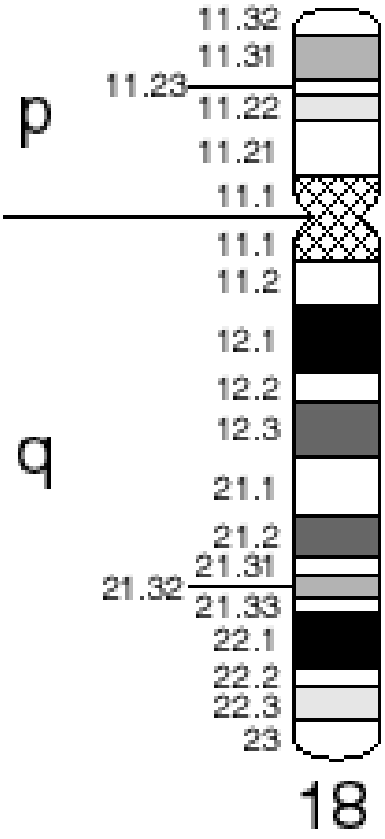
First Trimester
Interlaboratory Comparison Program

Distribution 2008 FT-A



Ideogram of chromosome 18

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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. Missing data may also result because some laboratories do not measure 'total or intact hCG' but instead measure another marker, e.g., free beta hCG. 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 interpretation of Down syndrome risk results.

Conversion of reported risks to first trimester risks. Almost all laboratories report first trimester risks, but some laboratories report second trimester or term risks. If the reported risks are not first trimester, then 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 x 0.74, or 1:740. Term risks are converted to first trimester risks by multiplying by 0.57.

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 "1st trim" column and are included in the final calculation of the consensus risk.

RESULTS

FT-01: Laboratories were asked to calculate an NT MoM value, given a CRL of 76 mm (about 13.6 weeks' gestation) and an NT value of 2.56 mm for a sonographer identified as JAC. Laboratories were previously given a series of NT/CRL measurements for sonographer JAC and asked to calculate a sonographer-specific median equation in distribution FT-C 2007 (sent again in this distribution for those laboratories needing to calculate a median equation). They may, or may not, have used that equation to calculate their MoM value, depending on 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 JAC to be: median NT = 10^(-0.303+0.00611*CRL) using the Excel calculator supplied to participants. This equation yields an expected median NT value of 1.45 mm for a CRL of 76 mm, and, therefore, a MoM value of 1.77 (2.56/1.45). The consensus NT MoM (calculated as the median value) value was 1.75. A few labs were on the low side. Some labs may not use the calculator supplied by the ICP, but instead use internal or commercial software that yields a different median equation. Overall, the results indicate that laboratories can derive a common median equation, given a set of paired sonographer NT/CRL values, and can use those results to provide clinical interpretations.

As was true for FT-11 in the FT-C 2007 distribution, gestational age was not provided as part of the clinical history for FT-01. Instead, a CRL was provided for that sample, requiring each lab to

estimate gestational age. This was done to assess the variability in assigning gestational age by participating laboratories. Assigned gestational ages for FT-01 ranged from 13.1 to 13.7 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), and includes a review of equations in common use. It is recommended that participants review this exercise, if there are questions.

The CVs of both the PAPP-A value and MoM were relatively high, which contributed to the relatively high CV of the log risk (32%). However, the high CV of log risk also reflects the fact that most laboratories reported risks as single digits. All labs interpreted the specimen as screen positive, and most recommended US/Amino.

FT-02: This specimen was targeted to have a moderately elevated PAPP-A and an elevated hCG. One laboratory had a PAPP-A value that was a low outlier, and also reported a relatively low PAPP-A MoM. In addition, one lab had an outlying high PAPP-A MoM, suggesting that the median value may be too low. The CVs for both PAPP-A and hCG were very good (11% and 9%, respectively). The CVs of the MoM values for the two markers were also reasonable, although the PAPP-A MoM value was higher than for hCG, which has been fairly consistently true over time. All but one lab reported the specimen as screen positive, and the CV of the log risk was very good (9%). One lab that offers sequential screening checked two answers (US/counsel for amnio and Collect new sample and retest for the recommended action, and this has been listed as Other in the report (also true for subsequent specimens).

