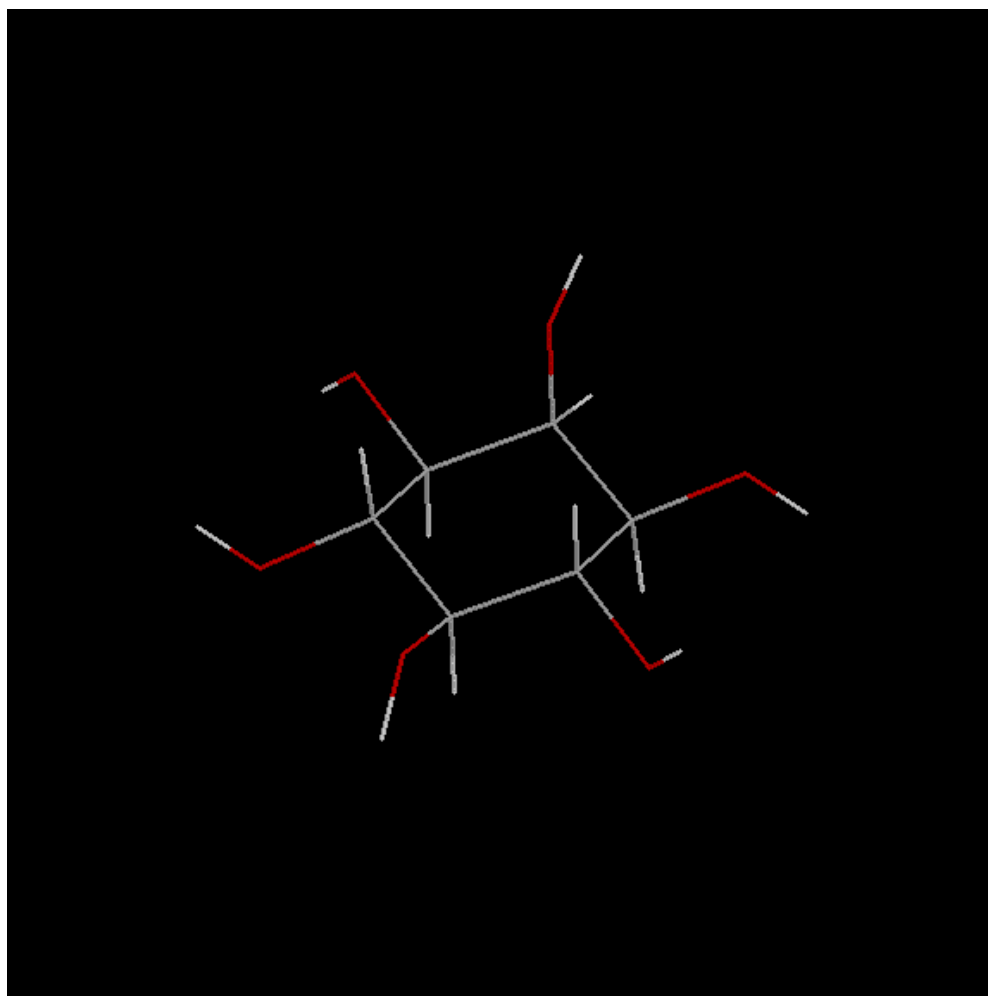


# First Trimester Interlaboratory Comparison Program

Distribution 2007 FT-C



**Three dimensional structure of myo-inositol**

Researchers from the Institute of Psychiatry at King's College London have identified a molecule that could be targeted to treat the cognitive impairment in people with Down syndrome. The study, published in Archives of General Psychiatry found that people with Down syndrome have higher levels of myo-inositol in their brains than people without the condition, and that increased levels of this molecule are associated with reduced intellectual ability. (Science Daily, Dec 6, 2005)

Sponsored by:  
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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. 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 0.78. This accounts for fetal loss between the first and second trimesters (40% from first trimester to term, and 27% from second trimester to term). For example, if the second trimester risk is 1:1000, the first trimester risk is 1:1000 x 0.78, or 1:780.

