

Fetal Defect Marker Proficiency Test Mailout October, 2008

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from September 9, 2008, for Fetal Defect Markers, which included samples for first and second trimester screening, as well as amniotic fluids. Your laboratory's results and grades are printed on a separate sheet; also included are the grades from the previous two PT events. Please review and sign your evaluation. Retain the signed packet in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

I. Graded Results Section:

Table 1: Second Trimester Maternal Serum: Summary of All Lab Results

Samples *N = 31	Sample #	MS 226	MS 227	MS 228	MS 229	MS 230
	Gestational Age (weeks)	20.0	15.0	18.0	19.0	16.0
Maternal Race	Ethnic Group	White	Black	Hispanic	White	Asian
Maternal Weight	Pounds (lbs)	145	180	155	139	150
Maternal Age	Years	30	22	32	28	40
Alpha-Fetoprotein (AFP)	Mean ng/ml \pm Std.Dev.	165.51 \pm 10.81	27.02 \pm 1.60	20.21 \pm 1.01	52.30 \pm 3.50	31.41 \pm 2.01
	MOM \pm Std.Dev.	2.75 \pm 0.19	0.93 \pm 0.08	0.47 \pm 0.03	0.98 \pm 0.07	0.95 \pm 0.07
Unconjugated Estriol (uE3)	Mean ng/ml \pm Std.Dev.	2.55 \pm 1.22	0.93 \pm 0.40	0.83 \pm 0.32	2.17 \pm 1.00	1.13 \pm 0.45
	MOM \pm Std.Dev.	0.75 \pm 0.21	0.94 \pm 0.32	0.42 \pm 0.15	0.80 \pm 0.24	0.86 \pm 0.26
human Chorionic Gonadotrophin (hCG)	Mean IU/ml \pm Std.Dev.	18.71 \pm 1.80	37.42 \pm 3.81	45.33 \pm 4.30	49.01 \pm 4.81	28.57 \pm 1.72
	MOM \pm Std.Dev.	1.05 \pm 0.17	1.01 \pm 0.17	2.18 \pm 0.20	2.50 \pm 0.37	0.97 \pm 0.14
Dimeric Inhibin-A (DIA)	Mean pg/ml \pm Std.Dev.	270.21 \pm 29.30	163.70 \pm 16.91	375.81 \pm 37.40	237.71 \pm 25.50	165.31 \pm 17.92
	MOM \pm Std.Dev.	1.39 \pm 0.19	0.97 \pm 0.11	2.27 \pm 0.27	1.30 \pm 0.18	0.95 \pm 0.12
Neural Tube Screen (Positive, Negative) percent	Pos (+) or Neg. (-)	Pos. (+) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Further Action R,U,A	G = 74% U = 77% A = 74%	NFA	NFA	NFA	NFA
	NTD Risk 1 in	103	10,000	10,000	6,850	8,655
Trisomy-21 Screen (Positive, Negative) percent 1. <u>Triple test</u>	Pos. (+); Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Neg. (-) (81%)	Neg. (-) (67%)
	Recommended Action**	NFA	NFA	G = 77% U = 55% A = 78%	U = 50% A = 50%	U = 44% A = 50%
	Risk Est. 1 in	3,050	3,170	12	452	233
2. <u>Quad Test</u>	Pos. (+); Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Neg. (-) (81%)	Neg. (-) (67%)
	Recommended Action**	NFA	NFA	G = 71% U = 64% A = 86%	U = 17% A = 21%	U = 24% A = 31%
	Risk Est. 1 in	6,388	5,000	7	871	380
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+)/Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (55%)	Neg. (-) (100%)
	Risk Est. 1 in	10,000	10,000	681	10,000	2,130

*N=total numbers may vary since some labs do not test all analytes. The values represent the all-lab consensus based on the arithmetic mean \pm Std.Dev.; (B) = borderline positive or negative, risk reflects central tendency (Median number for Down positive/borderline screen). NFA = no further action; FA = further action; G = genetic counseling; U = ultrasound, and A = amniocentesis. **This percentage is normalized to labs requesting further action. † Insulin Dependent Diabetic pregnancy.

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 31 all-lab Consensus Values.