Table 1. Risk cut-offs used by ICP Participants

Number of Laboratories	Trimester of Risk cut-off	Risk cut-off level (1:n)	
		Reported	1st Trimester equivalent
1	1	190	190
2	1	200	200
3	1	220	220
5	1	230	230
1	1	250	250
1	1	270	270
1	2	230	170[1]
1	2	250	185
1	2	260	190
2	2	270	200
1	3	300	170[2]
1	3	350	200
1	3	385	220
	Median Value		**220**

[1] Second trimester risks converted to first trimester risks by multiplying by 0.74
[2] Term risks converted to first trimester risks by multiplying by 0.57.

FT-03: This specimen had a first trimester consensus risk (1:210), close to screening cut-offs used by many screening laboratories (Table 1). It would therefore be expected that about one-half would call the specimen screen positive and one-half would call the specimen screen negative, by chance. In fact, nine of 17 reporting labs call the specimen screen positive (53%) while the remainder call it screen negative. It is inevitable that the split between screen positive

and screen negative will approach 50:50 when the risk is equal to the screening cut-off, regardless of how closely risks agree (in this case, a CV of 9%).

FT-04: This specimen was targeted to have a high PAPP-A value, and the trimmed mean consensus value of 7.2 mIU/ml was consistent with expectation. Overall, the results were good, with a CV of 13%, with only one outlying value (4.5 mIU/ml). The high consensus PAPP-A MoM of 3.25, the consensus hCG MoM of 1.90, and the low NT MoM of 0.85, along with a maternal age of 28.6 years would be expected to yield a low risk. The consensus risk was 1:8500, with a low CV of 3% for log risk. All labs called the specimen screen negative and all but one recommended no further action. One lab called for a repeat test because it offers sequential testing.

FT-05: This sample was targeted to have a low hCG, as might occur with trisomy 18 or fetal demise. The consensus value was 42.1 IU/ml with a CV of 12%. This contrasts with FT-05 in the 2007 FT-C distrubtion, where the consensus value of 19.6 IU/ml had a CV of 50%. This high CV resulted because the distribution of hCG values was bimodal, i.e., systematic differences existed between the DPC and Beckman kits at these low levels. This bimodal distribution is still present for the 2008 FT-05 but is less pronounced. This improvement may result because the hCG value is higher for FT-05 in 2008 than for FT-05 in 2007. Alternatively, the lab may be retesting at a lower dilution to bring the measured value into a more reliable part of the calibration curve, as we suggested in 2007 FT-C. Low hCG values will have little or no impact on the final Down syndrome risk calculation because of the truncation limits placed on the MoM value.

Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by four participants (Table 2). All reported using the same method (Di-0). The following table provides 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 somewhat higher DIA values and MoM levels.

Table 2. Dimeric Inhibin-A measurements for FT-A 2008

Sample Number	Laboratory	Value	MoM	DS Risk (1:n)	DIA LR[1]
FT-01	A	**212.0**	**0.69**	**10**	**<1.00**
	B	**164.0**	**0.75**	**26**	**0.23**
	C	**179.0**	**0.63**	**24**	**0.17**
	D	**180.3**	**0.76**	**19**	**0.26**
FT-02	A	**321.0**	**1.19**	**350**	**0.46**
	B	**255.0**	**1.22**	**210**	**0.32**
	C	**278.0**	**0.99**	**308**	**0.28**
	D	**283.4**	**1.16**	**342**	**0.42**
FT-03	A	**372**	**1.34**	**210**	**1.00**
	B	**287**	**1.37**	**230**	**0.61**
	C	**301**	**1.06**	**526**	**0.36**
	D	**304**	**1.24**	**324**	**0.53**
FT-04	A	**843.0**	**3.23**	**5800**	**1.38**
	B	**747.0**	**3.26**	**4410**	**>2.27**
	C	**805.0**	**2.55**	**5280**	**1.32**
	D	**723.3**	**2.48**	**1870**	**1.32**
FT-05	A	**195.0**	**0.97**	**1500**	**1.07**
	B	**145.0**	**0.58**	**2350**	**0.44**
	C	**149.0**	**0.46**	**2770**	**0.37**
	D	**146.3**	**0.43**	**1910**	**0.39**

[1] 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).

