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Collaborative Study of Maternal Urine β-core Human Chorionic Gonadotrophin Screening for Down Syndrome

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Several studies have shown that second-trimester maternal urine β-core human chorionic gonadotrophin (hCG) levels are raised on average in Down syndrome pregnancies. However, in all but one, testing was retrospective after extended sample storage and so we carried out a large international multicentre prospective study. 16 centres provided 6730 samples from 14–19 week pregnancies: 39 with Down syndrome, 12 with Edwards' syndrome, 42 with other aneuploidies, 52 unaffected twins and 6585 singleton unaffected pregnancies. Samples were from those having routine maternal serum screening in 6 centres and invasive prenatal diagnosis for reasons unrelated to maternal serum screening in 10 centres. Normalized levels of β-core hCG (nmom/mmol creatinine) were expressed as multiples of the gestation-specific normal median (MoMs). The median β-core hCG level in Down syndrome was 1.70 MoM (95 per cent confidence interval, 1.26–2.30); 14 (36 per cent) exceeded the normal 90th centile and 9 (23 per cent) the 95th centile. The median level in Edwards' syndrome was 0.23 MoM.

On the basis of our results alone it is unlikely that urinary β-core hCG will be a useful marker in Down syndrome screening practice. But the considerable variability in results between studies means that further research is needed before a reliable conclusion can be drawn.

KEY WORDS: Down syndrome; maternal urine; screening; β-core hCG

INTRODUCTION

The β-core fragment is the major metabolic product of human chorionic gonadotrophin (hCG) in maternal urine. Several studies have shown that second-trimester maternal urine β-core hCG levels are raised on average in Down syndrome pregnancies (Cuckle *et al*., 1994, 1995; Canick *et al*., 1995; Spencer *et al*., 1996; Kellner *et al*., 1997; Isozaki *et al*., 1997; Lam *et al*., 1997; Hallahan *et al*., 1998; Hsu *et al*., 1999; Cole *et al*., 1999a). Whilst the overall results are encouraging, there are large differences between studies in the magnitude of the effect, possibly due to assay method and gestational range. Also there are reasons to believe that the results found in the published studies might not be achieved in normal screening practice. All but one of them was based on retrospective analysis of samples frozen and mostly stored for an extended period, and all used Down syndrome cases largely derived from high-risk pregnancies.

In early 1996 a large international multicentre study was launched with the aim of overcoming some of these problems. A reproducible commercial assay method was used and a large proportion of samples was from relatively low-risk pregnancies. It was also intended that samples be tested unfrozen, but in the event assay throughput was too slow to achieve this and most samples had to be frozen prior to testing, albeit mostly for a short period. We report the results obtained for samples tested at 14–19 weeks of pregnancy, the gestational range at which maternal serum screening is currently practised.

METHODS

There were two sources of samples. The first was a series of women in six centres having routine maternal serum screening for Down syndrome who were asked to provide a random urine sample at the same time as their blood sample. The second were women in 10 centres having invasive prenatal diagnosis for reasons unrelated to maternal serum screening, who provided a

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Table 1—Median β-core hCG level (nmol/mmol creatinine) at each week of gestation according to laboratory

	14	15	16	17	18	19
Leeds						
Women	630	1587	1271	433	174	53
β-core hCG	10.88	8.33	5.71	4.73	4.60	4.04
Providence						
Women	113	634	898	504	188	100
β-core hCG	10.86	8.62	6.57	5.02	4.21	3.07
Total						
Women	743	2221	2169	937	362	153
β-core hCG	10.86	8.43	6.02	4.89	4.39	3.42
Regressed value[a]	10.99	8.26	6.23	4.78	4.00	3.87

[a]$123.40 - 1.7882 \times \text{days} + 0.0066864 \times \text{days}^2$.

sample immediately before or after the invasive procedure. The results of the urinalysis were not used clinically.

