

# First Trimester Interlaboratory Comparison Program

Distribution 2008 FT-C



If you are planning a pregnancy or are pregnant they you should take folic acid supplement to help prevent babies from being affected by such disease as spina bifida. It is currently recommended that a daily dose of 400mcg (0.4mg) folic acid tablet for at least three months before becoming pregnant and for 12 weeks into your pregnancy.

Sponsored by:  
Department of Pathology and Laboratory Medicine  
Women & Infants Hospital  
Providence, RI

## INTRODUCTION

### Explanation of Data Listing and Analysis

Reading the Data Listing: The five page data listing (attached) contains a summary of reported results for all participants, with each page summarizing one specimen. Your lab ID is listed at the beginning of the row with your results. Missing data (blanks) are likely due to participants who are manufacturers rather than screening labs, or to laboratories that are not yet offering screening services. Outliers for gestational age (or maternal age) are identified as those outside +/- 0.2 weeks (or years) of the correct answer. For the assay results (in mass units or MoM) and Down syndrome risks, outliers are defined as being outside of +/- 2 trimmed standard deviations, after accounting for rounding. A logarithmic transformation is used for the analysis of Down syndrome risks.

*Conversion of reported risks to first trimester risks.* Most all laboratories report first trimester risks, but some laboratories report second trimester or term risks. If the reported risks are not first trimester, these risks are displayed in the column labeled "Report" under the "Down S Risk (1:n)" heading. To allow all risks to be evaluated by a single statistic, second trimester risks are converted to first trimester risks using the factor 0.74. This accounts for fetal loss between the first and second trimesters (43% from first trimester to term, and 23% from second trimester to term). For example, if the second trimester risk is 1:1000, the first trimester risk is  $1:1000 \times 0.74$ , or 1:740. Term risks are converted to first trimester risks by multiplying by 0.57. Risks from labs that use free beta subunit rather than hCG in their screening protocol are also listed in the data sheets, but risks are not included in the calculation of the consensus risk.

*Maternal Age Reporting:* Maternal age can be reported as either as a decimal or as completed years (integer). Although the difference in risk is small for most ages, use of decimal age rather than completed years can be more significant for women whose age falls close to a whole year (e.g, 34.1 versus 34.9 years). Both women would be called 34 completed years even though they are almost one year different in age. Laboratories commonly calculate risk using a maternal age equation rather than a table of risks, and it is straightforward to use the more accurate risk to obtain better precision. Almost all labs in the ICP report decimal age. Currently, lab(s) that report integer maternal ages are listed separately on the data summary results, but in the future results will be listed along with decimal ages, but will not be included in the calculations.

*NT MoM Reporting:* The ICP provides a NT MoM for most of the challenges. Participants need to generate the MoM values provided in the histories by trial and error, usually by entering various CRL or GA values. Approximate CRL values (in mm) and GA values (in weeks and days) are provided only as an aid for this process. Participants are asked to report the MoM value that they actually obtained to serve as check on how reliably they could reproduce the targeted MoM value. Almost all participants report NT MoM values that closely match the targeted value. If participants are having difficulty generating a reliable MoM, we can assist.

The ICP also includes at least one challenge that does not provide a target NT MoM. Instead, we provide a patient CRL and NT value, along with a set of NT and CRL values from a hypothetical sonographer (identified by initials) who provided the NT measurement. Participants are asked to use the set of sonographer-specific NT/CRL values to generate an appropriate NT median equation for converting the NT values (in mm) to MoM. This NT MoM then used with their chemistry results to calculate the Down syndrome risk. We also provide an Excel spreadsheet that can be used to calculate the CRL/NT median equation with accompanying QA parameters (e.g. slope and logarithm standard deviation)

*Greater than and less than risks:* Risks that are reported as less than (<) or greater than (>) are displayed in the “Report” column under the “Down S Risk (1:n)” column. These risks are listed as the actual numeric risk in the “1<sup>st</sup> trim” column and may be included in the final calculation of the consensus risk.