Interpretive Question: First trimester trisomy 18 testing

Of the 24 participating laboratories, three do not screen patients (manufacturers). Of the remaining 21 clinical laboratories, two do not provide first trimester trisomy 18 risks. The analyses will focus on the 19 clinical laboratories reporting first trimester trisomy 18 risks.

1. **What markers do you use for interpreting results for trisomy 18?** Table 3 shows the serum and ultrasound marker combinations used for trisomy 18 risk. All define the prior risk using maternal age. Eight of the 19 laboratories then add NT and PAPPA measurements into their risk algorithm; another eight laboratories use hCG along with NT and PAPP-A measurements. One adds only measurements of PAPP-A. Laboratories should be aware that the parameters used to compute trisomy 18 risks are likely to be less reliable than those used for Down syndrome. Ascertainment bias is not accounted for in several published reports, resulting in over-estimating performance and systematically assigning inappropriate risks. This bias has the largest impact on the NT MoM parameters. This may be one reason behind the variability in marker combinations used.

Table 3. Trisomy 18 marker combinations for 19 participating laboratories

Marker Combination (maternal age and)	Participating Laboratories Number	(%)
NT and PAPP-A	8	42
NT, PAPP-A and hCG	8	42
PAPP-A and hCG	1	5
NT, PAPP-A, free beta hCG	1	5
PAPP-A	1	5
Total	19	99

2. **How do you interpret results for trisomy 18?** Table 4 shows the ways in which participants reported trisomy 18 test results. The choices for this question did not allow laboratories to report both risks and screen positive/screen negative interpretations on all reports, requiring multiple answers (two laboratories). The results are thus somewhat confounded, but almost half of laboratories report risk on all pregnancies, and about one-third report risk on only screen positives.

Table 4. Reporting trisomy 18 test results

Trisomy 18 reporting	Participating Laboratories Number	(%)
Calculate and provide interpretation on all reports	9	47
Calculate and provide interpretation on screen pos only	8	42
Calculate and provide interpretation (pos/neg) all reports	2	11
Any	19	100

3. What is your risk cut-off level (and trimester of risk)? Table 5 shows the trisomy 18 risk cut-off level for participating laboratories, along with the trimester of risk. Thirteen laboratories report using a 1:100 risk, but eight of these say it is a first trimester risk, five others indicate it is a second trimester risk. Data to suggest how to modify risk for trisomy 18 in the first trimester are sparse in the literature. Laboratories should verify that they are actually reporting first trimester trisomy 18 risks, and not just reporting trisomy 18 risks in the first trimester.

Table 5. Trisomy 18 risk cut-off level and trimester of that risk.

Trisomy 18 risk cut-off level		Participating Laboratories	
(1:n)	**Trimester**	**Number**	**(%)**
1:100	First	10	52
1:100	Second	6	32
1:200	Term	2	11
1: 67	First	1	5
Any		19	100

4. What is the source of your algorithm for trisomy 18 risks? Table 6 shows the source of the trisomy 18 risk for the 19 participating laboratories. Fourteen of the laboratories rely on commercial software. .

Table 6. Source of trisomy 18 risk algorithms

	Participating Laboratories	
Source of trisomy 18 risks	**Number**	**(%)**
Commercial software (Alpha)	8	42
Commercial software (Benetech)	3	16
Commercial software (Maciel)	3	16
Internal	4	21
Other	1	5
Any	19	100

5. What interpretation would you give to specimen FT-01? Two screening laboratories provided neither a risk nor an interpretation. Table 7 shows the trimester of risk, the trisomy 18 risk and interpretation for the remaining laboratories. Given the relatively small numbers of respondents, the large number of confounders (trimester of risk, number of markers, whether or not risk is reported), the analysis is limited. The one consistent result is that all participants identified the specimen as screen positive.

