

First-Trimester Down Syndrome Screening Using Dried Blood Biochemistry and Nuchal Translucency

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Objective: To assess the effectiveness of free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency in a prospective first-trimester prenatal screening study for Down syndrome and trisomy 18.

Methods: Risks were calculated for Down syndrome and trisomy 18 based on maternal age and biochemistry only ($n = 10,251$), nuchal translucency only ($n = 5809$), and the combination of nuchal translucency and biochemistry ($n = 5809$).

Results: The study population included 50 Down syndrome and 20 trisomy 18 cases. Nuchal translucency measurement was done on 33 Down syndrome and 13 trisomy 18 cases. Down syndrome screening using combined biochemistry and ultrasound resulted in a false-positive rate of 4.5% (95% confidence interval [CI] 3.9%, 5.2%) and detection rate of 87.5% (95% CI 47%, 100%) in patients under age 35 years. In older patients, the false-positive rate was 14.3% (95% CI 12.7%, 15.8%) and detection rate was 92% (95% CI 74%, 99%). For trisomy 18 screening, the false-positive rate was 0.4% (95% CI 0.24%, 0.69%) and detection rate was 100% (95% CI 40%, 100%) in younger patients, whereas in older patients the false-positive rate was 1.4% (95% CI 0.9%, 2.0%) and detection rate was 100% (95% CI 66%, 100%). Using modeling, at a fixed 5% false-positive rate, the Down syndrome detection rate was 91%. Conversely, at a fixed 70% Down syndrome detection rate, the false-positive rate was 1.4%.

Conclusion: First-trimester screening for Down syndrome and trisomy 18 is effective and offers substantial benefits to clinicians and patients. (Obstet Gynecol 2000;96:207–13. © 2000 by The American College of Obstetricians and Gynecologists.)

second trimester of pregnancy with protocols that include two or more of the biochemical markers alpha-fetoprotein (AFP), hCG, free β -hCG, and unconjugated estriol (E3). Those screening protocols have detection rates for Down syndrome in young, apparently healthy families in the range of 38%¹ to 75% (Macri JN, Spencer K. Toward the optimal protocol for Down syndrome screening [letter]. Am J Obstet Gynecol 1996;174:1668–9). Previous studies^{2–4} have shown that free β -hCG, a second-trimester marker, and pregnancy-associated plasma protein A, a biochemical marker not effective in the second trimester, are both productive screening markers for Down syndrome in the first trimester of pregnancy. More recently, ultrasound measurement of fetal nuchal translucency also has been found to be effective in screening for Down syndrome.⁵

Because these biochemical and biophysical screening approaches are relatively independent, first-trimester screening for Down syndrome can be optimized beyond the capabilities of either approach alone by combining the two. Several recent studies^{6–11} with small data sets or studies based on modeling have shown that such combined screening could detect 76–89% of Down syndrome cases in the first trimester. A recent opinion by the ACOG Committee on Genetics¹² stated that first-trimester screening for chromosome abnormalities offers many advantages over second-trimester screening, and it suggested further studies to confirm the efficacy of nuchal translucency screening with or without serum markers. In light of this suggestion, we

Maternal serum Down syndrome and trisomy 18 screening is conducted in the United States during the

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prospectively collected data from first-trimester pregnancies to evaluate the effectiveness of maternal serum biochemistry (free β -hCG and pregnancy-associated plasma protein A), ultrasound measurement of fetal nuchal translucency, and their combination in screening for Down syndrome and trisomy 18.

Materials and Methods

Maternal blood specimens were collected prospectively between September 1995 and June 1998, from 10,251 women and dried as spots on specialized filter paper (#903 paper; Schleicher Schuell, Keene, NH) using methods previously published.¹³ Blood was collected by fingerstick or venipuncture into red-top vacutainer tubes. Diff-Safe blood dispensers (Alpha Scientific Corporation, Southeastern, PA) were used to spot whole blood collected by venipuncture before clotting.

Dried blood specimens were analyzed for free β -hCG and pregnancy-associated plasma protein A by using previously described enzyme-linked immunosorbent assay procedures.⁶ Of 10,251 specimens, 7801 were assayed at NTD Laboratories (Huntington Station, NY) and 2450 were assayed at Centro Di Diagnosi Prenatale (Palermo, Italy) using identical assay reagents and procedures.