Samples were either sent immediately or temporarily stored locally at −20°C or cooler and then transported in batches to two central laboratories. Samples from centres in the screening series were tested in Leeds, those in the prenatal diagnosis series in Providence. The method of transportation in the screening series varied according to the centre, but to ensure uniformity this was done in such a way that the samples which had been frozen locally arrived frozen at the testing centre. Normally this was achieved by sending on dry ice by airmail. The method of transportation in the prenatal diagnosis series was overnight air express of cold, but not frozen, samples. Urine samples were not treated in any way prior to transportation to a testing centre. For example, they were not centrifuged and preservatives were not added.

The samples were assayed for β-core hCG using the Chiron manual assay, mostly with an initial dilution of 1 in 10 000 or 15 000, depending on the laboratory. A repeat test was carried out at a different dilution if the concentration fell outside the operating range. All samples were tested for creatinine by the Jaffe method. Quality assessment samples from a centrally produced pool were included in each analytical run. There was good comparability between the laboratories (means of 93.9 and 95.6 nmol/l in Leeds and Providence, respectively) and the between-run coefficient of variation was 14 per cent.

Normalized levels of β-core hCG (nmol/mmol creatinine) were expressed as multiples of the gestation-specific normal median (MoM). Gestational age was based on ultrasound if available, otherwise calculated from menstrual dates. The medians for each day of gestation were obtained by regression analysis on the observed medians for each completed week of gestation. Maternal weight was found to be a significant co-variable which was allowed for by log–linear regression of MoM on weight and by dividing the observed MoM by the regressed value.

RESULTS

In total, samples from 6730 pregnancies were tested: 39 with Down syndrome, 12 with Edwards' syndrome, 42 with other aneuploidies, 52 unaffected twins and 6585 singleton unaffected pregnancies. The screening series comprised 4218 pregnancies, including 6 with Down syndrome, whereas there were 2512 from the prenatal diagnosis series, including 33 with Down syndrome.

Table 1 shows the observed median β-core hCG level, expressed in terms of nmol/mmol creatinine, according to gestational age and laboratory. There was a tendency for the levels in Leeds to be lower, but this was a small effect and so all results were pooled in order to carry out the regression analysis. Four different regression models were compared. A simple quadratic equation (see footnote to Table 1) was a reasonable fit and as more complex models did not show great improvement this was used to express results in MoMs.

Information on maternal weight was available for 5683 (86 per cent) of the unaffected singleton pregnancies. Table 2 shows that there was a strong positive

Table 2—Median creatinine and β-core hCG level according to maternal weight

Maternal weight (kg)	Women	Creatinine (mmol/l)	β-core hCG (MoM)	
			Observed	Regressed[a]
<50	172	7.20	1.22	1.21
50–	514	7.65	1.19	1.14
55–	976	7.60	1.11	1.08
60–	1189	7.34	1.01	1.03
65–	977	8.00	0.93	0.99
70–	637	8.10	0.92	0.95
75–	396	8.08	0.90	0.91
80–	290	8.60	0.89	0.88
85+	532	9.49	0.89	0.82

[a]$0.407027 + 38.7492/\text{weight}$.

