

First Trimester Interlaboratory Comparison Program



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INTRODUCTION

Explanation of Data Listing and Analysis

Specimen Options: The ICP offers two choices for specimens for analysis. One specimen set is designed for those participants using hCG in their screening marker combination (**hCG sample set**). The other set is designed for those participants using free beta subunit in their screening marker combination (**free beta sample set**). The specimens may consist of: 1) unmodified patient pools, 2) patient pools diluted with normal human serum and spiked with recombinant hCG or recombinant free beta subunit (but not both), recombinant inhibin, and a PAPP-A concentrate, or 3) normal human serum spiked with recombinant hCG or recombinant free beta subunit (but not both), recombinant inhibin-A and PAPP-A concentrate.

A limited number of additional samples sets are available upon request (free of charge) so that laboratories considering switching from hCG or vice versa can receive both the hCG and the free beta sample sets.

Reading the Data Listing: The five page data listing (in a separate pdf file) contains a summary of reported results for all participants, with each page summarizing one specimen. Your laboratory identification number (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 +/- 0.2 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 standard deviations, after accounting for rounding. A logarithmic transformation is used for the analysis of Down syndrome risks.

Conversion of Reported Down Syndrome Risks to First Trimester Risks: Most 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] x 0.74, or 1:740. Term risks are converted to first trimester risks by multiplying by 0.57.

Down syndrome risks from participants using the free beta sample set are listed in the data sheets, and may be included in the calculation of summary statistics if the target levels are similar to those for hCG. Otherwise, the risks are listed but not included in the analysis. When sufficient numbers are available, a separate analysis will be performed.

Maternal Age Reporting: Maternal age can be reported 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 important for an older woman, especially one whose age falls close to a whole year (e.g., 34.1 versus 34.9 years). Each of these women would be considered to be 34 completed years, even though they are almost one year apart. Laboratories commonly calculate risk using a maternal age equation rather than a table of risks, and it is straightforward to use the more accurate age to obtain better precision. Almost all labs in the ICP report decimal age. Currently, the lab(s) that report integer maternal ages are listed separately on the data summary results. In the future, such results will be listed along with decimal ages but will not be included in the calculations.

NT MoM Reporting: The ICP provides a target NT MoM for most challenges. Participants need to generate the MoM values provided in the histories by trial and error, usually by entering various combinations of CRL/NT/GA values. Approximate CRL values (in mm) and GA values (in weeks and days) are provided as an aid. Participants are asked to report the MoM value that they actually obtained to serve as a check on how reliably they can 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 provide assistance.

The ICP also includes at least one challenge that provides a patient CRL and NT value (in mm), along with a set of NT and CRL values from the submitting 'hypothetical' sonographer (identified by initials) who provided those measurements. Participants can then use that set of sonographer-specific NT/CRL values to generate NT medians for use in converting the NT values (in mm) to MoM. That NT MoM is then used along with maternal age and the chemistry results to calculate the patient-specific Down syndrome risk. We also provide an Excel spreadsheet that can be used to calculate the CRL/NT median equation with accompanying quality assurance parameters (e.g., slope and log standard deviation).

Labs that do not use the MoM for interpretation of NT will only be evaluated for analyte values.

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 may be included in the final calculation of the consensus risk.

Free Beta Subunit Results: The data listings include the analyte and MoM values for the free beta measurements for those laboratories using the free beta specimen set. A median is reported, but a comprehensive analysis is not performed, due to the small number of participating laboratories. However, each of these participants can review their own results by inspection of the data listing.