<u>Sample #</u>	<u>Summary Comments (Mock specimens):</u>
MS 226 Wk 20.0	This specimen was obtained from a 30 year old white woman (Gravida = 2, Parity = 0) in her 20 th week gestation with a body weight of 145 lbs. She had a personal history of pregnancy loss. Her specimen, a second pregnancy sample, was a positive screen for NTD (100% consensus; MOM=2.75). Her screen was negative for both Trisomies with all labs in agreement. Recommendations of further action from labs performing the NTD screen were: genetic counseling, 74%, ultrasound, 77% and amniocentesis, 74%; The MS226 specimen had an amniotic fluid counterpart which was also elevated (MOM = 3.39).
MS 227 Wk 15.0	This specimen was obtained from a 22 year old black woman (Gravida = 1, parity = 0) in her 15 th week gestation with a body weight of 180 lbs. She had no family history of pregnancy complications or adverse outcomes. Her sample screened negative for NTD and her aneuploidy screen was negative for both Trisomy-21 and for Trisomy-18. Although no recommendation of further action was reported from participating labs, a body weight correction was warranted. This specimen was not paired to an amniotic fluid specimen.
MS 228 Wk 18.0	This specimen was obtained from a 32 year old hispanic woman (Gravida = 3, parity = 1) in her 18 th week gestation with a body weight of 155 lbs. She had a family history of pregnancy complications and adverse outcomes. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100%). Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 71%; ultrasound, 64%; and amniocentesis, 86%; while the triple tests were: genetic counseling, 77%; ultrasound 55%, and amniocentesis, 78%. This specimen was paired to an amniotic fluid specimen which had a normal AFAP level (MOM = 1.47). The T18 screen was also negative in all labs.
MS 229 Wk 19.0	This specimen was procured from a 28 year old white woman (Gravida = 3, parity = 0) in her 19 th week gestation with a body weight of 139 lbs. She had a family history of pregnancy complications. To date, her pregnancy appeared to follow a favorable course of gestation, and her specimen resulted in a negative screen for NTD. The labs were also in agreement that both trisomy-21 screens were negative (81%), as was the T18 screen. Specimen MS229 was not paired with an amniotic fluid sample.
MS 230 Wk 16.0	Specimen MS230 represented a specimen obtained from a 40 year old asian woman in her 16 th week (Gravida = 5, Parity = 4) with a body weight of 150 lbs. She had no family history of adverse pregnancy outcomes in any relatives. Her MS sample was deemed screen negative (100%) for NTD with no further action recommended. Specimen MS230 also screened negative for trisomy-21 (67%; see critique) and 100% negative for Trisomy-18. This specimen had no amniotic fluid counterpart.

Notice of Gravida/Parity Clarification for Present and Future Mail outs;

Instructional Note:

This notice regards the demographic data provided for the mock patients in the FEDM program. For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of times pregnant including the present one; a gravida = 2 indicates that the women was pregnant once before in addition to her present pregnancy. Parity = 1 indicates the patient already has one child; however, multiple birth is also considered as a single parity.

Example: A woman of gravida = 3, parity = 2 indicates that the pregnant woman has been pregnant twice before, and has two children.

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=31; all-lab Consensus Values

