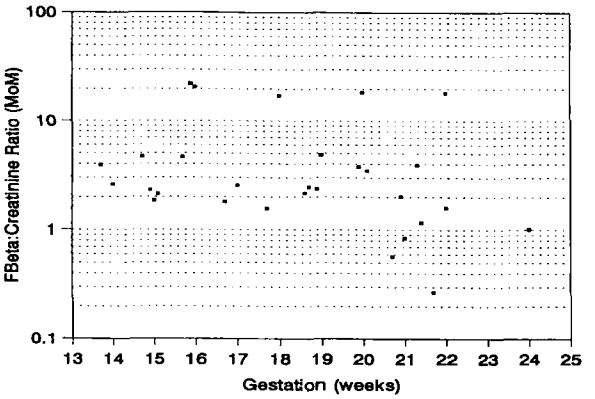

Fig. 5—Urine free beta hCG:creatinine ratios as MOM in 29 cases of Down's syndrome in the second trimester

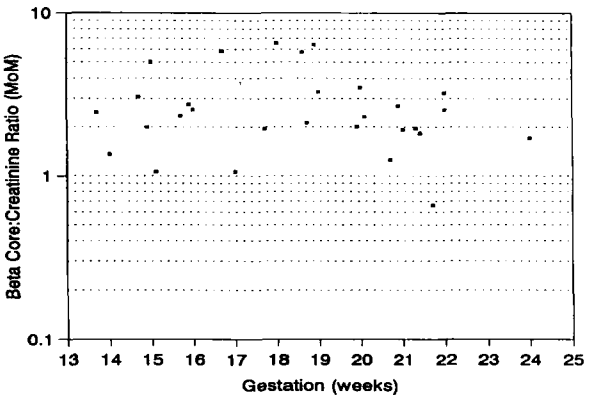

Fig. 6—Urine beta core:creatinine ratios as MOM in 29 cases of Down's syndrome in the second trimester

interval for the median in the affected group was 2·47 (1·85–3·92). For beta core, the $\log_{10}$ mean was 0·4072 with a $\log_{10}$ SD of 0·262. The tenth, 50th, and 90th centiles were 1·25, 2·35, and 5·86, respectively. The 95 per cent confidence interval for the median in the affected group was 2·35 (1·94–3·07). There was thus a significantly tighter distribution of beta core values in the affected population compared with the unaffected population.

When the Down's syndrome cases were examined on the basis of method of ascertainment, i.e., serum screening or advanced maternal age, the median values for the free beta:creatinine ratio were 2·20 and 3·50, and for the beta core:creatinine ratio 2·00 and 2·70.

From the distribution of the individual analytes alone in the affected and unaffected population, beta core at a 5 per cent false-positive rate (4·10 MOM cut-off) would have identified 6 of 29 (21 per cent) Down's syndrome cases, whilst urine free beta hCG (3·00 MOM cut-off) would have identified 12 of 29 (41 per cent) Down's syndrome cases. In this respect, the performance of free beta hCG is very similar to that in serum, where this marker alone has been shown to detect 45 per cent of cases at a 5 per cent false-positive rate (Spencer *et al.*, 1992).

Table III shows the predicted detection rate and false-positive rate when the distributions of beta core and urine free beta hCG in the affected and unaffected populations were used to model the detection rates with the maternal age distribution of England and Wales. At a 5 per cent false-positive rate, the 95 per cent confidence interval for

Table III—Expected detection rates and false-positive rates using urine free beta hCG or beta core as the screening marker

False-positive rate (%)	Free beta hCG	Beta core
1	36·8	21·2
2	45·7	28·2
3	51·0	33·7
4	54·9	37·2
5	58·4	41·1
6	61·1	44·1
7	63·7	46·5
8	65·7	49·2
9	67·8	52·1
10	69·2	54·0

the urine free beta hCG detection rate was 58·4 per cent (52·8–64·0), and for beta core 41·1 per cent (34·9–47·3).