Currently, all participants receiving the free beta sample set report risks as term risks, and these are listed in the "Report" column under the "Down S Risk" heading. Term risks are converted to first trimester risks and listed in the 1st trim column next to each free beta user's reported term risk. If the consensus MoM for free beta is similar to the hCG MoM, the reported risks for free beta users are included in the summary statistics (after converting term to first trimester risks). A close approximation in MoM values is possible for most manufactured specimens (but not all) because advantage is taken of the high correlation between hCG and free beta values (r values of ~0.8). Roughly, absolute free beta values are approximately 50% to 60% of the absolute values of hCG for patient specimens, e.g., 100 IU/mL hCG will typically have a free beta value of 50-60 ng/mL. For some specimens the relationship for manufactured specimens does not hold, and these are not included in the risk summary statistics. Note also that the impact of hCG and free beta MoM values on the final risk may differ even for identical MoM values because the parameters used in the risk calculation differ for the two analytes.

PAPP-A Values: Many laboratories are now using the Beckman Dxl (or Access) assay for measuring PAPP-A. The Beckman assays are calibrated in ng/mL, which gives absolute values that are approximately 300 times higher than those reported in mIU/mL. Beckman Dxl results are now listed separately in the data sheets for each specimen. Also, some laboratories using the Beckman Dxl assay clinically report out PAPP-A results in ug/mL (ng/mL divided by 1000). These results are converted to ng/mL to avoid introducing further complexity in the report. Finally, most laboratories using the PerkinElmer assay report results in mIU/mL, but some report in mIU/L, which yields values 1000 times higher than those reported in mIU/mL. These values are converted to mIU/mL by dividing by 1000 in the report, again to avoid complexity.

Values in Boxes: The ICP uses two types of boxes in the individual data listings:

- Thin lined boxes are used to call attention to values that are significantly different from the consensus but are not considered outliers (e.g., 1.07). For example, a group of laboratories appears to use only a single set of median NT values (rather than sonographer-specific reference ranges) for calculating MoM values. These differ significantly from the results reported by participants using sonographer-specific medians, but cannot be considered as outliers.
- Thick lined boxes identify values that are outliers as compared to the consensus (e.g., 25.0).

RESULTS

PAPP-A and MoM values (All specimens):

Values. Among the 28 participants, approximately half report in mIU/mL and half in ng/mL. There is no constant relationship between these units (e.g., the conversion factor varies from 248, 379, 400, 348, and 385 ng/mL per mIU/mL for the five specimens, respectively). Separate analyses are therefore performed for each group. The CVs for the participants reporting in mIU/mL are higher (18 to 32%, excluding results for FT-08 which has a very low value with mIU/ml results reported only in one digit) than for those reporting in ng/mL (6 to 9%). This discrepancy is probably because these latter reported are from a single manufacturer. In contrast, three separate manufacturers' reagents are used to report in mIU/mL; insufficient numbers of participants are available to perform method-specific analyses here. However, it is evident by inspection that the Beckman Elisa results (Di-01) are more variable than those from Perkin Elmer (Pe-01). These between-method differences likely account for much of the high CVs for participants reporting in mIU/mL. Therefore, participants using one of these methods should also compare their own results to the other users of the same kit.

MoM. Medians should account, at least in part, for the differences observed for PAPP-A values. However, the CV of MoM values for PAPP-A in this distribution range from 17% to 36%, with higher CVs for lower values. The CV of PAPP-A MoM values have historically been relatively high compared to corresponding values observed for hCG. This may change as the newer, higher precision methods begin to dominate, assuming laboratories generate their own median values. However, methods with high imprecision will continue to inflate the CV. It will also be interesting to see whether differences in between-kit mass values are proportional over the range of values, (e.g., differences in values attributable only to calibration differences). We suggest that ICP participants also review their MoM results in the context of other users of the same method.

hCG mass and MoM values (All specimens):

Values: The all method CVs for the hCG values are typically low as compared to PAPP-A, and this distribution is no different (range 8% to 13%). Systematic between-kit differences may exist, but are likely to be small.

MoM: The all-method CVs for MoM values range from 8% to 13% for the five specimens in this distribution, which are almost as precise as the mass values themselves. This indicates that collectively, laboratories have developed reliable kit-specific population-specific medians.