Gestational ages ranged from 9 weeks 0 days to 13 weeks 6 days and were based on ultrasound or last menstrual period if ultrasound was not done. All pregnancies were apparently healthy, singleton, and not complicated by diabetes. All nuchal translucency measurements were conducted according to the protocol of the Fetal Medicine Foundation, London, United Kingdom. If gestational age was between 10 weeks 4 days and 13 weeks 6 days and an ultrasonographer who was trained by the Fetal Medicine Foundation was available, a nuchal translucency measurement was determined. Of the 10,251 women in the study, 5809 met this criterion and nuchal translucency measurement was successfully done in all in addition to biochemical analysis.

Risk calculations were determined for biochemical markers alone ($n = 10,251$), nuchal translucency alone ($n = 5809$), and for both ($n = 5809$). For statistical purposes only, risks were also calculated based on nuchal translucency alone and biochemistry alone. Risks were calculated based on biochemistry only for women on whom nuchal translucency measurement was not performed. As part of risk assessment, free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency values were divided by their respective day-specific median level to determine the multiples of the median (MoM) for each marker. Each laboratory developed separate analyte medians to ac-

count for interlaboratory assay variation. Likelihood ratios were calculated from multivariate log-gaussian distributions of MoM values in unaffected, Down syndrome, and trisomy 18 cases. Risks were determined by multiplying the likelihood ratio by the women's risk for Down syndrome and trisomy 18 before screening, which was based on maternal age¹⁴ and gestational age, using the formula of Snidjers et al.¹⁵ If blood collection and nuchal translucency measurement were not done on the same day, the later of the two gestational ages was used to determine prior risk. Women with risks greater than that of a 35-year-old at the same gestational age were considered to be at increased risk for Down syndrome. For trisomy 18 screening, a risk cut-off of one in 150 was used.

Exact confidence intervals (CI) based on binomial distribution were determined for false-positive and detection rates. The yield was determined by dividing the sum of the Down syndrome and trisomy 18 cases that were at increased risk by the total number of women who were at increased risk after excluding women with other outcomes. False-positive and detection rates were modeled using observed likelihood ratios and the maternal age distribution of live births. For each maternal age 14–49 years, age-specific false-positive and detection rates were determined on the basis of the observed likelihood ratios, the prior risk at that maternal age, and a cut-off risk. The overall false-positive rate was then determined by taking a weighted average of the age-specific false-positive rates, where the weights were equal to the number of unaffected pregnancies in the United States at each maternal age divided by the total number of unaffected pregnancies in the United States. Similarly, the overall detection rate was determined by taking a weighted average of the age-specific detection rates, where the weights were equal to the number of Down syndrome pregnancies at each maternal age divided by the total number of Down syndrome pregnancies in the United States. The number of Down syndrome pregnancies was estimated by multiplying the number of live births at each maternal age by the incidence rate of Down syndrome. The cut-off risk was varied until it reached a 5% false-positive rate and the detection rate at a 5% false-positive rate was determined. Similarly, a separate analysis was done to determine the false-positive rate at a 70% detection rate.

We also present distribution parameters for free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency based on the samples in the study plus 58 Down syndrome and eight trisomy 18 cases analyzed previously. A total of 108 Down syndrome and 28 trisomy 18 cases were used to determine these parameters.

Table 1. Summary of Maternal and Gestational Age Distribution of Study Population

	Unaffected	Down syndrome	Trisomy 18	Other
Patients screened with biochemistry	10,106	50	20	75
Mean maternal age (y)	31.6 ± 5.4	37.2 ± 4.9	37.4 ± 6.3	35.3 ± 6.2
Mean gestational age (wks)	11.65 ± 1.06	11.73 ± 1.16	11.46 ± 1.05	11.47 ± 1.09
Patients screened with biochemistry and nuchal translucency	5718	33	13	45
Mean maternal age (y)	32.1 ± 5.7	37.5 ± 4.4	37.5 ± 7.0	35.4 ± 6.2
Mean gestational age (wks)	12.07 ± 0.85	12.07 ± 0.88	11.68 ± 0.96	12.00 ± 0.88

Data are given as *n* or mean ± standard deviation.