## RESULTS

FT-11: Laboratories were asked to calculate an NT MoM value, given a CRL of 50 mm (~ 11.7 weeks' gestation) and an NT value of 1.24 mm for sonographer FST. Participants were provided with a set of NT/CRL measurements for this sonographer and may have already calculated a sonographer-specific median equation (sent again in this distribution for those who may need to recompute the median equation). Participants may, or may not, have used those medians to calculate their MoM value, depending on existing laboratory protocols. The expectation is that the resulting MoM values reported by laboratories that use sonographer-specific medians should be similar. We calculated the median equation for sonographer FST to be: median NT =  $10^{(-0.373+0.00613*CRL)}$  using the EXCEL calculator supplied to participants. This equation yields an expected median NT value of 0.86 mm for a CRL of 50 mm, and, therefore, a MoM value of 1.44 (1.24/0.86). The consensus NT MoM (calculated as the trimmed mean value) value is 1.42, in close agreement. Two results were outliers (0.86 and 0.89 MoM). Some labs may not use the calculator supplied by the ICP, but instead use internal or commercial software that may yield a slightly different set of medians. Also, some labs use a single set of medians rather than sonographer-specific medians. Overall, the results indicate that laboratories can derive a common median equation, given a set of paired sonographer NT/CRL values, and usually can use those results to provide clinical interpretations.

A CRL was provided for this sample, requiring each lab to calculate gestational age. This was done to assess the variability in assigning gestational age by participating laboratories. Assigned gestational ages for FT-11 ranged from 11.4 to 11.7 weeks. As pointed out previously, differences reflect the 'CRL to decimal weeks' equation selected by laboratories. The Supplemental Question in the 2005 FT-C report addresses this issue (accessible at <http://www.ipmms.org/ICP/FT-C%202005%20Final%20Report.pdf>), and includes a review of equations in common use. It is recommended that participants review this exercise if there are questions.

The CVs of the PAPP-A and hCG values were similar (14% and 15%, respectively) but the PAPP-A MoM was higher than for the corresponding hCG MoM (23% and 13%, respectively). The CV of log risk was low (6%). This specimen had a first trimester consensus risk (1:240), close to screening cut-offs used by many screening laboratories (cut-offs listed in Table 1 in the FT-A 2008 report). Six of 18 reporting labs call the specimen screen positive (37%) while the remainders call it screen negative. It is expected that when reported risks are close to screening cut-offs that the split between screen positive and screen negative approaches 50:50.

FT-12: This specimen is a pool of sera from 12 week pregnancies, and should, therefore, more accurately reflect actual between-lab and between-kit differences. The CV for the PAPP-A value (10%) is lower than our manufactured samples. However, the CV of the MoM (23%) is similar to other samples in this distribution, suggesting that median values are more variable. All labs reported the specimen as screen negative, and the CV of the log risk was low (4%).

FT-13: THE CVs of risk for values and MoM for both markers were in line with typical performance. The trimmed mean consensus risk (1:133) is relatively high, and 85% called the specimen screen positive.

FT-14: This specimen was targeted to have a low PAPP-A value, and the trimmed mean consensus value of 0.54 mIU/mL was consistent with that target. The CV of 19% was in line with the CVs of samples with lower values, which indicates that the PAPP-A assays used by ICP participants measures this analyte with reasonable precision with concentrations important for detecting the low levels of PAPP-A associated with Down syndrome. However, the CV of log risk (17%) was significantly higher than is typical for other samples. This may reflect the fact that the PAPP-A consensus MoM of 0.16 is very low, falling at the extreme of the population distribution of PAPP-A MoM values. Small differences in MoM values can yield relatively large differences in the likelihood ratios that are used in the risk calculation.

FT-15: This sample was targeted to have a low PAPP-A and a low hCG MoM value, and the consensus MoM values were in line with expectation (0.67 and 0.50 MoM, respectively). The consensus risk was low, and all participants called it screen negative.

## Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by four participants (Table 1). All reported using the same method (Di-01). Table 1 lists the reported DIA values and MoM levels for each of the five samples. Included also are the DIA likelihood ratios (LR) in the context of the other markers. Overall, the laboratories reported reasonably equivalent DIA values, MoM levels and likelihood ratios, with some indication that Laboratory A has higher DIA values and MoM levels.

**Table 1. Dimeric Inhibin-A measurements for FT-B 2008**

Sample Number	Laboratory	Value	MoM	DS Risk (1:n)	DIA LR <sup>1</sup>
FT-11	A	787.0	2.25	290	0.93
	B	603.1	2.15	1600	0.81
	C	617.6	1.66	101	0.76
	D	605.1	1.78	266	0.76
FT-12	A	387.0	1.20	540	0.94
	B	289.9	1.17	1600	0.30
	C	281.1	0.84	1570	0.43
	D	307.1	1.03	1190	0.52
FT-13	A	348.0	1.49	210	0.52
	B	262.2	1.31	300	0.32
	C	274.8	1.03	275	0.28
	D	286.0	1.08	597	0.36
FT-14	A	769.0	2.84	10	6.8
	B	639.8	3.21	6	48
	C	663.7	2.39	8	20
	D	683.5	2.59	12	48
FT-15	A	210.0	0.57	16	<1.0
	B	166.0	0.56	6	<1.0
	C	179.0	0.46	4	0.65
	D	171.0	0.49	5	0.49

<sup>1</sup> For each participant, the DIA LR is computed by dividing the reported risk for NT, PAPP-A and hCG by the risk that also includes DIA measurements. If blanks are shown, the likelihood ratio cannot be reliably determined, usually because one (or both) of the reported risks are very high (e.g., >1:10) or very low (e.g., <1:10,000).