In the small number of trisomy 18 cases, both the beta core and the urine free beta hCG values were significantly reduced. The median value for beta core was 0·15 MOM (range 0·13–0·18), and for urine free beta hCG 0·22 MOM (range 0·16–0·30).

DISCUSSION

Our preliminary report (Spencer *et al.*, 1995) contained an error in the calculations of the medians which resulted in us reporting a slightly inferior performance for both markers in that abstract. In our present extended study, we have shown that urine beta core levels in pregnancies affected by Down's syndrome are elevated with a median of 2·35 (95 per cent confidence interval 1·94–3·07). Whilst this observation confirms the general observations of Cuckle *et al.* (1994, 1995) and Canick *et al.* (1995), the average levels of 6–7 MOM observed in those studies cannot be confirmed. Our median MOM in affected cases is much higher than that observed by Hayashi and Kozu (1995) in a much smaller study using an assay that has recently been criticized as being non-specific for beta core (Cole, 1995). The assay used in our present study was confirmed by Cole to be specific for beta core and to show no cross-reactivity with free beta hCG (Cole, 1995).

Although we used the same assay as that used in the study by Canick *et al.* (1995), our median data are some two-fold higher than those found by Canick *et al.* (Table IV). The median data from our study are consistent with those obtained in the original Cuckle *et al.* (1994) study using an in house assay and also consistent with those obtained by Cuckle using the same commercial beta core assay (H. Cuckle, 1995, personal communication). However, even the two published studies by Cuckle *et al.* (1994, 1995) using their research assay showed inconsistency in the median data. These differences between the median in studies using the same assay are therefore of some concern and highlight the difficulty in measuring

Table IV—Variation in the urine beta core median values from various studies

Gestation (weeks)	This study	Canick *et al.* (1995)*	Cuckle *et al.* (1994)†	Cuckle *et al.* (1995)‡	Cuckle—personal communication (1995)§
14	12·23	9·7	13·1	24·89	13–14 weeks=13·6
15	11·04	5·9	10·6	19·53	
16	9·84	4·0	8·56	15·32	15–16 weeks=9·2
17	8·65	3·0	6·92	12·02	
18	7·46	2·4	5·59	9·43	17–18 weeks=5·1
19	6·27	2·2	4·52	7·40	
20	5·07	2·0	3·66	5·81	>19 weeks=4·6

*Calculated from the quoted regression formula: beta core median $= 57{\cdot}475e^{-0{\cdot}09438\,(GA\ days\ -\ 100)} + 16{\cdot}667$ and further divided by 8·84 to convert pmol/mg creatinine to nmol/mmol creatinine.

†Calculated from the quoted regression formula: beta core median $= 10^{(2{\cdot}411\ -\ 0{\cdot}01320\ \times\ GA\ days)}$.

‡Calculated from the quoted regression formula: beta core median $= 10^{(2{\cdot}871\ -\ 0{\cdot}01505\ \times\ GA\ days)}$.

§Personal communication from Professor Cuckle, using the Ciba Corning assay.

beta core. As Cole (1995) has pointed out, not all beta core assays have the necessary specificity. Further, the need to pre-dilute samples 1 in 15 000 before analysis is a potential source of error. These conflicting results from the various studies cast doubt on the potential value of beta core as a marker of Down's syndrome, and any consideration of beta core as a potential Down's marker must be considered cautiously.

In our small number of trisomy 18 cases, we, like Cuckle *et al.* (1994, 1995) and Canick *et al.* (1995), observed very low values of beta core in such cases but unlike those other studies we did not see values above 0·2. This difference between the two studies is probably a reflection of the small number of cases in each study.