Results

Of 10,251 fetuses, 10,106 were unaffected, 50 had Down syndrome, and 20 had trisomy 18. Using each women's incidence rate based on maternal age and gestational age, the expected number of Down syndrome and trisomy 18 cases was 48.9 and 21.6, respectively. There were 75 additional cases with adverse fetal outcomes, including three cases of trisomy 13, five cases of Turner syndrome, and four cases of triploidy. Among the 5809 women who had nuchal translucency measurement, there were 33 Down syndrome and 13 trisomy 18 cases. In this subset, the expected number of Down syndrome and trisomy 18 cases was 31.7 and 13.6, respectively. In this subset there were 45 additional cases with adverse fetal outcomes, including three cases of trisomy 13, five cases of Turner syndrome, and two cases of triploidy. Table 1 shows the distribution of gestational and maternal ages.

Table 2 shows results of prospective screening using the combined screening protocol for the 5809 women who had both nuchal translucency and biochemical analysis done. In women under 35 years old, the observed false-positive rate for Down syndrome screening was 4.5% (95% CI 3.9%, 5.2%) with a detection rate of 87.5% (95% CI 47%, 100%). The false-negative rate was 12.5% (95% CI 0%, 53%). In older women the

observed false-positive rate was 14.3% (95% CI 12.7%, 15.8%) and detection rate was 92% (95% CI 74%, 99%). The false-negative rate was 8% (95% CI (1%, 26%). For trisomy 18 analysis in the younger women, the observed false-positive rate was 0.4% (95% CI 0.24%, 0.69%) and observed detection rate was 100% (95% CI 40%, 100%). The false-negative rate was 0% (95% CI 0%, 60%). In the older women the observed false-positive rate was 1.4% (95% CI 0.9%, 2.0%) and observed detection rate was 100% (95% CI 66%, 100%). The false-negative rate was 0% (95% CI 0%, 34%). The yield of either Down syndrome or trisomy 18 for every increased-risk result was 11 of 195 (one in 18) in younger women and 32 of 343 (one in 11) in older patients. Including other adverse outcomes, the yield would be 19 of 203 (one in 11) in younger patients and 45 of 356 (one in eight) in older women. For results that were within normative range, the negative predictive value was 99.97% for younger women and 99.88% in older women after excluding other outcomes. Both cases of triploidy, all five cases of Turner syndrome, and two of three cases of trisomy 13 were detected with combined screening. Table 3 lists the median MoM values for the other adverse outcomes in the study.

Table 4 shows projected Down syndrome detection rates based on a general United States pregnancy pop-

Table 2. Results of Prospective Screening Using a Combined Biochemical and Ultrasound Protocol

Screening result	Unaffected	Down syndrome	Trisomy 18	Other	Total
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Patients under 35 y					
Within normative range	3552 (95.1)	1 (12.5)	0 (0.0)	10 (55.6)	3563 (94.6)
Increased risk for Down syndrome	168 (4.5)	7 (87.5)	0 (0.0)	8 (44.4)	183 (4.9)
Increased risk for trisomy 18	16 (0.4)	0 (0.0)	4 (100)	0 (0.0)	20 (0.5)
Total	3736 (100)	8 (100)	4 (100)	18 (100)	3766 (100)
Patients 35 y and older					
Within normative range	1671 (84.3)	2 (8.0)	0 (0.0)	14 (51.9)	1687 (82.6)
Increased risk for Down syndrome	283 (14.3)	23 (92.0)	0 (0.0)	10 (37.0)	316 (15.5)
Increased risk for trisomy 18	28 (1.4)	0 (0.0)	9 (100)	3 (11.1)	40 (2.0)
Total	1982 (100)	25 (100)	9 (100)	27 (100)	2043 (100)

A cut-off equal to the age-related risk of a 35-year-old was used for Down syndrome screening. A cut-off of one in 150 was used for trisomy 18.