## Down syndrome screening in twin pregnancies

### Background

Methods for calculating a second trimester Down syndrome risk for twin pregnancies using multiple serum markers was described in 1991 (1). The method can only yield a *pregnancy-specific* risk because it is not possible to distinguish the contribution of each fetus to the measured concentration of each marker. In the first trimester, however, screening typically combines serum measurements with a NT measurement that could be used to yield a fetal-specific Down syndrome risk (2). Alternatively, risks can be added and reported as a *pregnancy specific* risk.

Calculating the most reliable first trimester risks for a twin pregnancy requires information on the zygosity of the twins. If the twins are dizygous (non-identical) the risk for each fetus having Down syndrome is independent of the risk for the other. The *pregnancy specific* risk is then calculated by adding the two *fetal specific* risks together. If the twins are monozygous (identical) both fetuses are usually affected or unaffected. Risk for monozygotic twins can be calculated by either averaging the NT measurements (using the geometric mean because NT measurements are logarithmically distributed), or by using one of the largest measurements [the largest is preferred because one of the twins may be growth retarded (3)].

Zygosity cannot be definitely determined in pregnancy by a non-invasive procedure. Between 10 and 14 weeks gestation, however, zygosity can be established about 90% of the time (4) by determining the number of chorions (chorionicity) via ultrasound. Chorionicity is an indicator of the number of placentas and, therefore, the number of fetuses. The chorion is the outer, and the amnion the inner, membrane that surrounds the fetal sac. Dizygous twins are always in separate sacs, each with its own chorion and amniotic sac (dichorionic, diamniotic). Historically, two out of three pregnancies are dizygous (and dichorionic), but with assisted reproductive techniques, this proportion is increasing. Two thirds of monozygous twins have one chorion, indicating a single placenta and two amnions (monochorionic, diamniotic). However, the other third of monozygous twins are dichorionic and diamniotic, and will not be distinguishable from a dizygotic pregnancy by ultrasound. Rarely, a monozygotic twin pregnancy can be monochorionic and monoamniotic.

A method has been published for calculating “pseudo risks” for both a dichorionic and monochorionic pregnancies (2). [This latter publication provides in detail the methods for calculating twin risk and is attached as a PDF file]. The term “pseudo risk” was introduced to acknowledge that the risks calculated for twin pregnancies are less reliable than those in a singleton pregnancy. However, by using this methodology, one can expect false positive rates similar to that found for singleton pregnancies (at a given risk cut-off level). Other methods have been published and are commonly used (3).

Many people have trouble adding fractions or odds. This is important consideration when reporting only fetal-specific risks, as the pregnancy specific risk is the sum of the two fetal-specific risks. For example, consider the Down syndrome risks for twins A and B to be 1:350 and 1:460, respectively. A physician might consider this pregnancy ‘screen negative’ as both risks are below a common cut-off level of 1:250. In fact, some people assume that the sum of the risks must lie between the two risks (e.g., about 1:400 for this example). This is not the case; this pregnancy is screen positive.. The sum of two risks must always be higher than the highest one. Two methods can be used to add risks expressed as odds.

**Common denominator:** express risks as fractions and add with a common denominator  
(this example assumes that 1:460 is about 1/460, for simplicity)

$$\begin{aligned} 1/350 + 1/460 &= 460 / (350*460) + 350 / (350*460) \\ &810 : 350*460 \\ &1:(350*460)/810 \\ &1:199 \text{ and screen positive} \end{aligned}$$

**Conversion to decimal:** convert to decimal, add, and convert back to odds.

$$\begin{aligned} 1:350 + 1:460 &= 1/351 + 1/461 \\ &= .00285 + .00217 \\ &= .00502 \\ &= 1:(1-.00502)/.00502 \\ &= 1:198 \text{ and screen positive} \end{aligned}$$