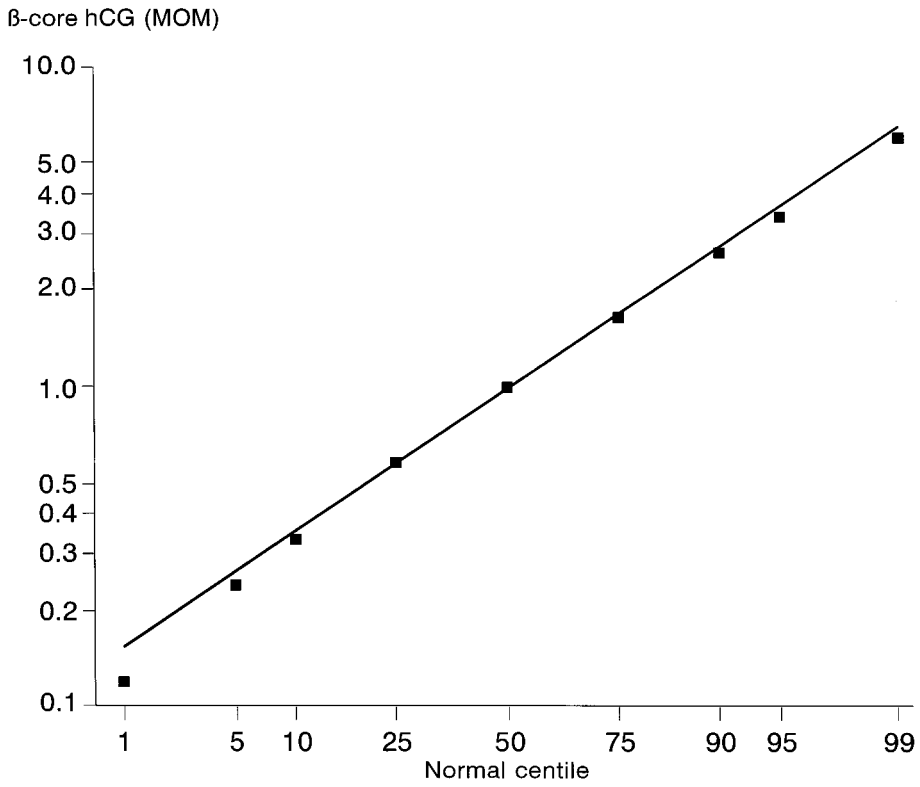

Fig. 1—Probability plot of log β-core hCG showing selected centiles and a line corresponding to a log–Gaussian distribution

association between weight and creatinine level. Since creatinine is produced by muscles and, in so far as body weight reflects muscle mass, the positive association we have seen might be expected. Table 2 also shows that there is a negative association between weight and creatinine-corrected β-core hCG levels. Multivariate regression analysis showed that this is due to both an independent effect of weight on uncorrected β-core hCG as well as the positive association between creatinine and weight. When weight was available, all MoMs were subsequently adjusted for using an inverse regression equation, which fit the data well (see footnote to Table 2).

The distribution of marker levels in unaffected pregnancies approximated reasonably well to a log–Gaussian distribution. Fig. 1 is a probability plot showing selected centiles. A straight line would imply a log–Gaussian distribution and the line shown is the theoretical distribution with median 0.00 and standard deviation 0.35 (the 10th–90th centile range divided by 2.563). The observed values are close to the expected above the median but there is a small deviation from expected at low levels. Overall, the 95th centile was 3.40 MoM, although there was a tendency for this to increase with gestation: 2.98, 3.33, 3.39, 3.59, 4.99 and 3.27 MoM at 14–19 weeks, respectively. The 90th centile was 2.63 MoM.

Table 3 shows the median MoM value at each centre. Some of the between-centre differences in centres with under 200 samples will be due to chance. However, differences between the larger centres require explanation. The duration of sample storage prior to testing may have contributed, causing a reduction in levels with increased storage. For example, the median level from the centre in Leeds was 1.09 MoM for samples tested within one month but 0.92 MoM for those with delayed testing. As expected, the maternal age was more advanced in centres from the prenatal diagnosis series compared with the low-risk series. We found a negative association between maternal age and creatinine (correlation coefficient, $r = -0.14$, $p<0.0001$) presumably due to diminishing bladder control. This resulted in a small positive correlation with normalized β-core hCG ($r=0.05$, $p=0.001$). Creatinine levels were, on average, very low in some centres from the prenatal diagnosis series where women were expected to present with a full bladder. This will not have been important provided normalization by creatinine fully corrected for concentration. This was largely achieved, with median levels of 0.99, 0.98, 1.03, 0.97, 0.96, 0.99, 1.01, 1.03 and 1.07 MoM for women with creatinine <2, 2–, 4–, 6–, 8–, 10–, 12–, 14– and ⩾16 mmol/l, respectively. We considered the possibility that levels may vary during the day even after normalization with creatinine. Information on the time of voiding was not routinely collected but towards the end of the study we were able to obtain data on 1222 pregnancies. Fig. 2 shows that this is a strong confounding variable with β-core hCG levels highest in the morning and lowest in the late afternoon. It was not possible to assess the effect on between-centre differences in this study.

