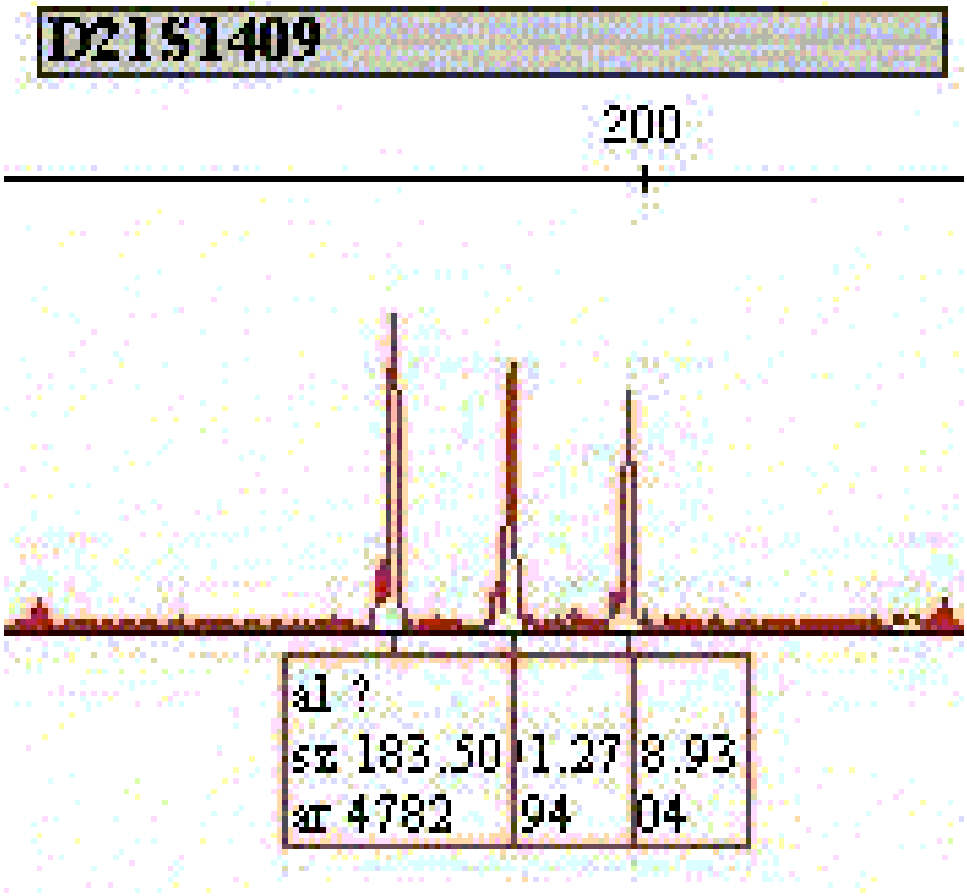
First Trimester
Interlaboratory Comparison Program



An electrophoretogram showing abnormal qfPCR results. The short tandem repeat (STR) D21S1409 located on chromosome 21, is heterozygous over the three copies of the chromosome, indicated by relatively equal heights of the peaks. The result indicates the sample was from a pregnant woman whose fetus is affected with Down syndrome. Diagnostic testing using qfPCR is widespread in Europe and Canada, but relatively uncommon in the United States. Figure courtesy of the University of Leeds (www.leedsteachinghospitals.com).

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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.

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 DxI (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 DxI 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 mass and MoM values (All specimens):

Values. Among the 29 participants, approximately half report in mIU/mL and half in ng/mL. There is no consistent relationship between these units (*e.g.,* the conversion factors for the five current specimens are 325, 306, 266, 321, and 328 ng/mL per mIU/mL, respectively). Separate analyses are performed for each group. The CVs for the participants reporting in mIU/mL are higher (20% to 29%) than for those reporting in ng/mL (6 to 8%). Some of this difference is attributable to the fact that values reported in ng/mL are from a single manufacturer. In contrast, three separate manufacturers' reagents report in mIU/mL. Insufficient numbers of participants are available to perform method-specific analyses.

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 19% to 37%, with higher CVs being associated with lower PAPP-A mass 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 of interest 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 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 compared to PAPP-A, and this distribution is no different (range 10% to 17%). Although systematic between-kit differences may exist, they are likely to be small.