Table 3. Median Multiples of the Median in Cases With Adverse Outcomes Other Than Down Syndrome and Trisomy 18

Outcome	Cases	Median MoM free β -hCG	Median MoM PAPP-A	Cases with NT	Median MoM NT
Spontaneous abortion	5	0.77	1.00	1	1.20
Fetal death	2	0.95	0.62	1	3.96
Anencephaly	3	1.28	0.96	0	
Trisomy 13	3	0.12	0.23	3	2.91
Turner syndrome	5	1.26	0.75	5	2.47
Triploidy	4	1.17	1.83	2	2.88
Other chromosomal anomalies	26	0.85	0.94	19	1.20
Chromosome mosaics	12	1.38	1.09	6	0.93
Other anomalies	15	1.10	0.91	8	1.13

MoM = multiples of the median; PAPP-A = pregnancy-associated plasma protein A; NT = nuchal translucency.

ulation (ages 14–49 years) for each marker at a fixed 5% false-positive rate and the false-positive rate at a fixed 70% detection rate. At a fixed 5% false-positive rate, the detection rates of biochemistry, ultrasound, and the combination were 63%, 74%, and 91%, respectively. Conversely, to detect 70% of Down syndrome cases, the false-positive rates of biochemistry, ultrasound, and both were 6.8%, 3.4%, and 1.4%, respectively. A receiver operating characteristic (ROC) curve (Figure 1) shows the relationship between detection and false-positive rates for the combined protocol. Table 5 shows the incremental detection rate of all three screening markers in achieving a 91% detection rate for Down syndrome. Table 6 shows false-positive and detection rates increasing with maternal age. For trisomy 18, a combined screening approach can achieve a detection rate of 96% at a 1.1% false-positive rate using a one in 100 cut-off or a 97% detection rate at a 1.2% false-positive rate using a one in 150 cut-off.

Table 7 shows distribution parameters based on 108 cases of Down syndrome and 28 cases of trisomy 18. Among Down syndrome pregnancies, the Spearman rank correlation coefficients of MoM values compared with gestational age were 0.10 ($P = .302$), 0.26 ($P = .007$), and 0.27 ($P = .134$) for free β -hCG, pregnancy-

associated plasma protein A, and nuchal translucency, respectively.

Discussion

The data show that when used alone, biochemical screening (63% detection rate) or ultrasound screening (74% detection rate) each had Down syndrome detection rates similar to or better than currently available second-trimester methods, but the combination of biochemical and sonographic screening protocols resulted in a significantly improved detection rate of 91%. Further, each of the individual markers, free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency, significantly improved the screening process (Table 5). In addition to increased detection, first-trimester screening offers inherent advantages of early detection. Most women are found to be at low risk of chromosomal abnormalities and thus can be reassured much earlier in pregnancy. For patients found to be at increased risk, more time is available to decide on diagnostic options. Finally, for patients who choose to terminate an affected pregnancy, safer and earlier procedures are available than those used at or around 20 weeks' gestation.

Table 4. Efficiency of First-Trimester Down Syndrome Screening Protocols

Protocol	At a fixed 5% false-positive rate		At a fixed 70% detection rate	
	Cut-off	Detection rate	Cut-off	False-positive rate
Free β -hCG	1/145	46%	1/355	15.8%
PAPP-A	1/105	38%	1/395	19.0%
Free β -hCG + PAPP-A	1/140	63%	1/195	6.8%
NT	1/195	74%	1/90	3.4%
Free β -hCG + NT	1/240	80%	1/55	2.3%
PAPP-A + NT	1/185	81%	1/35	2.3%
Free β -hCG + PAPP-A + NT	1/270	91%	1/15	1.4%

Abbreviations as in Table 3.

All risks are in terms of first-trimester risk. All protocols include maternal age. Data are modeled based on observed likelihood ratios and distribution of live births in the United States (maternal age 14–49 years).