Table 3—Median β-core hCG level according to centre (number of unaffected pregnancies in parentheses)

Centre	β-core hCG (MoM)	Type	Tested within month[a]	Age	Creatinine (mmol/l)
Cambridge, U.K.	0.77 (38)	Screen	53%	33	6.4
Dusseldorf, Germany	0.78 (35)	PND	100%	36	6.1
Chicago, IL, U.S.A.	0.84 (34)	PND	100%	36	6.8
Paris, France	0.84 (81)	Screen	90%	31	8.4
Esch, Luxembourg	0.86 (602)	Screen	4%	29	9.6
East Lansing, MI, U.S.A.	0.95 (16)	PND	100%	38	1.2
Rochester, NY, U.S.A.	0.98 (280)	PND	100%	37	1.8
Leeds, U.K.	0.98 (2937)	Screen	40%	34	8.4
Bronx, NY, U.S.A.	1.01 (543)	PND	100%	36	7.9
Philadelphia, PA, U.S.A.	1.01 (365)	PND	100%	36	6.3
Hull, U.K.	1.03 (398)	Screen	79%	28	10.0
New Haven, CT, U.S.A.	1.03 (416)	PND	100%	37	6.8
Birmingham, AL, U.S.A.	1.19 (553)	PND	100%	37	6.6
Cleveland, OH, U.S.A.	1.27 (94)	PND	100%	37	2.6
Providence, RI, U.S.A.	1.29 (101)	PND	100%	37	3.2
Hamburg, Germany	1.33 (92)	Screen	47%	28	8.0

[a]From the date of sample collection.
Screen=screening series; PND=prenatal diagnosis series.

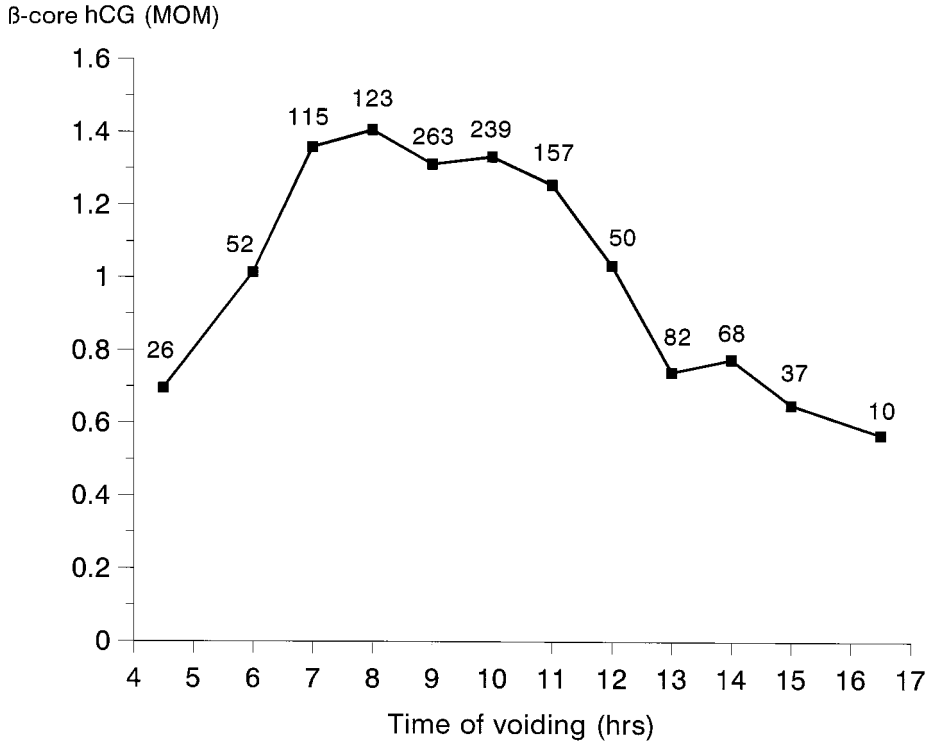

Fig. 2—Median β-core hCG level according to time of voiding (numbers of women tested are shown)