MoM: The all-method CVs for MoM values range from 11% to 18% 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.

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 suggests that the between-lab agreement is excellent.

Down Syndrome Risk (All specimens):

The consensus trimmed risks for the five specimens in this distribution, ranked from highest to lowest are **1:16** (FT-14), **1:34** (FT-12), **1:140** (FT-13), **1:610** (FT-15), and **1:710** (FT-11). The CVs of the log risk for these ranked risks are **41%, 27%, 25%, 13%, and 8%,** respectively. The CVs monotonically decrease as risks increase. The very high CV of 41% for FT-14 reflects the fact that the PAPP-A trimmed mean MoM of 0.25 is very low and the hCG trimmed mean of 3.79 is very high. These values fall at the extremes of the population distributions for these two markers. Small differences in MoM values at these extremes can yield relatively large differences in the likelihood ratios used in the risk calculation. In addition, participants may employ algorithms that use different truncation limits. Correlation coefficients used in the algorithms can also become quite important in these outlying regions.

Free beta results: Down syndrome risks for free beta subunit users are therefore included in the summary statistics (for most samples they are within the +/- 2 SD limits). However, one free beta user reports some risks that are discrepant from other free beta users as well as with the overall consensus.

Calculation of gestational age and NT MoM Exercise (FT-13 and FT-13fb):

Participants were asked to calculate an NT MoM value, given a CRL of 48 mm (about 11.6 weeks' gestation) and an NT value of 0.9 mm, assuming the values were submitted by sonographer identified as "GNG". Participants were provided with a set of 150 NT/CRL measurements for sonographer GNG (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 GNG to be:

$$\text{median NT} = 10^{(-0.239+0.00724*\text{CRL})}$$

using the ICP Excel calculator. This equation yields an expected median NT value of 1.28 mm for a CRL of 48mm, which results in a NT MoM value of 0.70 (0.9/1.28). The trimmed mean NT MoM value is 0.70, identical to the expected value.

In contrast to previous exercises (*e.g.,* 2010 FT-01) the FT-13 NT challenge found that all of the reported NT MoM values showed good agreement. 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 a likely explanation why they had outlying NT MoM results compared to the consensus. We specifically designed data for sonographer GNG in an attempt to have agreement between all participants in computing the NT MoM. GNG's measurements correspond to the median values advocated by the Fetal Medicine Foundation. That group assumes that all credentialed sonographers would achieve the same NT measurements for any given women. Although a laudable goal, it is not always achieved in practice. The use of sonographer specific medians could be viewed as an interim step in for sonographers who have not yet met this goal.

Using the CRL of 48 mm, most labs reported a gestational age of 11.6 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 (see the 2009 FT-A report that includes an analysis of the 'CRL to decimal weeks' equation reported by each laboratory).

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Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by four participants (Table 1). DIA mass values show excellent between-laboratory agreement. MoM levels more variable. Included in Table 1 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-11	A	Beckman DxI	280	1.09	360	310	0.86
	B	Beckman DxI	308	1.28	600	600	1.00
	C	Beckman DxI	251	0.80	3740	1740	0.47
	D	Beckman DxI	289	0.84	2160	1380	0.64
FT-12	A	Beckman DxI	211	1.32	29	12	0.41
	B	Beckman DxI	217	1.09	130	36	0.28
	C	Beckman DxI	191	0.83	540	63	0.12
	D	Beckman DxI	214	0.90	153	26	0.17
FT-13	A	Beckman DxI	426	1.29	110	68	0.62
	B	Beckman DxI	451	1.68	40	30	0.75
	C	Beckman DxI	436	1.14	269	158	0.59
	D	Beckman DxI	419	1.20	56	43	0.77
FT-14	A	Beckman DxI	273	1.58	20	10	0.50
	B	Beckman DxI	277	1.37	20	7	0.35
	C	Beckman DxI	253	1.16	44	12	0.27
	D	Beckman DxI	261	1.04	33	7	0.21
FT-05	A	Beckman DxI	266	1.20	210	160	0.76
	B	Beckman DxI	283	1.46	490	390	0.80
	C	Beckman DxI	244	1.09	2060	991	0.48
	D	Beckman DxI	262	1.10	3590	2250	0.63

1 Rounded value in ng/mL

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: Polices with regard to use of NT measurements for calculation of Down syndrome risk.

