First Trimester
Interlaboratory Comparison Program

Distribution 2007 FT-B

RAFFAELLO

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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; one page summarizing each of the five specimens. 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 rather another marker. 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 Down syndrome risk.

Variance of Analyte Results: The coefficients of variation (CV) for PAPP-A values range from 13% to 27% for the five challenges. Although data are insufficient to directly compare results between kits, it appears that the PE and DPC kits yield lower CVs than DSL for PAPP-A values, especially values that are relatively low (<2.0 mL). The CVs for hCG are lower than those for PAPP-A, ranging from 11% to 16%. The tighter CVs for hCG have been a consistent finding over time.

Variance of MoM Results: The CVs for PAPP-A MoM values range from 20% to 33%. The highest CV of 33% was observed for FT-06, which likely reflects the variability in computing a gestational age (see discussion on the next page of this report). The CVs for the hCG MoM range from 11% to 15% for four of the specimens, almost as good as the values themselves. This is considered exceptional performance. Specimen FT-07 yields a higher CV of 26%, which reflects the nearly bimodal distribution of responses (eight laboratories reporting MoM levels between 0.84 and 1.01, one laboratory at 1.28 and five laboratories between 1.50 and 1.71). These differences do not seem to be kit associated.

Variance of Risk Results: Most participants in the FT survey report first trimester risks. The two laboratories that report second trimester risks have been converted to first trimester risks, assuming a 45% fetal loss rate between the late first trimester and term. The CVs of the log risk range from 12% to 23%. As expected, the most variable risks occurred for specimen FT-06.

SAMPLE FT-06:

Estimating Gestational Age

For the first time, gestational age was not provided as part of the clinical history for one of the FT samples (FT-06). Instead, a CRL was provided for that sample (and an approximate gestational age in decimal weeks), requiring each lab to estimate gestational age. This was done in order to determine the variability in assigning gestational age by participating laboratories. In contrast to previous distributions and samples FT-07, 08, 09, and 10 in this distribution, more variability is observed. Assigned gestational ages range from a low of 13.1 weeks (three laboratories) to 13.7 weeks (eleven laboratories). The three remaining responses were between these limits. The differences likely reflect the choice of the source of the 'CRL to decimal weeks' equation selected by laboratories. The Supplemental Question in the 2005 FT-C report addresses this issue (accessible at www.ipmms.org), and includes a review of equations in common use. It is recommended that participants review this exercise if there are questions.

Calculating NT MoM

Laboratories were asked to calculate an NT MoM value for a CRL of 76 mm (about 13.7 weeks) and an NT value of 2.0 mm for sonographer HFW. Laboratories had previously been given a set of NT measurements for sonographer HFW and were asked to calculate a sonographer specific equation. This equation can then be used to calculate the MoM value. The expectation is that the resulting MoM values reported by laboratories should be similar, if the median equation is derived correctly given the common set of data. We calculate the median equation for sonographer HFW to be: median NT = 10^(-0.209+0.0050*CRL). One way to compute this equation is to use the Excel calculator supplied to participants over the last two distributions. Using this equation yields a median value of 1.48 mm for a CRL of 76 mm, and a corresponding MoM value of 1.35 (2.0 / 1.48). The consensus NT MoM value was 1.33, close to this expectation. Most laboratories reported MoM values close to 1.33, but three deviated significantly from the consensus value (1.07, 0.98 and 1.15 MoM). These labs are either not using sonographer specific medians, or are deriving the median equation incorrectly. A group of three other laboratories reported MoM values of 1.20, 1.20, and 1.24. This may result from using a source of median values that is higher than the majority of the group. Overall, the results indicate that most laboratories can derive a common median equation, given a set of sonographer NT values, which is the requirement for calculating reliable sonographer specific medians.

Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by three participants (Table 1). All reported using the same method (Di-01 or DSL). The following table provides the reported DIA values and MoM levels for all three samples. Included also is the likelihood ratio (LR) for DIA in the context of the other two markers. Overall, the laboratories reported reasonably equivalent DIA values and MoM levels. However, the changes in Down syndrome risk for Laboratory B are, on average, not consistent with either the other two laboratories or with the reported DIA MoM. Laboratory B should review data entry to ensure that the correct risk results were reported and, if so, that the algorithm was properly implemented.