Fig. 3 shows the individual β-core hCG level in the 39 cases of Down syndrome according to gestation and series. The median level was 1.70 MoM (95 per cent confidence interval, 1.26–2.30). 14 (36 per cent) exceeded the 90th centile among unaffected pregnancies and 9 (23 per cent) the 95th centile. There was no obvious difference in levels across the gestational range and among the cases that were identified in the screening compared with the prenatal diagnosis series.

The median β-core hCG level in the 12 cases of Edwards' syndrome was 0.23 MoM. For the other common types of simple aneuploidy the median values were: Patau syndrome 0.98 MoM (six cases); Turner syndrome 1.33 MoM (five cases); 48,XXX 1.48 MoM

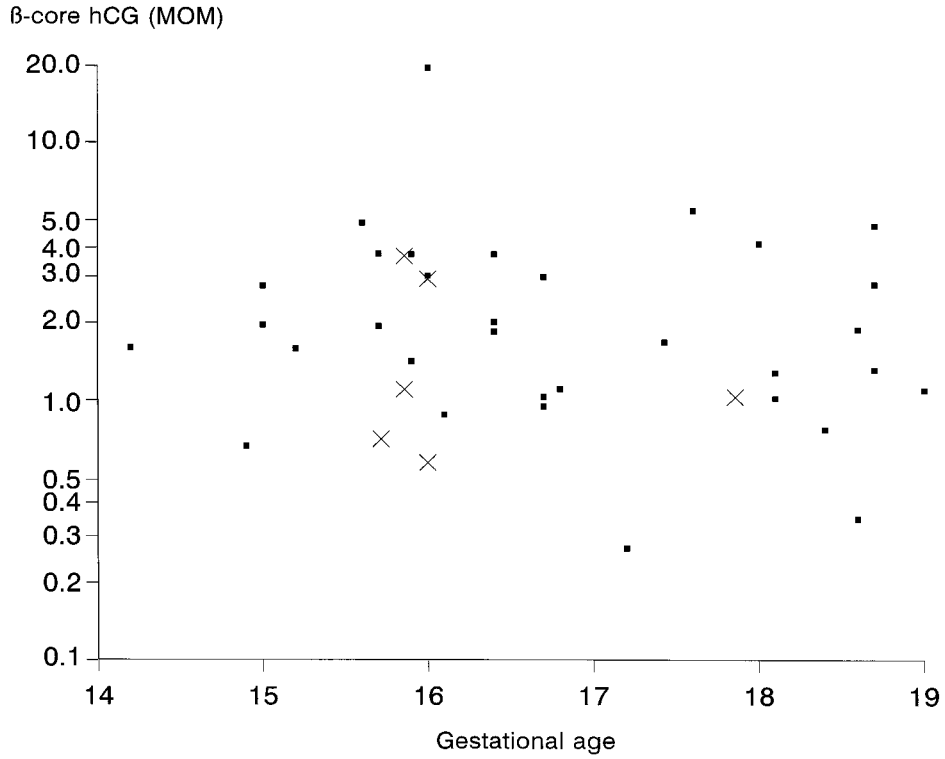

Fig. 3—Individual β-core hCG values in 39 Down syndrome pregnancies from the low-risk (×) and prenatal diagnosis (■) series

(four cases). In addition there were: two cases of 47,XXY, 0.42 and 0.66 MoM; two of 47,XYY, 0.23 and 1.30 MoM; a triploidy, 2.64 MoM; and 22 other chromosomal defects, with a median level of 1.09 MoM. The median level in the 52 unaffected twins was 2.54 MoM.