Q1. Does your laboratory use NT measurements in computing Down syndrome risks as part of a clinical service?
28 participants responded "Yes". One of these did not report any further answers. The following analyses are restricted to the remaining 27 participants.

Q2. Do you use NT MoM values or delta NT values?
All 27 participants (100%) reported using the NT MoM, rather than delta NT for interpretation.

Q3. To convert NT measurements to MoM values, are your medians..
5 (18%) always use a single median equation for all sonographers
6 (22%) sometimes use center or sonographer-specific medians
15 (56%) nearly always use center or sonographer-specific medians
1 (4%) reported using some other method

Q4. Given that you use sonographer or center-specific medians are these..
11 (41%) use the existing capability in their interpretative software
5 (18%) aren't sure (these are the same five reporting using only a single set of medians)
5 (18%) use combinations of these and other methods (*e.g.,* from FMF)
3 (11%) use the Excel spreadsheet routinely distributed as part of the ICP
2 (8%) compute the medians off-line and enter them into the software
1 (4%) uses an off-site service (NT Monitor)

Q5. Does your laboratory require proof of successful training/credentialing for sonographers submitting NT measurements used for Down syndrome screening?
All 27 participants (100%) report requiring proof of training/credentialing.

Q6. If yes, indicate what organizations provide acceptable training/credentialing.
18 (67%) allow both FMF and NTQR training/credentialing
5 (18%) allow only FMF
3 (11%) allow FMF, NTQR and at least one other training/credentialing group
1 (4%) allows only NTQR

Q7. Do you routinely send all NT results submitted to your laboratory to a credentialing organization?
13 (48%) do send sonographer information to the credentialing organization
12 (44%) do not send sonographer information to the credentialing organization(s)
1 (4%) selectively sends data to one, but not the other, credentialing organization
1 (4%) sends information on selected sonographers to the credentialing organization(s)

Q8. Is a minimum number of CRL/NT measurements (or images) required to be submitted to the laboratory before a sonographer's NT results can be incorporated in the Down syndrome risk?
16 (59%) do have a required minimum number of images/measurements
9 (33%) do not have a required minimum number of images/measurements
2 (8%) state that the requirement may, or may not, be required, depending on other factors

Fourteen of the 16 participants requiring a minimum number of images reported the number to be…
6 (43%) 10-20 images
4 (29%) >60 (the largest requirement was 300-400 images/measurements)
2 (14%) 21-40
2 (14%) 41-60
0 (0%) < 10 images

Q9. Do you routinely monitor NT QA parameters for each sonographer (prenatal center)?
22 (82%) do regularly monitor NT QA parameters
5 (18%) do not regularly monitor NT QA parameters

Q10. How is your monitoring accomplished (may sum to more than 100%, as many reported multiple methods of monitoring).
Overall, 23 participants (85%) reported their methods of monitoring
19 (83%) In-house
5 (22%) Credentialing agency (e.g., NTQR, FMF)
5 (22%) Other (e.g., commercial software, collaboration of laboratories)
3 (13%) External service (NT monitor)

Q11. What parameters are monitored and what is considered an acceptable range?
Overall, 19 participants (70%) reported monitoring at least one parameter.
11 (58%) monitor all three parameters (slope, median MoM and log SD of NT MoM)
5 (26%) monitor one parameter (4 monitor the median MoM, and one monitors the slope)
3 (16%) monitor two parameters (slope and median MoM)

Slope (acceptable range of percent increase in NT measurement per week)
6 (46%) 15-35%
3 (19%) 15-25%
2 (15%) 14-50%
2 (15%) 15-40%

Median NT MoM (acceptable range)
13 (73%) 0.90 to 1.10
3 (17%) 0.92 to 1.08
1 (5%) 0.91 to 1.09
1 (5%) Other (95% confidence interval)

Standard deviation of the log NT MoM (acceptable range)
5 (45%) 0.08 to 0.13
2 (18%) <0.18
2 (18%) 0.08 to 0.14
1 (9%) 0.07 to 0.11
1 (9%) 0.09 to 0.14

Q12. On average, how often are NT QA parameters monitored?
Overall, 23 participants (85%) reported how often they monitor QA parameters.
3 (13%) Annually
7 (30%) Bi-annually
9 (39%) Quarterly
4 (17%) Other (2 are monthly, 2 are unknown)

Q13. Do you share individual results of your monitoring with each sonographer on a routine basis (with or without comparison to a reference distribution of NT values)?