Table 7. Trisomy 18 results for specimen FT-01

Trimester of risk[1]	Trisomy 18 risk (1:n)	Interpretation
First	> 5	Pos
Second	5	Pos
First	7	NR
First	10	Pos
First	10	Pos
First	>10	Pos
First	>10	Pos
First	>10	Pos
Second	12	Pos
Second	30	Pos
First	34	Pos
First	36	Pos
Second	75	Pos
Second	75	Pos
Second	75	Pos
First	85	Pos
First	85	Pos
Median	**12**	

NR = not reported

[1] At this time, it is not clear how to convert first trimester risks to second trimester risks, or vice versa.

Interpretive Questions – Integrated Screening for Down Syndrome

1. **Does your laboratory provide clinical Down syndrome screening services?** Nineteen of 21 respondents answered yes.

2. **Does your laboratory perform integrated risk interpretations?** Among the 19 laboratories, six reported that they do not offer integrated screening. Of the remaining 13, all reported that they offer integrated testing as part of a formal integrated screening program. All laboratories used the same 'trimester of risk' for their quadruple and integrated Down syndrome risks (second trimester or term risks). All 13 laboratories could provide (including NT) integrated risks using all four second trimester markers (AFP, uE3, hCG and DIA).

3. **Report the Down syndrome risks from FP-05 (CAP FP-A 2008 Survey). Report integrated risks using FT-05 results (with modifications to the draw date).** The consensus risk (median value) from the FP survey for the quadruple markers was 1:147 (second trimester). Given the PAPP-A consensus MoM of 1.58, one would expect each laboratory to calculate a serum integrated test risk that is lower than its quad risk. The second trimester consensus risk for the serum integrated test was 1:935 This reduction can be expressed as a likelihood ratio (LR), obtained by dividing the quad risk by the serum integrated risk (column 2 in Table 8). The consensus LR for these two risks is 0.22 (145/650), consistent with the LR for all labs (0.21) reported in the table. Two laboratories reported LR greater than one, indicating an increased risk, (5.50, 1.00). These labs reported using different parameter sets for calculating second trimester risks versus integrated risks. One lab reported a very low LR of 0.01 (Lab M), and this lab also indicates that it uses separate parameter sets for quad versus integrated testing. The consensus risk (median) for the full integrated test is 1:300, yielding a consensus LR of 0.49 (147/300), again consistent with the consensus LR of 0.52 in Table 8. Three laboratories found increases in risk compared to the quad test result (1.09, 2.50, 18.33). The lab with the very high LR of 18.33 (Lab H) reported using different parameter sets. Lab M, which also uses different parameter sets for quad versus integrated testing reported a relatively low LR of 0.03, as was true for the serum integrated test.

4. **Analysis of the integrated risks.** The maternal age for this sample is 38.5 years. Consensus median MoMs for PAPP-A and NT are 1.58 and 1.62, respectively (FT-05 data listing). Consensus MoMs for AFP, uE3, hCG and DIA are 1.23, 0.80, 3.76, and 1.43, respectively (CAP FP-05 summary report). The second trimester risks calculated using this maternal age and consensus results (using ultrasound-based SURUSS parameters) are: 1:78 (quadruple test); 1:350 (serum integrated test), and 1:130 (full integrated test). Table 8 summarizes important findings of the risks reported for combinations of these results. The first seven laboratories used the same parameter set (SURUSS) to compute all three risks and, therefore, it is instructive to examine the likelihood ratios between the estimates. Results from the 13 laboratories are summarized at the bottom of Table 8.

Table 8. The Down syndrome risks (1:n) for quadruple, serum integrated and full integrated testing for 13 laboratories performing integrated screening

	Down syndrome risk (likelihood ratio)		
Laboratory	**Quadruple**	**Serum Integrated**	**Full Integrated**
A	95	870 (0.11)	280 (0.34)
B	380	1000 (0.38)	410 (0.93)
C	18	224 (0.08)	106 (0.17)
D	190	1800 (0.11)	680 (0.28)
E	116	716 (0.21)	223 (0.52)
F	293	1694 (0.22)	693 (0.42)
G	578	1309 (0.57)	531 (1.09)
H	103	7052	3152
I	165	30	9
J	830	3800	1500
K	147	NR	300
L	100	100	40
M	25	130	45
Expected	**58**	**259**	**95**
Geo Mean	**145**	**651**	**251**
Log SD	**0.48**	**0.70**	**0.69**
CV	**22%**	**25%**	**29%**

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