Information was available on the level of at least one maternal serum marker in 4499 of the unaffected pregnancies. Table 4 shows the median urinary β-core hCG level according to the level of each maternal serum marker in six groups. As expected, there is a strong positive association with hCG or free β-hCG ($r=0.47$, $p<0.0001$). The small positive correlation with α-fetoprotein ($r=0.07$, $p<0.0001$) and negative correlation with unconjugated oestriol ($r=-0.03$, $p=0.02$) could be a result of their known correlations with hCG and free β-hCG. The oestriol result could also be secondary to a positive association with creatinine; the median creatinine in the six groups was 7.40, 8.05, 8.60, 8.40, 8.70 and 8.70 mmol/l, respectively.

Table 4—Median β-core hCG level according to maternal serum marker level (number of unaffected pregnancies in parentheses)

Serum marker (MoM)	α-fetoprotein	Unconjugated oestriol	hCG or free β-hCG
<0.50	0.74 (89)	1.03 (157)	0.48 (445)
0.50–	0.92 (669)	1.01 (691)	0.67 (780)
0.75–	0.92 (1341)	0.97 (1360)	0.87 (834)
1.00–	1.00 (1809)	0.95 (1799)	1.12 (1191)
1.50–	1.07 (538)	0.94 (386)	1.38 (905)
2.50+	1.09 (50)	0.88 (23)	2.12 (343)

DISCUSSION

This study has confirmed that maternal urine β-core hCG levels are raised on average in second-trimester Down syndrome pregnancies. However, the median level in affected pregnancies was lower than in any other published study (see Table 5) and the discriminatory power was poor with less than one-quarter of cases exceeding the normal 95th centile. There are a number of possible explanations, including chance, the assay method, gestational age and study design.

None of the published series, including the present one, included large numbers of affected pregnancies, and some of the between-study differences could be due to chance alone. If so, the best estimate of the average β-core hCG value in Down syndrome will be obtained by meta-analysis. The geometric mean of the value in each study weighted for the number of cases in each is 3.75 MoM (95 per cent confidence interval, 3.21–4.37). However, there is significant heterogeneity between the studies—the confidence intervals in our study and those of Spencer *et al.* (1996) do not overlap with four of the other studies—so chance is unlikely to be the only explanation.

We used the same commercial assay (Triton® UGP EIA kit, Chiron Diagnostics Inc., Alameda, CA,

Table 5—Results from published second-trimester studies

Study	Assay	⩾19 weeks	Serum indication	Design	Down syndrome	Median (95 per cent CI) (MoM)
Cuckle *et al.* (1994, 1995)	In-house	62%	50%	Retrospective	24	6.0 (4.2–8.8)
Canick *et al.* (1995)	Chiron	29%	14%	Retrospective	14	5.3 (4.0–7.2)
Spencer *et al.* (1996)	Chiron	45%	38%	Retrospective	29	2.4 (1.8–3.1)
Kellner *et al.* (1997)	Chiron	44%	17%	Retrospective	18	5.0 (4.0–7.4)
Lam *et al.* (1997)	Chiron	52%	10%	Retrospective	29	3.4 (2.3–5.1)
Hallahan *et al.* (1998)	Toagosei	62%	Not known	Retrospective	8	2.2 (0.7–6.8)
Hsu *et al.* (1999)	Toagosei	59%	55%	Retrospective	69	5.0 (3.3–7.4)
Cole *et al.* (1999a)[a]	In-house	13%	0%	Prospective	23	5.4 (2.0–8.3)
Current	Chiron	3%	0%	Prospective	39	1.7 (1.3–2.3)

[a]Seven of the cases from this study were included in an earlier publication (Isozaki *et al.*, 1997).
CI=confidence interval.