Free beta mass and MoM values (All specimens):

Values: The number of participants using free beta subunit measurements (all use PerkinElmer assays) is too few to allow a separate analysis (mean, SD, CV). However, from visual inspection of the data it is evident that the between-lab agreement is very good for this analyte with the exception of specimen FT-07. FT-07 is a pool of patient sera drawn at 12 weeks of gesta-

tion, and therefore contains the concentrations of hCG found in clinical practice. It has been well established that spontaneous dissociation of hCG into its component α and β subunits can result in an increase in the concentration of free beta subunit after blood is drawn. Consequently, specimens that are shipped at ambient temperature can demonstrate significant increases in free beta subunit depending on the time in transit and storage conditions. This spurious increase does not manifest in specimens that are manufactured by spiking recombinant free beta subunit in non-pregnant off-clot serum that contains negligible amounts of intact hCG. Those ICP participants using free beta subunit should review results for the specimens other than FT-07. An excellent article discussing this issue for both whole blood and serum was recently published (Cruz J, *et al.* Effect of temperature on free beta human chorionic gonadotrophin and PAPP-A concentration. *Ultrasound Obstet Gynecol* 2010;36:141-6).

MoM: As is true for free beta mass values, the number of participants using free beta subunit measurements is insufficient to allow a separate analysis (mean, SD, CV). However, the limited data suggest that the between-lab agreement is excellent, with the exception of FT-07 (see above).

Down Syndrome Risk (All specimens):

The consensus risks for the five specimens in this distribution, ranked from highest to lowest are **1:14** (FT-08), **1:16** (FT-09), **1:550** (FT-06), **1:3300** (FT-07), and **1:4000** (FT-10). The CVs of the log risk for these ranked risks are **31%**, **15%**, **9%**, **5%**, and **2%**, respectively. The CVs steadily decrease as risks go lower. The atypically very high CV of 31% for FT-08 reflects the fact that the PAPP-A trimmed mean MoM of 0.24 is very low, with the corresponding CV of 33% falling at the extremes of the population distributions. Small differences in MoM values can yield relatively large differences in the likelihood ratios that are used in the risk calculation. In addition, different laboratories may employ algorithms that use different truncation limits for calculating risks. Correlation coefficients used in the algorithms become quite important in these outlying regions.

Free beta results: Down syndrome risks for free beta subunit users are not included in the summary statistics but are instead listed for examination by participant laboratories.

Calculation of gestational age and NT MoM Exercise (FT-06 and FT-06fb):

Participants were asked to calculate an NT MoM value, given a CRL of 48 mm (~ 11.6 weeks' gestation) and an NT value of 0.9 mm submitted by sonographer with initials "GNG". Participants were provided with a set of 150 NT/CRL measurements for sonographer GNG to calculate a sonographer-specific median equation (participants may have already calculated a sonographer-specific median equation in previous exercises). However, participants may or may not have used those medians to calculate their MoM value, depending on their own laboratory protocols. The expectation is that the resulting MoM values reported by laboratories that use sonographer-specific medians should be similar, while those using a single fixed set of NT medians might be different. We calculated the median equation for sonographer FST to be: median NT = $10^{(-0.234+0.00715 \cdot \text{CRL})}$ using the ICP Excel calculator. This equation yields an expected median NT value of 1.285 mm for a CRL of 49 mm, which results in a NT MoM value of 0.70 (0.9/1.285). The consensus NT MoM (calculated as the trimmed mean value) value is 0.69, almost identical to the expected value.