<u>Sample#</u>	<u>Values</u>	<u>Summary Comments:</u>
AF 226 Wk 20.0	AFP= 21.0 ± 2.5 µg/ml MOM= 3.39 ± 0.47	This sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs called AF226 an elevated specimen for NTD. This AFAFP sample was matched to a maternal serum specimen, MS226 which was also elevated (MOM = 2.75).
AF 227 Wk 19.0	AFP= 9.50 ± 1.41 µg/ml MOM= 1.23 ± 0.16	This sample was targeted for an NTD screen negative AFAFP value in the upper gestational age range. All labs reported this specimen as a screen negative (low) AFAFP value. The AF227 specimen was not paired with a maternal serum sample.
AF 228 Wk 18.0	AFP= 14.02 ± 2.01 µg/ml MOM= 1.47 ± 0.16	This sample was targeted for a negative NTD screen AFAFP value in the routine gestational age screening range. All labs categorized this as an NTD screen negative specimen. This sample was matched to a maternal serum specimen, MS228, which was low (MOM = 0.47).
AF 229 Wk 19.0	AFP= 6.00 ± 0.71 µg/ml MOM= 0.78 ± 0.08	This sample was targeted as an NTD screen negative AFAFP value in the upper gestational age screening range. All labs categorized AF229 as a negative NTD screen specimen, and it had no maternal serum counterpart.
AF 230 Wk 17.0	AFP= 5.4 ± 0.80 µg/ml MOM= 0.46 ± 0.04	This sample was targeted for an NTD screen negative AFAFP value in the routine gestational age range. All labs classified AF230 as an NTD screen negative (non-elevated) specimen. This AFAFP specimen was not matched to an MS AFP specimen.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples N = 16	Sample #	FT 226	FT 227	FT 228	FT229	FT230
	Gestational Age (weeks)	13.0	10.9	11.9	11.5	12.4
Maternal Race	Ethnic Group	Hispanic	Black	White	Asian	White
Maternal Weight	Pounds (lbs)	160	155	150	130	140
Maternal Age	Years	21	32	25	28	33
Nuchal Translucency (NT)-Associated	Crown Rump Length (mm)	67	41	53	47	60
	NT Thickness (mm)	1.55	1.10	2.90	1.20	1.40
	NT - MOM	1.05	1.10	2.36	1.09	1.03
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	67.90 ± 8.40	89.80 ± 8.31	158.51 ± 17.90	81.70 ± 9.71	71.61 ± 6.60
	MOM ± Std. Dev.	1.09 ± 0.15	1.09 ± 0.12	2.16 ± 0.22	0.99 ± 0.15	1.01 ± 0.13
Pregnancy-Associated Plasma Protein-A (PAPP-A)	Mean mIU/mL ± Std. Dev.	5.54 ± 2.71	3.40 ± 1.69	1.98 ± 0.95	4.11 ± 2.33	4.74 ± 2.30
	MOM ± Std. Dev.	2.90 ± 1.34	3.78 ± 2.12	1.48 ± 0.74	3.21 ± 1.76	2.67 ± 1.30
Trisomy-21 Screen (Positive/Negative) percent	Pos. (+) or Neg. (-)	Neg. (100%)	Neg. (100%)	Pos. (99%)	Neg. (100%)	Neg. (100%)
	Recommended Action	NFA	NFA	G = 78% U = 57% C = 71%	NFA	NFA
	Risk Estimate	10,000	10,000	45	10,000	10,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	10,000	10,000	2,800	10,000	10,000

(B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; C = chorionic villus sampling; N = number of labs participating; FT = First Trimester

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

N = 16 all-lab Consensus Values.

<u>Sample#</u>	<u>Summary Comments:</u>
FT 226 Wk 13.0	This specimen was obtained from a 21.0 year old hispanic woman of medium body weight (160 lbs). Her gestational age at time of screening was 13.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs (N=16) were in agreement. The FT226 risk estimate for Trisomy-21 was 1 in 10,000, and the Trisomy-18 risk was 1 in 10,000.
FT 227 Wk 10.9	This specimen was procured from a 32 year old black woman of average body weight (155 lbs.). Her gestational age at time of screening was 10.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement (see Critique). The FT227 risk estimate for Trisomy-21 was 1 in 10,000, while the Trisomy-18 risk was 1 in 10,000.
FT 228 Wk 11.9	This specimen was obtained from a 25 year old white woman of average body weight (150 lbs.). Her gestational age at time of screening was 11.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen positive and all testing Labs were in agreement (see Critique). The FT228 risk estimate for Trisomy-21 was 1 in 45; while the all-lab Trisomy-18 risk was 1 in 2,800. All labs were in agreement that FT228 was a high risk Trisomy-21 pregnancy.
FT 229 Wk 11.5	This specimen was procured from a 28 year old white woman with a body weight of 130 lbs. Her gestational age at time of screening was 11.5 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative and all labs were in agreement. The risk estimate for FT229 was 1 in 10,000, and the T18 risk was 1 in 10,000. All labs were in agreement with the FT229 negative screen assessment.
FT 230 Wk 12.4	This specimen was procured from a 33 year old white woman with a body weight of 140 lbs. Her gestational age at time of screening was 12.4 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative and all labs were in agreement. The risk estimate for FT230 was 1 in 10,000, and the T18 risk was 1 in 10,000. All labs were in agreement with the FT230 negative screen assessment.