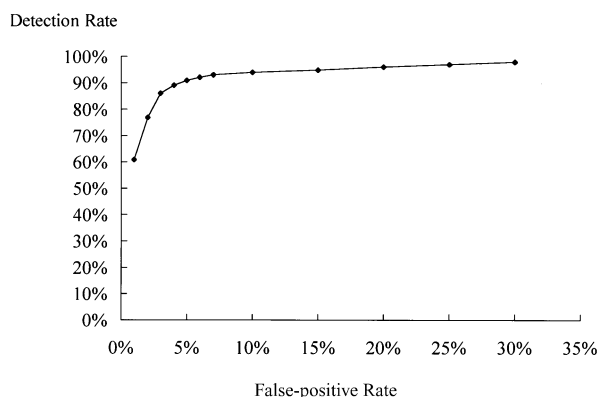


Figure 1. Receiver operating characteristic (ROC) curve shows false-positive rate and detection rate using a combined free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency protocol.

Our experience with second-trimester screening indicates that there is significant maternal anxiety after an increased-risk result. It is essential, therefore, that diagnostic procedures be available and offered as quickly as possible after screening results have been reported. Chorionic villus sampling (CVS) and early amniocentesis are the primary diagnostic procedures used between 11 and 14 weeks' gestation. After the initial introduction of CVS, reports indicated an increased incidence of fetal complications, including specific limb defects.^{16,17} However, more recent studies showed lower rates of limb-reduction defects if CVS is done at 10 weeks or later.¹⁸ Similarly, there are reports concerning the safety of early amniocentesis.^{19,20} On balance, it is likely that both CVS and early amniocentesis will result in similar low but acceptable risks for patients who have been given appropriate preprocedure counseling. Randomized studies are currently under way to assess this issue further.

Our detection rate of 91% at a 5% false-positive rate based on combined ultrasound and biochemical screening in the first trimester is similar to or slightly greater than the detection rate of 76–89% reported by other studies^{6–11} using smaller data sets or modeled data

Table 6. Results of Combined Screening Using Free β -hCG, Pregnancy-associated Plasma Protein A, Nuchal Translucency, and Maternal Age

Age (y)	False-positive rate (%)	Detection rate (%)
≤24	3.2	88
25–29	3.7	88
30–34	5.3	90
35–39	10.3	91
≥40	24.7	97
All ages	4.8	91

Results are based on modeling observed likelihood ratios and the age distribution of live births. A cut-off equal to the age-related risk of a 35-year-old was used.

using the same protocol, and it is unlikely that bias could have artificially enhanced screening results. One potential bias could result from not accounting for the fetal loss rate for Down syndrome, which could overestimate the detection rate because of cases of Down syndrome that are undetected and spontaneously abort before term. This overestimation affects both first- and second-trimester screening studies, although the overestimation will tend to be greater in the first trimester where the loss rate is greater. Two recent studies by Snijders et al¹⁵ and Morris et al²¹ found that the fetal loss rate between late first trimester (when this screening is done) and term was approximately 31%. Using the model of Dunstan and Nix,²² which addresses the issue of fetal loss, true detection rate in our study might be adjusted to 88% (based on an average gestational age of 12 weeks and the fetal loss data of Snidjers et al).¹⁵ This detection capability still surpasses commonly used second-trimester protocols that yield detection rates of 38–75%. Further, the first-trimester detection of affected cases destined to miscarry would mean that treatment can be offered in a medically controlled setting, and the diagnosis can alert patients to increased risks in future pregnancies. Another bias could result from failure to ascertain live-born Down syndrome cases that were not identified by the screening process, causing an overestimation of detection efficiency. In the current study,

Table 5. Relative Contribution of Biochemistry and Nuchal Translucency in First-Trimester Screening at a 5% False-Positive Rate

Initial protocol	PAPP-A + NT	Free β -hCG + NT	Free β -hCG + PAPP-A	NT
A. % detected	81	80	63	74
B. % undetected	19	20	37	26
Additional marker(s)	Free β -hCG	PAPP-A	NT	Free β -hCG + PAPP-A
C. Combined detection (%)	91	91	91	91
D. % detection with inclusion of additional marker(s) = (C – A)	10	11	28	17
E. % of remaining undetected cases detected with inclusion of additional marker(s) = (D ÷ B)	53	55	76	65

Abbreviations as in Table 3.