In contrast to previous exercises (*e.g.*, 2010 FT-01) the FT-06 NT challenge found that all of the reported NT MoM values agreed. Those participants with outlying NT MoM values in the earlier distribution had indicated that either they do not use sonographer-specific medians, or they did not know the source of their NT medians. The use of a single set of medians is the likely explanation why they had outlying NT MoM results compared to the consensus (NT MoM

of 1.08) We had specifically designed data for sonographer GNG in an attempt to have agreement between all participants in computing the NT MoM. These GNG median values actually correspond to those used by a group in Europe that advocates the use of a single set of median. That group focuses on standardizing NT measurement protocols such that all sonographers would achieve the same measurements for any given women (allowing for statistical variation). Although this is a laudable goal, it may require some sonographers years of feedback to achieve this goal. In some instances, it may not be achievable. The use of sonographer specific medians could be viewed as an interim improvement in the interpretation of NT results from sonographer who have not yet met these goals.

A CRL of 48 mm was provided for this sample, requiring each participant to calculate gestational age. Most labs reported a gestational age of 11.6 or 11.7 weeks. Two participants reported lower gestational ages (11.3 weeks and 11.4 weeks). This is most likely due to the use of different conversion equations. For more information, see the 2009 FT-A report that includes an analysis of the 'CRL to decimal weeks' equation reported by each laboratory.

Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by four participants. Table 1 lists the reported DIA values and MoM levels for each sample. DIA values show excellent between-method and between-lab agreement. MoM levels are somewhat more variable. Included in the table are the DIA likelihood ratios (LR) in the context of the other markers.

Table 1. Dimeric Inhibin-A results and comparison of DS risks including, and omitting DIA

Sample No.	Lab	Method	Value ¹	MoM	DS Risk (1:n) including DIA	DS Risk (1:n) omitting DIA ²	DIA LR ³
FT-01	A	Beckman Dxl	321	1.13	700	400	0.57
	B	Beckman Dxl	350	1.26	500	320	0.64
	C	Beckman Dxl	375	1.04	2670	2130	0.80
	D	Beckman Dxl	322	0.95	666	427	0.64
FT-02	A	Beckman Dxl	209	1.21	2100	1900	0.90
	B	Beckman Dxl	217	0.85	8300	3800	0.46
	C	Beckman Dxl	231	0.88	7960	3460	0.43
	D	Beckman Dxl	199	0.66	7380	2380	0.32
FT-03	A	Beckman Dxl	101	0.40	17	10	0.59
	B	Beckman Dxl	114	0.40	25	12	0.48
	C	Beckman Dxl	115	0.31	27	35	1.30
	D	Beckman Dxl	98	0.27	13	9	0.69
FT-04	A	Beckman Dxl	196	1.10	10	10	1.00
	B	Beckman Dxl	207	0.91	49	16	0.33
	C	Beckman Dxl	196	0.88	58	16	0.28
	D	Beckman Dxl	189	0.75	110	23	0.21
FT-05	A	Beckman Dxl	339	1.16	3300	2900	0.88
	B	Beckman Dxl	347	1.21	6400	4000	0.63
	C	Beckman Dxl	353	0.91	6440	4270	0.66
	D	Beckman Dxl	329	0.92	9630	6450	0.67

¹ Rounded value

² DS risk reported for NT, PAPP-A, and hCG

³ For each participant, the DIA likelihood ratio (LR) is computed by dividing the reported risk for NT, PAPP-A and hCG by the risk that also includes DIA measurements. If blank, the LR cannot be reliably determined, usually because one (or both) of the risks are very high (e.g., >1:10) or very low (e.g., <1:10,000).

Interpretive Questions: Serum Sample submitted for a first trimester screen drawn in the second trimester.

Q1/Q2. Does your laboratory provide clinical results for Down syndrome in the first trimester?

Analysis is restricted to the 25 participants responding “Yes”. Manufacturers were not included.

Q3. Given NT and CRL measured at 12 weeks 3 days, but serum submitted with a draw date yielding a GA of 15 wks 3 days (based on CRL) what would your laboratory do?