U.S.A.) as four previous studies. This assay is a highly sensitive method originally designed to detect extremely low levels of β-core hCG found in oncology patients, and requires a 10–20 000-fold sample dilution for use in pregnancy. When our study was launched it was intended that samples were to be collected and tested prospectively using a new high-throughput assay which required minimal dilution. However, in the event, the improved assay was not available. Nonetheless, the geometric mean for Down syndrome from this and the four other studies combined was 3.22 MoM (95 per cent confidence interval, 2.78–3.72) which is similar to the result obtained in the four laboratories using other methods. Furthermore, a comparison of all four assays in the same set of samples has demonstrated that they produce similar results (Cole *et al.*, 1996).

The Collaborative Study Group (Cuckle *et al.*, 1996) and others (Macintosh *et al.*, 1997; Korman *et al.*, 1997; Spencer *et al.*, 1997; Hallahan *et al.*, 1998) have found that in the first trimester of pregnancy the median level of β-core hCG in Down syndrome is not greatly increased. Whilst this report excludes first-trimester Collaborative Study Group cases, there are a great proportion in the early second trimester than in the other studies. Nonetheless, the published second-trimester studies, including the present study, do not show any obvious increase in MoM values among affected pregnancies with increasing gestation.

Women tested because they had abnormal maternal serum biochemistry could bias urinary β-core hCG levels because of the correlation between maternal serum hCG or free β-hCG and urine levels. This potential bias was not applicable to our results but is likely to have enhanced the effect seen in the other published studies to some degree, although only three included a large proportion of such samples (Cuckle *et al.*, 1994, 1995; Spencer *et al.*, 1996; Hsu *et al.*, 1999).

Whilst sampling in this study was prospective, much of the testing was carried out after the sample had been frozen. This may have seriously affected the results by artefactual alteration of the analyte. There is only one published prospective study where testing was done on fresh samples. This reported a median value of 4.1 MoM in 13 Down syndrome pregnancies (Isozaki *et al.*, 1997) and 5.4 MoM in 23 affected pregnancies, including 7 from the earlier publication (Cole *et al.*, 1999a).

In addition to chance, assay method, gestation and study design there may be other explanations for the observed inconsistency in results between studies. We found that the time of day when the urine was voided is a potential confounding factor. However, there is no reason to believe that the samples from Down syndrome pregnancies were systematically collected later in the day than unaffected samples. If not, then the only contribution of this factor to our results will have been to increase variance, and so reduce the overlap in MoMs between affected and unaffected pregnancies. Sample stability and integrity remain issues that for the most part are unresolved. Cole *et al.* (1999a) have shown that urine samples from Down syndrome cases may be less stable than those from unaffected pregnancies. However, nothing from the published literature, which in most cases involved assays for β-core hCG in long-term frozen samples, indicates that stability is a problem. In our own study, samples which gave very high β-core hCG results at one point in time, gave almost identical results when the same samples were re-assayed on average two years later. Sample integrity, which includes possible microbial contamination has not yet been examined in a systematic manner.

Based on the results of this study it is unlikely that urinary β-core hCG will be a useful marker in Down syndrome screening practice. However, because of the considerable variability of published results, some of which continue to show a large effect, we cannot say definitely that it will not be of value. Further research is needed to clarify the matter.

Although the early promising results of β-core hCG have not been fulfilled by more recent studies there is some evidence that there are better urinary markers for Down syndrome. Maternal urine hyperglycosylated hCG has been reported to be markedly raised on

average in 10 affected pregnancies with a median level of 5.7 MoM (Cole *et al*., 1998). The series has now been expanded to include 23 cases with a median of 7.3 MoM (Cole *et al*., 1999b). In a second study of 45 affected pregnancies, the median level was 3.6 MoM (Cuckle *et al*., 1999).

Further aliquots of urine samples from this report, as well as those from about 3000 samples collected but not tested by the Collaborative Study Group, are available for further analysis if an improved assay becomes available or if previously undetermined assays or sample-related issues are resolved.

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