### Supplemental Questions:

- Q1. Does your laboratory provide clinical Down syndrome screening services?** Of the 25 participants in the 2008 FP-C survey, 20 reported that they did, one reported that they didn't, and four did not answer the question (most likely manufacturers). The next three questions focus on the responses from these 20 clinical laboratories.
- Q2. Does your laboratory requisition slip collect chorionicity for twin pregnancies?** Of the 20 participants, 10 reported that they did collect chorionicity (50%) while 10 others did not (40%).
- Q3. Does your laboratory use chorionicity to modify the Down syndrome risks and/or interpretation?** All 10 that have chorionicity on their laboratory slip use the information.
- Q4. If chorionicity is missing, what do you do?** Focusing on the 10 participants with the question on their requisition slip, six (60%) assume dichorionicity, one wasn't sure, and the others would attempt to obtain the information by phone.
- Q5. The CRL measurements for the twins differ. What gestational age is assigned to this pregnancy?** All but one of the participants assigned a gestational age, and the average was 84 days (range 82 to 86) or 12 week, 0 days. The differences in gestational age in this example is not expected to be great because the CRL measurements for the twins are similar (50mm versus 56 mm)
- Q6. If you assigned a gestational age, what CRL did you use?** Of the 19 participants responding, 10 use the largest (53%), eight use the mean or geometric mean (42%) and one used the smallest (5%).
- Q.7 Provide the following Down syndrome risks: for the pregnancy, for twin A and for twin B (provide responses for only what you routinely report).** The median first trimester risk after logarithmic transformation is 1:310 and the CV is 12%. The CV is greater than that observed for typical singleton pregnancy samples in the ICP (usually 3% to 10%). Risks for the remaining participants are not summarized but provided in table 2. Interesting, four laboratories would have provided fetus-specific risks and one of these also provided an overall pregnancy risk. For the three laboratories that reported only fetus-specific risk, the pregnancy risks have been computed (in parenthesis).
- Q.8. Report the interpretation.** The entries have been sorted in order of decreasing risks, and the interpretations follow that trend. The interpretations are consistent with the use of a risk cut-off level of about 1:270.

**Table 2. 2008 FTC – Results for the twin pregnancy supplemental exercise**

No	Gestational Age		Trim.	Down syndrome Risk 1:n				
	Days	CRL used		Preg.	Twin A	Twin B	Interp	Action
1	85	Largest	First	75			positive	US/Amnio
2	84	Mean	First	126			positive	US/Amnio
3	85	Largest	First	143			positive	US/Amnio
4	82	Smallest	First	204			positive	US/Amnio
5	84	Largest	First	251			positive	US/Amnio
6	84	Mean	First	285			Neg	None
7	84	Mean	First	300			Neg	None
8	84	Mean	First	390			Neg	Other
9	83	Mean	First	400			Neg	None
10	86	Largest	First	470			Neg	None
11	83	Mean	First	470			Neg	None
12	85	Largest	First	545			Other	By MD
13	85	Largest	First	726			Neg	None
14	82	Mean	First	1000			Neg	None
				<b>311</b>				
15	ND	NR	Second	(290)	406	962	Neg	NR
16	82	Mean	Second	660			Neg	None
17	85	Largest	Term	(1100)	1400	5100	Neg	None
18	85	Largest	Term	(910)	1200	3800	Neg	None
19 <sup>2</sup>	85	Largest	Term	(750)	880	5000	Neg	None
20	83	Largest	NR	380			NR	NR

**84**

ND=not done/calculated, NA=not applicable, NR=not reported

<sup>2</sup> Uses free beta and PAPP-A to compute Down syndrome risks

### References

1. Wald NJ, Cuckle HS, Wu T, George L. 1991. Maternal serum unconjugated oestriol and human Gonadotrophin levels in twin pregnancies: implications for screening for Down's syndrome. *Br J Obstet Gynaecol* **98**:905-908.
2. Wald NJ, Rish S, Hackshaw AK. 2003 Combining nuchal translucency and serum markers in prenatal screening for Down syndrome in twin pregnancies. *Prenat Diagn* **23**:588-92.
3. Spencer K, Kugan KO, Nicolaides KH. 2008 Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers. *Prenat Diagn* **28**:49-52
4. Fisk NM, Bennett PR. 1995. Prenatal determination of chorionicity and zygosity. In *Multiple Pregnancy*. Ward RH, Whittle W (eds). RCOG Press: London;**56-66**.

## Interpretive Questions – Integrated Screening for Down Syndrome

**Question 10. Does your laboratory perform integrated risk interpretations?** Among the 21 responding laboratories, 14 reported that they offer integrated screening as part of a formal screening program, and the remaining seven reported that they do not offer integrated screening. All laboratories used the same ‘trimester of risk’ for their quadruple and integrated Down syndrome risks (second trimester or term risks). All 14 laboratories could provide integrated risks using all four second trimester markers (AFP, uE3, hCG and DIA), along with NT.