Table 1. Dimeric inhibin-A (DIA) measurements for FT-B, 2007

Sample Number	Laboratory	Value	MoM	DS Risk (1:n)	DIA LR[1]
FT-06	A	144.0	0.54	810	0.49
	B	119.6	0.51	<10000	
	C	144.0	0.54	810	0.22
FT-07	A	174.0	0.72	510	0.73
	B	132.1	0.54	3900	1.00
	C	178.8	0.72	1620	0.37
FT-08	A	255	1.08	200	0.46
	B	206.9	0.93	546	0.21
	C	220.5	0.95	187	0.36
FT-09	A	860.0	3.26	30	2.33
	B	711.3	2.87	200	1.04
	C	747.2	2.90	160	1.58
FT-10	A	605.0	1.95	26	0.73
	B	493.7	1.86	57	1.00
	C	551.3	1.97	142	0.82

[1] For each participant, the increase/decrease in risk from the combination of NT, PAPP-A and hCG, is divided by the risk that includes DIA measurements. Blanks indicate that the likelihood ratio cannot be reliably determined, usually because both reported risks are very high (e.g., >1:10) or very low (e.g., <1:10,000).

Interpretive Question: First trimester trisomy 18 testing

1 & 2. Of the 21 participating laboratories, three do not screen patients (i.e., they are manufacturers). Of the remaining 18 clinical laboratories, two do not provide first trimester trisomy 18 risks. One of these laboratories mainly provides integrated screening, and a trisomy 18 risk is provided in the second trimester. The analyses will focus on the 16 clinical laboratories reporting first trimester trisomy 18 risks.

3. **What markers do you use for interpreting results for trisomy 18?** Table 2 shows the serum and ultrasound marker combinations used for trisomy 18 risk. Eleven of the 16 utilize NT measurements (69%) in the risk algorithm, while all use measurements of PAPP-A. Laboratories should be aware that the parameters used to compute the trisomy 18 risks are less reliable than those used for Down syndrome. An important ascertainment bias is present in several published reports that will result in over estimating performance of trisomy 18 algorithms and systematically assigning inappropriate risks. The bias has the largest impact on the NT MoM parameters and is likely to have contributed to the wide variability in marker combinations used.

Table 2. Trisomy 18 marker combinations for 16 participating laboratories

Marker Combination (maternal age and)	Participating Laboratories	
	Number	(%)
NT and PAPP-A	8	50
PAPP-A and hCG	4	25
NT, PAPP-A and hCG	3	19
PAPP-A	1	6
Any	16	100

4. **How do you interpret results for trisomy 18?** Table 3 shows the ways in which participants reported trisomy 18 test results. The choices for this question did not allow for the possibility that laboratories might report both risks and screen pos/neg on all reports. Two laboratories interpreting results in this way checked two responses. Almost half of laboratories report risk on all pregnancies, and about one-third report risk only on screen positives.

Table 3. Reporting trisomy 18 test results

Trisomy 18 reporting	Participating Laboratories	
	Number	(%)
Calculate and report risks for all	7	44
Calculate and report only for screen positives	5	31
Calculate and report risks for all and report screen pos	2	2
Calculate and report screen pos/screen neg for all	1	6
Other??	1	6
Any	16	100

5. What is your risk cut-off level (and trimester of risk)? Table 4 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 check to be sure that they are actually reporting first trimester trisomy 18 risks, and not just that they are reporting second trimester risks for trisomy 18 in the first trimester.

Table 4. Trisomy 18 risk cut-off level and trimester of that risk.

Trisomy 18 risk cut-off level		Participating Laboratories	
Risk (1:n)	**Trimester**	**Number**	**(%)**
1:46	First	1	6
1:67	First	1	6
1:100	First	8	50
1:100	Second	5	32
1:200	Term	1	6
Any		16	100

6. What is the source of your algorithm for trisomy 18 risks? Table 5 shows the sources of the trisomy 18 risk for the 16 participating laboratories. Twelve of the laboratories rely on their commercial software. The remaining four laboratories did not respond.

Table 5. Source of trisomy 18 risk algorithms

	Participating Laboratories	
Source of trisomy risks	**Number**	**(%)**
Commercial software (Alpha)	6	38
Commercial software (Benetech)	4	25
Commercial software (Maciel)	2	12
No response	4	25
Any	16	100

7. What interpretation would you give to specimen FT-06? Two laboratories provided neither a risk nor an interpretation. Table 6 shows the risks and interpretation for the remaining laboratories. The trimester of risk is included as well. Given the relatively small numbers of respondents, the large number of confounders (trimester of risk, number of markers, whether or not risk is reported), no analysis was performed. The one, most consistent, result is that all but one lab called the specimen screen negative.