Overall, 23 participants (85%) reported whether or not they contacted sonographers.
12 (52%) Did not contact each sonographer
11 (48%) Did contact each sonographer

Q14. Do you take any action of QA parameter results are unacceptable for an individual sonographer?

Overall, 22 participants (82%) reported their action/inaction.
20 (91%) reported taking action
2 (9%) reported taking no action

Q15. What are those actions taken by these 20 participants? (may sum to more than 100%, as many reported multiple actions taken).

16 (80%) Recalculate medians
15 (75%) Inform the sonographer / center of the findings
7 (35%) Offer/request that the sonographer talk with/visit with an expert
5 (25%) Other (*e.g.,* retake on-line course/resubmit images, provide findings to supervisor)
3 (12%) Send results to credentialing organization for action
1 (4%) Cease providing DS risk until credentialing organization gives OK

These data are provided as feedback to the participants without comment from the ICP organizers. They may form the basis of a short communications regarding the quality assurance of NT measurements by participating laboratories.

Interpretive question: Integrated screening

Twenty-one participants reported integrated risks using first trimester markers (FT-13) 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 can now report integrated risks using the second trimester quadruple test. Some laboratories report a risk for both the triple and the quadruple test, but only the quadruple test results are analyzed. Eighteen laboratories report risks in the second trimester while three report risks at term.

Table 2 lists these risks, along with the trimester of risk. The last column contains the risk after adjustment to the second trimester, to allow for direct comparison. Laboratories using free beta subunit in their risk are therefore included in the summary statistics. One participant was identified as an outlier for both serum and full integrated risks.

Table 2. Summary of Integrated Down Syndrome Risks

Trimester of Risk	Quad Risk (FP-15) Reported	Quad Risk (FP-15) Adjusted	Serum Integrated Risk Reported	Serum Integrated Risk Adjusted	Full Integrated Risk Reported	Full Integrated Risk Adjusted
2	660	660	420	420	1700	1700
2	520	520	46	46	300	300
2	1100	1100	20	20	75	75
2	890	890	30	30	230	230
2	637	637	200	200	210	210
2	1900	1900	26	26	149	149
2	630	630	15	15	91	91
2	870	870	75	43	790	450
2	580	580	16	16	120	120
2	1010	1010	20	20	145	145
2	620	620	25	25	210	210
2	550	550	18	18	110	110
2	390	390	11	11	85	85
2	110	110	9	9	50	50
2	448	524	2200	2200	3700	3700
3	920	920	25	14	140	80
2	400	400	10	10	50	50
3	2400[1]	1368	170	97	1400	798
3	2500[1]	1425	370	211	3300	1881
3	740	422	50	29	310	177
3	3200[1]	1824	450	257	2500	1425
Trimmed		110		2200		3700
Geo Mean		740		36		210
CV(log risk)		7%		27%		19%
Mean-2SD		320		8		46
Low		390		9		50
High		1900		257		1881
Mean+2SD		2200		740		3800

[1] Laboratories using free beta subunit rather than total/intact hCG measurements

Among the ICP participants, the trimmed geometric mean risk for the second trimester quadruple test for ICP participants is 1:740 (CAP specimen FP-15). The trimmed mean PAPP-A MoM value for specimen FT-13 is 0.23. The cross-over point (likelihood ratio of 1.0) for affected and unaffected PAPP-A distributions is approximately 0.6 MoM. Thus, the expectation is a large increase in the serum integrated risk compared to the quadruple risk. The geometric mean for the serum integrated risk of 1:36 meets this expectation (LR of 20.5). The trimmed mean NT MoM value calculated by ICP participants for FT-13 is 0.70. The cross over point for the affected and unaffected distributions of NT MoM is approximately 1.45 MoM. The expectation is, therefore, that full integrated test would be reduced, and the risk of 1:210 meets expectation (LR of 0.17).

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