**Question 11 and 12. Using the Down syndrome risks from FP-16 (CAP FP-C 2008 Survey), report integrated risks using FT-15 results** (after appropriate modifications to the draw date). These analyses are restricted to the 14 participants reporting integrated risks (Table 3). Although we found unexpectedly heterogeneity for serum integrated test likelihood ratios (when compared to the quadruple test), the results for full integrated test, and for the impact of adding just NT measurements were more consistent. In summary:

- The median reported quadruple markers risk (column 1) was **1:16**. Given the consensus MoM values and associated maternal age, this is reasonable. One participant reported a 1:172 and was deemed an outlier.
- The median reported serum integrated risk (column 2) was **1:47**. Given a consensus PAPP-A MoM value of 0.67, the risk would be expected to be slightly reduced. Although this is true in general, several participants report larger increases in risk (or large reductions) and these might want to check their calculations.
- The median reported full integrated risk (column 3) was **1:355**. Given a consensus NT MoM of 0.69, the full integrated risk would be expected to be greatly reduced. This is true for most participants.

The next three columns explore how the individual laboratories modified the risk based on the addition of markers. The “Q/SI” column examines the impact of adding PAPP-A to the quadruple test, the “Q/FI” column examines the impact of adding both PAPP-A and NT measurements to the quadruple test. The last column “SI/FI” looks only at the impact of NT on the quadruple test.

- The trimmed likelihood ratio (LR) for Q/S is **0.73** (median 0.69) with no outliers. The log standard deviation is quite large, due to the combination of both large (7.5) and small (0.06) LRs. The consensus LR is consistent for a PAPP-A consensus MoM of 0.66.
- The trimmed LR for Q/FI is **0.06** (median 0.12) with one outlier (7.5). Given the addition of an NT MoM of 0.69 to the SI test, it is reasonable that the LRs are reduced compared to Q/SI5).
- The trimmed LR for SI/FI is **0.11** (median 0.13) with one outlier (1.0). Given that nearly all laboratories had an NT MoM of 0.69, all should have reported an important reduction in risk. All but the one outlier did report LRs of 0.23 or lower, indicating a reduction in risk of 4 fold or greater.

**Question 13. Do you use the same parameter sets for both the quadruple and integrated test?** Fourteen participants responded. Of these 10 (71%) reported that they do use the same parameter set (the preferred methodology). Three reported that they did not (21%) and one did not know (7%). More laboratories are using consistent parameter sets than three years ago. This was not the reason for the one laboratory with several outlying values in Table 3.

**Table 3. Comparison of quadruple risks to integrated risks.**

Down syndrome risk (1:n)			Likelihood ratio <sup>1</sup>		
Quadruple (Q) (FP-16)	Serum Integrated (SI) (FP-16 & FT-15)	Full Integrated (SI) (FP-16 & FT-15)	Q/SI	Q/FI	SI/FI
16	5	110	3.20	0.15	0.05
16	8	91	2.00	0.18	0.09
29	510	2800	0.06	0.01	0.18
11	65	520	0.17	0.02	0.13
13	29	199	0.45	0.07	0.15
172	75	1238	2.29	0.14	0.06
>10	>10	35			
15	16	121	0.94	0.12	0.13
12	110	490	0.11	0.02	0.22
20	200	860	0.10	0.02	0.23
15	2	2	7.50	7.50	1.00
40	95	1700	0.42	0.02	0.06
55	25	220	2.20	0.25	0.11
16	180	2200	3.20	0.15	0.05
<b>16</b>	<b>47</b>	<b>355</b>	← <b>Median reported risk</b>		
		<b>outlier</b>	none	7.50	1.00
		<b>Trimmed LR</b>	.73	0.06	0.11
		<b>log SD of LR</b>	0.69	0.49	0.24
		<b>Mean - 2sd</b>	0.03	0.01	0.03
		<b>low</b>	0.06	0.01	0.05
		<b>high</b>	7.50	0.25	0.23
		<b>Mean + 2sd</b>	17	0.57	0.32

<sup>1</sup> Derived by dividing the associated Down syndrome risks.

George J. Knight, Ph.D.  
Jacob A. Canick, Ph.D.

Glenn E. Palomaki, B.S.  
Geraldyn M. Messerlian, Ph.D.

(207) 657-7888  
(401) 453-7650

Department of Pathology and Laboratory Medicine  
Women & Infants Hospital  
Providence, Rhode Island