Table 6. Trisomy 18 results for specimen FT-06

Trisomy 18 risk (1:n)	Trimester of risk	Interpretation
	First	Neg
5200	Second	Neg
90	First	Positive
	First	Neg
<100	First	Neg
5480	First	Neg
5860	Second	Neg
10000	First	Neg
345	First	Neg
	First	Neg
847	Second	Neg
521	Second	Neg
450	First	Neg
325	First	Neg

Interpretive Questions – Integrated Screening for Down Syndrome

1. **Do you offer clinical testing for Down syndrome?** As part of an ongoing effort to expand the usefulness of the ICP, we asked whether participants offer integrated screening, and whether they could provide a Down syndrome risk using results from the current ICP distrubtion and the CAP FP-B 2007 survey. Among the 18 clinical laboratories, one did not respond to these interpretive questions. The following analyses are based on the 17 laboratories that responded.

2. **Does your laboratory perform integrated risk interpretations?** Seven reported that they did not. Of the remaining 10, nine reported that they did so as part of a formal integrated screening program; one reported performing integrated risk calculations upon request.

   Laboratories were then asked to combine the information from the FT-07 first trimester sample with the information obtained from sample FP-09 distributed through the CAP FP-B survey. The only changes imposed by us were the draw date of the first trimester sample, and the maternal weight (slightly higher). The different draw date was selected so that it would appear as though the first trimester sample was obtained prior to the receipt of the second trimester sample. Laboratories also were asked to report the triple or quadruple risk reported as part of the CAP FP survey, so that LRs could be computed. As part of this exercise, we also asked laboratories to produce as many different integrated combinations as possible (e.g., serum and full integrated testing with the triple test and/or with the quadruple test) even if they did not offer all of those combinations clinically. For all respondents, the trimester of risk (i.e., second, term) reported as part of the CAP FP survey (e.g., triple, quadruple) were the same as for integrated. To allow direct comparison of the results, reported term risks were converted to second trimester risks, using a fetal survival coefficient of 0.77.

<u>Laboratories that could incorporate quadruple markers into their integrated test</u>.

- *Serum integrated*. Nine laboratories reported both the original quadruple test results for FP-09 and serum integrated, PAPP-A +quadruple test, risks. The consensus PAPP-A result for FT-07 was 1.68 MoM (trimmed MoM 1.60), and, therefore, the serum integrated

risk should be lower than the quad test risk. Likelihood ratios attributable to integrating PAPP-A with the quad test were calculated by dividing the risk reported for the quad test by the risk reported for the serum integrated test. Seven laboratories yielded LRs between 0.16 and 0.48 (geometric mean 0.32), consistent with the expectation of reducing the Down syndrome risk by incorporating PAPP-A (high MoM value) into the risk calculation. However, two laboratories had likelihood ratios consistent with an *increase* in risk (LR of 1.2 and 9.0). These laboratories should review their serum integrated algorithm.

- *Full integrated.* Eight of the nine laboratories reported both the original quadruple test result and full integrated, NT + PAPP-A + quadruple test, risk. The consensus NT MoM result for FT-07 was 1.90 MoM (trimmed mean 1.91 MoM). This elevated MoM would result in a somewhat higher risk, even though the PAPP-A result was elevated. The LRs ranged widely from 1.00 to 8.2 (geometric mean LR of 2.9).

Laboratories that could incorporate triple testing into their integrated test.

- *Serum integrated.* Four participants reported both the original triple test result from FP-09 and the serum integrated (maternal age, PAPP-A, and the triple test). The fifth laboratory did not provide integrated risks for comparison. The consensus PAPP-A result for FT-07 was 1.68 MoM, and, therefore, the resulting serum integrated risk should be lower. The likelihood ratios for the four laboratories responding were 0.31, 1.68, 0.45 and 0.39. The laboratory reporting a higher, rather than lower likelihood ratio and risk, should check its results.
- *Full integrated.* The consensus NT MoM result for FT-07 was 1.90 MoM. This elevated MoM would result in a higher risk, even though the PAPP-A result was relatively high. The four LRs for full integrated triple testing were 2.6, 2.8, 11.8, 3.3 and 2.8. Although the LRs were consistent with increased risks, the 11.8 value appears to be a high outlier and should be checked.

The results from this exercise indicate that integrated testing is being performed by a sufficient number of laboratories to justify continuing to offer inter-laboratory comparison as an ongoing activity of the ICP FT survey. Overall, integrated screening test results were reasonable for most participants. However, a few laboratories appeared not to derive reasonable answers, given the PAPP-A and NT MoM consensus values. These laboratories should review their risk algorithms.

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