## RESULTS

FT-11: Laboratories were asked to calculate an NT MoM value given a CRL of 58 mm (about 13.6 weeks' gestation) and an NT value of 0.89 mm for a new sonographer identified as JAC. Laboratories were given a series of NT/CRL measurements for sonographer JAC and asked to calculate a sonographer-specific 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 who use sonographer-specific medians should be similar. We calculate the median equation for sonographer JAC to be: median NT =  $10^{(-0.303+0.00611*CRL)}$  using the Excel calculator supplied to participants. Using this equation yields an expected median NT value of 1.126 mm for a CRL of 58 mm, and, therefore, a MoM value of 0.79 (0.89/ 1.126). The consensus NT MoM value was 0.79. Most laboratories reported MoM values close to 0.79, but two deviated significantly from the consensus value (0.59 and 1.12 MoM). The lab that reported a MoM of 0.59 successfully completed the exercise for calculating a median equation, but earlier reported using only one set of NT medians. The lab that reported an NT MoM of 1.12 also successfully calculated a median equation for sonographer JAC, but likely also does not implement sonographer specific medians as part of clinical practice. Overall, the results indicate that most 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 also true for FT-06 in the FT-B distribution, gestational age was not provided as part of the clinical history for FT-11. 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-11 were similar, ranging from 12.1 to 12.3 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.

FT-12: The original date of birth (DOB) for sample FT-12 sent out on the FT-C data form was given as 12/12/75, which would have yielded a maternal age of **32.3 years**. In a follow-up email from us, this DOB was changed to 03/26/69 to match the DOB provided for sample FP-18 in the CAP FP-C distribution. This was done so that labs could perform an integrated screening exercise. The updated DOB yielded a maternal age of **39.0 years** and almost all laboratories reported this maternal age. However, two labs appear to have used the original DOB as they reported an age of 32.3 years, and one lab reported a truncated age of 31 years; results from these labs are highlighted in the data listing. The lab that reported an age of 32.3 had an outlying risk of 1:25,500, but use of an age of 39.0 would increase this risk by a factor of five, yielding a risk of approximately 1:5000, and it would no longer be an outlier. We apologize for any confusion that the change in DOB created.

Two laboratories that use the DPC kit reported PAPP-A values consistent with the other two DPC users. However, their reported MoM values were significant outliers, suggesting that their median values may be too low.

FT-13: The PAPP-A value for this specimen was targeted to be low, and the consensus value of 0.45 mIU/ml was close to expectation. The CV of 26% was reasonable given this low value. The hCG value was targeted to be high, and the consensus value of 295 IU/ml was close to expectation. Not unexpectedly, given a low PAPP-A, a high hCG, and a maternal age of 33.6 years, the Down syndrome risk was expected to be high (the NT MoM did not have much of an effect on the final risk because the consensus NT MoM of 1.42 was close to the crossover point on the overlapping distributions, which yields a likelihood ratio close to 1.0). The consensus risk was 1:7 with a CV of log risk of 27%. The high CV likely results from the relatively extreme PAPP-A and hCG MoM values where small differences cause relatively large differences in the likelihood ratio.

FT-14: This specimen was targeted to have a high PAPP-A value, and the trimmed mean consensus value of 8.36 mIU/ml was consistent with expectation. Overall, the results were good, with a CV of 11%, with only one outlying value (5.20 mIU/ml). The high consensus PAPP-A MoM of 2.37, the consensus hCG MoM of 1.32, the NT MoM of 0.75, along with a maternal age of 28.6 years would be expected to yield a low risk. The consensus risk was 1:1100, with a low CV of 4% for log risk.

FT-15: This sample was targeted to have a low hCG, as might occur with trisomy 18 or fetal demise. The consensus value was 19.2 IU/ml, with a high CV of 50%. Note that the trimmed mean of 28.2 IU/ml differed from the consensus value even though no values were trimmed. This was because the median, rather than the trimmed mean was used, given that the distribution was essentially bimodal. This also explains the high CV. The bimodal distribution is attributable primarily to the systematic differences between the DPC and Beckman kits at these low levels (median value 44.1 IU/ml and 17.3 IU/ml, respectively). The CVs of the hCG MoM for the other PT specimens were much lower, indicating that these differences are primarily confined to very low hCG values. In practice, very 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. However, if the specimen also had a low PAPP-A value and/or a high NT value, then the patient might be identified at increased risk for trisomy 18. If a laboratory is reporting out risks for trisomy 18 they might consider retesting the specimen at a lower dilution to obtain a more reliable value. It is interesting that several laboratories recommended US/Amnio as the action,

even though they interpreted the Down syndrome risk as screen negative. Although this might be due to the sample being from a women over 35 years of age, this was not the action recommended for another specimen that was screen negative, again from a women over age 35.