Table 7. Unaffected, Down Syndrome, and Trisomy 18 Distributions

Parameter	<12 weeks		≥12 weeks		9–13 weeks
	Unaffected	Down syndrome	Unaffected	Down syndrome	Trisomy 18
Total samples	5994	67	4112	41	28
Samples with NT	2643	14	3075	19	13
Median free β -hCG	1.0	1.70	1.0	1.98	0.18
Median PAPP-A	1.0	0.50	1.0	0.65	0.31
Median NT	1.0	2.42	1.0	2.89	3.32
SD free β -hCG	0.4820	0.5109	0.5561	0.3454	0.7303
SD PAPP-A	0.5264	0.5582	0.5330	0.5476	0.8984
SD NT	0.3087	0.6310	0.3070	0.6077	0.7120
R FB compared with PAPP-A	.226 (<.001)	-.167 (.09)	.194 (<.001)	.217 (.09)	.111 (.29)
R FB compared with NT	-.026 (.09)	.303 (.15)	.060 (<.001)	.209 (.20)	.627 (.01)
R PAPP-A compared with NT	.006 (.38)	.040 (.45)	-.028 (.06)	.162 (.15)	.472 (.05)

NT = nuchal translucency; PAPP-A = pregnancy-associated plasma protein A, FB = free β -hCG; R = correlation of LOGe transformed variables; SD = standard deviation based on LOGe.

Numbers in parenthesis indicate *P* value for correlation coefficient being significantly different than zero.

however, the number of Down syndrome and trisomy 18 cases observed in the population was similar to that expected, based on maternal age and gestational age. Therefore it is unlikely that there was a significant underreporting of undetected affected cases.

The data indicate that the pregnancy-associated plasma protein A median MoM in Down syndrome pregnancies varies by gestational age, so separate distribution parameters might be needed at different gestational ages. We observed a similar effect for free β -hCG and nuchal translucency values. However, the variation for these markers was not significant.

A recent study¹¹ suggested that an alternative to first-trimester screening could include a protocol combining results from first-trimester and second-trimester screenings. Unfortunately, this protocol negates the substantial advantages of early screening and diagnosis and could unnecessarily increase anxiety among women who must wait to receive second-trimester screening results. Further, the improved screening performance of an integrated test relies mostly on the high detection capability of first-trimester markers, with small improvements obtained by second-trimester AFP, inhibin, and unconjugated E3, none of which are effective individual markers for Down syndrome. It is likely that further investigation will identify other first-trimester markers (either ultrasound or biochemical) that can make similar small improvements in screening performance while maintaining the substantial advantages of the first-trimester screen.

Results could be reported in terms of either first-trimester or term Down syndrome risk. Either method is valid as long as women are counseled properly about the meaning of the risks. In our Down syndrome screening protocol we used a first-trimester risk cut-off

equal to the risk of a 35-year-old at the corresponding gestational age and obtained an acceptable false-positive rate (Table 2). With trisomy 18 screening, a logical age-related cut-off would have resulted in a false-positive rate that was too high. Therefore we chose a first-trimester cut-off risk of one in 150, which, based on the maternal age distribution of live births, resulted in a false-positive rate of approximately 1%.

Previous studies^{6,23} have shown that nuchal translucency measurements are effective in screening for other chromosomal abnormalities, such as trisomy 13, Turner syndrome, and triploidy. The data in this study confirm those results. Undoubtedly, ultrasound examinations done during the first trimester of pregnancy will lead to the detection of other fetal abnormalities as well.²⁴ Although concerns have been raised about the cost of ultrasound, it has been argued that ultrasound examinations should be done on all gravidas before second-trimester triple screening to reduce false-positive rates.²⁵

We believe that first-trimester screening using a combination of biochemistry and nuchal translucency measurement is feasible, results in improved Down syndrome detection compared with currently used second-trimester protocols, and provides substantial advantages to clinicians and patients. Further studies will refine risk algorithms for Down syndrome and trisomy 18, reduce CIs for false-positive and detection rates, provide more information on detection of autosomal trisomies, determine the impact of first-trimester screening on second-trimester screening, and assess the correlation of free β -hCG, pregnancy-associated plasma protein A, and nuchal translucency with other congenital anomalies and perinatal complications.

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