Table 2 lists the 25 responses to Q3. More than half of laboratories would report out a Down syndrome risk using the results from a quad test performed on the maternal serum drawn at 15 weeks 3 days. One lab would also make the offer of a quadruple test to the client. The next most common interpretation would be to add the NT result to a quadruple test, resulting in an integrated risk. This option provides most accurate risk because it uses all of the information available. It is noteworthy that although 18 laboratories report integrated risks for the supplemental portion of this distribution less than half of them would opt to integrate the NT measurement with the quadruple test. One possible explanation for this approach is that some laboratory’s software does not allow the option of integrating results with incomplete information.

The remaining three participants would report that no interpretation is possible. These laboratories might want to consider modifying their protocol to at least allow reporting out a Down syndrome risk with quad test results.

Table 2. Responses of 25 participants to Q3

Content of Report	N (%)
DS risk based on quadruple only	14 (56)
Other (offer client quadruple test)	1 (4)
DS risk based on NT integrated with quadruple	7 (28)
No interpretation possible	2 (8)
No interpretation possible, add footnote explaining when blood must be collected	1 (4)
DS risk based only on NT and maternal age	0 (0)
All	25 (100)

Interpretive question: Integrated screening

Over the past three years, we have focused our integrated screening analysis on computing likelihood ratios showing the progression of risk from quad (or triple) to serum integrated (adding PAPP-A) to full integrated (adding NT). The serum and full integrated risks are now analyzed in the same way as the first trimester combined risks. Eighteen participants reported integrated risks using first trimester markers (FT-07) in combination with the second trimester quadruple test (FP-12). Six laboratories do not report integrated risks and one participant did not respond to this question. All laboratories now integrate risks using the second trimester quad results. Also, some laboratories report a risk for both the triple and the quad test but only the quad test results are analyzed. Eighteen laboratories report risks in the second trimester while the remaining five report risks at term.

Table 3 lists these risks, along with the trimester of risk. The last column contains the risk after adjustment to the second trimester (for purposes of comparison). Laboratories using free beta subunit in their risk computations did not differ from those laboratories using hCG. Therefore, these have been included in the summary statistics. Two participants were identified as outliers for both serum and full integrated risks.

Table 3. Summary of Integrated Down Syndrome Risks

Trimester of Risk	Serum Integrated Risk (with 2 nd trimester quad)		Full Integrated Risk (with 2 nd trimester quad)	
	Reported	Adjusted	Reported	Adjusted
2	125	125	79	79
2	180	180	120	120
2	680	680	180	180
2	550	550	200	200
2	420	420	290	290
2	580	580	390	390
2	600	600	400	400
2	857	857	556	556
3	1300	975	770	578
2	920	920	650	650
2	910	910	660	660
3	1700	1275	920	690
2	1472	1104	952	714
3	1000 ¹	750	1000 ¹	750
3	1100 ¹	825	1100 ¹	825
2	1280	1280	902	902
3	6900	5175	4900	3675
2	5800	5800	3900	3900
Data trimmed		125,180		79,120
Geo mean		1020		640
CV (log risk)		10%		12%
Mean - 2 SD		320		180
Low		420		180
High		5800		3900
Mean + 2 SD		5800		5000

¹Laboratories using free beta subunit in place of hCG

Among the ICP participants, the geometric mean risk for the second trimester quad test is 1:129 (CAP specimen FP-12). The consensus PAPP-A MoM for specimen FT-07 is 1.20. The cross over point (likelihood ratio of 1.0) for affected and unaffected PAPP-A distributions is approximately 0.6 MoM. Thus, the expectation with a PAPP-A of 1.20 MoM is a relatively large decrease in the serum integrated risk compared to the quadruple risk. The geometric mean for the serum integrated risk of 1:1016 meets this expectation (LR of 0.13). The NT MoM provided in the ICP history for FT-7 is 1.40. The cross over point for the affected and unaffected distributions of NT MoM is approximately 1.45 MoM. The expectation is, therefore, that combining the NT MoM value of 1.4 with the serum integrated test would slightly increase the risk for the full integrated test. The geometric mean for the full integrated risk of 1:640 also meets expectation (LR of 1.6).

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