### Dimeric inhibin-A (DIA)

First trimester DIA measurements were reported by four participants (Table 1). 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 1. Dimeric inhibin-A (DIA) measurements for FT-C, 2007**

Sample Number	Laboratory	Value	MoM	DS Risk (1:n)	DIA LR <sup>1</sup>
FT-11	A	472.0	1.43	3900	0.87
	B	326.0	1.29	7820	0.60
	C	358.3	1.17	8450	0.56
	D	348.2	1.13	839	0.56
FT-12	A	83.1	0.40	10000	0.06
	B	78.6	0.35	<10000	
	C	60.3	0.24	27500	
	D	77.0	0.31	3220	0.13
FT-13	A	1025	3.63	10	1.00
	B	905.6	3.32	4	1.00
	C	765.4	2.28	10	1.00
	D	873.7	2.44	5	1.00
FT-14	A	204.0	0.85	<10000	
	B	161.2	0.73	<10000	
	C	153.8	0.65	27500	0.47
	D	167.5	0.70	15800	0.20
FT-15	A	101.0	0.33	130	0.29
	B	70.7	0.32	<10000	
	C	67.6	0.27	27500	0.27
	D	71.2	0.30	37100	0.29

<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. Blanks indicate that 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 Questions – Review of new sonographer “JAC”

1. **Does your laboratory convert NT measurements (in mm) to multiples of the median (MoM) as part of a clinical service?** Among the 21 responding participants, 18 laboratories responded yes (one of these intends to begin screening in the near future). The following analyses are restricted to these 18 respondents.
2. **To convert NT measurements to MoM levels, are your medians ...** Five reported that only a single set of medians is used, three reported medians are sometimes based on sonographer- or center-specific medians, and nine reported that center- or sonographer-specific medians are nearly always used.
3. **Given that you can use sonographer- or center-specific medians, are these ....** Five reported that the clinical software does the computation, three computed medians off-line and entered the results into their clinical software, and four used the Excel program supplied by the FT survey. Among the five laboratories that do not currently use sonographer- or center-specific medians, one reported using the Excel program for computations and another reported that their software performed these calculations internally.
4. **Analyze the data for sonographer JAC.** Eighteen clinical laboratories reported results.
  - a. **Number of samples used.** A total of 74 samples were distributed in the spreadsheet. Ten participants trimmed one sample and analyzed 73, six used all 74, one said they used 75 (probably looked at the row number and forgot about the header row), and another reported using 84. The default upper CRL limit is set to 78 mm, and one observation is at 79 mm. Laboratories should enter their own limits for both CRL and NT as the default values may not be appropriate for all.
  - b. **Slope per week.** This question should have explicitly stated that the slope was to be expressed in percent per week as this is the common format. If one were to report the ‘raw’ slope (e.g., 1.2088, or 0.0824), these aren’t really in a ‘per week’ format and one would have to include the model that you are fitting. However, it is straightforward to convert these ‘raw’ slopes to percent per week. All but one laboratory reported a slope of about 21%. One laboratory reported 28.4% per week and they need to recheck their computations. A slope of 21% is right on the expected slope per week for the NT values in the late first trimester.
  - c. **Median NT MoM of the samples.** Within random error, this number should be 1.00. The overall median was 0.99 with an overall mean value of 1.00. Only one laboratory reported a value other than 0.99 or 1.00 (a 1.04). This laboratory should recheck their analysis.
  - d. **Log SD of the NT MoM levels.** All participants reported SD’s in the range of 0.1099 to 0.1112. This is a reasonable log SD for an individual sonographer.

## Interpretive Questions – Integrated Screening for Down Syndrome

5. **Does your laboratory provide clinical Down syndrome screening services?** Among the 21 responding participants, 16 laboratories responded yes.
6. **Does your laboratory perform integrated risk interpretations?** Among the 16 laboratories, six 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. All laboratories used the same ‘trimester of risk’ for their quadruple and integrated Down syndrome risks (second trimester). Based on the results of

a similar set of questions in FT-B, nearly all laboratories could provide both serum and full (including NT) integrated risks using all four second trimester markers (AFP, uE3, hCG and DIA). Relevant information includes: Maternal age of 39.0 years, median MoMs for PAPP-A and NT of 1.09 and 0.89, respectively (FT-12 data listing), and AFP, uE3, hCG and DIA consensus MoMs of 0.98, 0.59, 1.14, and 0.56. Table 2 summarizes important findings of the risks reported for combinations of these results.