Our studies with urine free beta hCG have confirmed that free beta hCG is cleared effectively into the urine and that median values decline with increasing gestational age after reaching a peak in early pregnancy. This fall mirrors that seen in serum and amniotic fluid (Spencer *et al.*, 1992, 1993b). When we compared serum free beta hCG and urine free beta hCG in matched samples collected at the same time, we found a high degree of correlation; these results are in contrast to the observations of Norman *et al.* (1987), who found no correlation between free beta hCG measured in the two fluids during the first trimester (6–14 weeks). Our data suggest that during the second trimester, urine free beta hCG levels certainly reflect serum levels and it would be surprising if this was not also the case in the first trimester.

In pregnancies affected by Down's syndrome, maternal serum levels of free beta hCG on average are elevated to a median of 2·64 MOM (Macri *et al.*, 1994). In amniotic fluid from Down's syndrome pregnancies, free beta hCG values are similarly elevated to 2·40 MOM (Spencer *et al.*, 1993b). Our present study in urine further shows that in such pregnancies similarly sized increases in free beta hCG occur, with values on average being 2·47 MOM (95 per cent confidence interval 1·85–3·92). Macri *et al.* (1990) showed that maternal serum screening using free beta hCG resulted in a higher detection rate prior to 16 weeks' gestation, compared with later gestation. This was confirmed by Spencer and Macri (1992) and shown by Spencer *et al.* (1993c) to relate to free beta hCG values being more elevated at this earlier gestation. As has been found in maternal serum, urine free beta hCG values in early gestation do seem to be higher, with the median value in affected pregnancies prior to 18 weeks being 2·59 MOM compared with 2·37 MOM at later weeks. Whilst this difference is not statistically significant, the observation parallels those observed in large maternal serum studies (Spencer *et al.*, 1993a).

In pregnancies affected by trisomy 18, as in maternal serum (Spencer *et al.*, 1993c), urine levels of free beta hCG are significantly depressed.

The distribution of urine free beta hCG in both unaffected and Down's syndrome pregnancies was very similar to that observed in maternal serum (Spencer *et al.*, 1992). Using mathematical modelling techniques, we have shown the feasibility of using urine free beta hCG:creatinine ratios as a screening test for Down's syndrome and have predicted that at a 5 per cent false-positive rate, detection rates of 58 per cent might be possible. This detection rate is as high as that observed using three maternal serum markers (triple test) and only 10 per cent less than that achieved using maternal serum AFP and free beta hCG (Spencer *et al.*, 1994; Macri *et al.*, 1994, 1996).

We were unable to confirm the projected 80 per cent detection rates suggested from two previous studies using urine beta core:creatinine ratios (Cuckle *et al.*, 1995; Canick *et al.*, 1995). At best, from the simulation from the data in this present study, we can only achieve a detection rate of 41 per cent at a 5 per cent false-positive rate. Further studies are required to ascertain the reasons for the difference between our study of beta core and those published previously; an investigation is also required of the anomalies between the data in previously published studies. Certainly the fact that we observed only four cases where the beta core:creatinine MOM ratio was greater than 5 MOM and the fact that the distribution of beta core in unaffected cases was twice as wide as that for free beta hCG would have contributed to this poor detection rate. Although suggestions (Canick *et al.*, 1995) have been made that urine beta core is stable in urine, other than the data from de Medeiros *et al.* (1991) there are few data published. Similarly, for free beta hCG, whilst extensive studies have been published with respect to serum there are few data available on urine. Our unpublished data suggest that in both urine and amniotic fluid, free beta hCG is stable at 4°C for at least 2 weeks and for at least 3 months at −20°C (Spencer, 1995, unpublished observations).

Our observations with respect to urine free beta hCG lead us to speculate that this may be a useful route for screening in the future; if our data can be

translated into the first trimester, then urine screening may be particularly valuable at this time when greater emphasis will need to be placed on screening in the community. Urine testing offers a less invasive approach to screening and one which may be more economical and more easily accepted in some cultures. Further studies are required to expand on these encouraging results with free beta hCG.

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