**7/8. Report the Down syndrome risks from FP-18 (CAP FP-C 2007 Survey). Report integrated risks using FT-12 results (with modifications to the draw date).** The consensus risk from the FP survey for the quadruple markers was 1:900 (second trimester). Given the PAPP-A consensus MoM of 1.09, one would expect each laboratory to calculate a reduced serum integrated test risk as compared to their quad risk. This reduction can be expressed as an LR ratio, obtained by dividing the quad risk by the serum integrated risk (column 2 in Table 2) This is generally true; seven of the nine labs reported risks reduced by 51% (LR of 0.49). However, two laboratories reported higher serum integrated risks than quadruple risks. One lab (indicated by the code E) reported a relatively low PAPP-A MoM that would likely increase the risk. Another (coded G) reported a PAPP-A MoM slightly under 1.0, inconsistent with nearly an 8-fold increase in Down syndrome risk. Given the addition of an NT MoM of 0.89 (found for all participants), the expectation is that the risk would be further reduced (column 3 in Table 2). This is generally true as well. The consensus reduction is 67% (LR of 0.33). Again, however, two laboratories found large increases in risk compared to the quad test result. One (coded G) did reduce the risk from a 7.9-fold increase to a 4.6-fold increase. Another (coded I) reported that the NT MoM of 0.89 resulted in a several fold increase in risk, which is not plausible.

**Table 2. The likelihood ratios (LR) for serum integrated (S-Int) and full integrated (F-Int) compared to quadruple testing (Quad) for 10 laboratories performing integrated screening**

Laboratory	LR (S-Int/Quad)	LR (F-Int/Quad)	Identical Parameters
A	0.67	0.31	Don't know
B	0.40	0.23	No
C	0.08	0.08	No
D	NA	0.09	No
E	2.5	0.77	No
F	0.93	0.52	Yes
G	7.9	4.6	Yes
H	0.73	0.34	Yes
I	0.41	6.1	Yes
J	0.20	0.30	Yes
All	0.49	0.33	

**9. Do you use the same parameters for the second trimester markers (e.g., uE3 mean and SD) for both the quadruple test and the integrated test?** Nine of the 10 laboratories providing integrated risks responded, and four reported that they used different parameters, five used the same parameters, and one did not know. This is important for the following reason. Suppose you interpret a quadruple test using parameter set A for Woman 1. Another woman (Woman 2) has a serum integrated test that includes PAPP-A and the

quadruple test interpreted using another parameter set (B). By chance, the AFP, uE3, hCG and DIA levels are identical for Woman 1 and 2. However, their risks will systematically differ for two reasons. First, they will differ because one interpretation includes PAPP-A measurements, but secondly they will also differ because of differences in the two parameter sets.

In order to see how different parameter sets can impact assigned Down syndrome risks, we used SURUSS parameters and another parameter set based on published quadruple markers and first trimester markers different from SURUSS. Table 3 contains a summary of these computations (all second trimester risks), created using the consensus MoM levels and maternal age reported above. The consensus CAP FP Survey risk of 1:900 for the quadruple test is considerably lower than the 1:380 or 1:240 predicted by the two parameter sets we tested. Within both sets, the changes in risks are similar (LR's of 0.72 and 0.55 for the addition of PAPP-A) and 0.38 and 0.16 for the addition of PAPP-A and NT measurements in the full integrated test. These LR's are also similar to the majority of LR's given in Table 2. Notice the problem that would occur if you used the quadruple test risk of 1:900 (based on whatever parameter set was most common in the FP survey) and the SURUSS parameters for serum integrated. It would appear that the risk increased (from 900 to 530), when the expectation is that the risk should be reduced. The use of a single parameter set will eliminate the occurrence of these 'odd' risk comparisons.

**Table 3. A comparison of Down syndrome risks generated using consensus values and selected parameter sets**

Parameter Set	Test combination	Down syndrome risk	Likelihood ratio
FP Survey/Unk	Quadruple	900	
SURUSS	Quadruple	380	
SURUSS	Serum Integrated	530	0.72 (380/ 530)
SURUSS	Full Integrated	1000	0.38 (380/1000)
Mixed	Quadruple	240	
Mixed	Serum Integrated	440	0.55 (240/ 440)
Mixed	Full Integrated	1500	0.16 (240/1500)

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