III. Critique and Commentary:

A) Fetal Defect Proficiency Test Mail out 9/9/08 of Second Trimester Maternal Serum and Amniotic Fluid Values:

In general, the all-lab results of the targeted values for the NTD and the Trisomy Screen attained the goals of our projected target values, risks, and outcomes. As displayed in the second trimester tables, maternal serum sample MS226 was targeted as an elevated specimen for NTD (Figs. 1 and 3), together with an elevated AF-AFP. Specimen MS226 was determined to be screen positive for NTD, but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS226 specimen, the NTD screen resulted in a 1 in 103 all-lab risk for open neural tube defects (ONTD) and achieved 100% screen consensus. The NTD-related recommended action for the MS226 specimen was as follows: genetic counseling, 74%; ultrasound; 77%; and amniocentesis, 74%. Sample MS228, a T21 screen positive specimen, was obtained from a hispanic woman with a prior family history of pregnancy problems. The T21 MOM results for specimen MS228 (MSAFP-MOM = 0.47, MSuE3-MOM = 0.42, MShCG-MOM = 2.18, DIA-MOM = 2.27) were all in accordance with a T21 positive screen; hence, all labs classified this specimen as T21 positive with all recommending further action. Samples MS227, MS229 and MS230 produced negative screens for NTD, Trisomy-21, and Trisomy 18. However, the MS229 specimen was obtained from a woman of caucasian descent and demonstrated an elevated hCG which served to contribute to a positive screen in 19% of the participating labs. Finally, the MS230 sample with advanced age (40 yrs.) triggered a positive T21 screen in 33% of the labs, because the AFP (MOM = 0.95), uE3 (MOM = 0.86), DIA (MOM = 0.95) and hCG (MOM = 0.97) showed decreased levels. Although a greater risk of T21 would be expected from the maternal age alone (1 in 55), the risk was lower due to the levels of the remaining serum analytes.

Specimen MS226, together with a matched AF-AFP sample, deserves comment in that the MSAFP specimen was NTD screen positive (100%; MOM = 2.75) and the paired AF-AFP sample was also elevated (MOM = 3.39) (Figs. 1 & 2). This mock patient had a family history of pregnancy loss; thus, paired maternal MS and AF samples had been obtained at time of amniocentesis. Since, her positive MSAFP MOM, was accompanied by the elevated AF-AFP MOM, it was consistent with an NTD positive screen. No demographic correction factors for this patient were indicated for the MS226 specimen screen assessment. An all-lab NTD risk assessment for MS226 was 1 in 103 for her maternal serum screen. A subsequent high definition Stage-II ultrasound of this mock patient together with an Ache analysis later confirmed the presence of an open NTD lesion in the spinal cord of the fetus.

The MS228 specimen (gravida = 3, parity = 1; maternal age = 32) produced a positive Trisomy-21 (T21) result with both the triple and quad testing platforms. All labs reporting either triple or quad testing concluded that sample MS228 was T21 screen positive (100% all-lab consensus). Further action recommended for the T21 screen was determined as genetic counseling, 77%, 55% ultrasound (US), and 78% amniocentesis (AM) for labs using the triple screen; while genetic counseling, 71%, 64% US and 86% AM was recorded for labs employing the quad screen. The recommended action on MS228 was consistent with the severity of the similar risk ratio assessments of 1 in 12 risk from the triple test versus a 1 in 7 risk from the quad test. Note from the point distribution graphs comparing the triple with the quad test (Figs. 5 and 6) that the MS228 point cluster in the quad assay was nearly the same but slightly lower and tighter (note scale difference) than the MS228 cluster in the triple test. As with previous mailouts, the quad test signaled a slightly higher risk (difference) for Down syndrome than the triple test; hence, both screens resulted in high risks for Down syndrome. The mid-term DS risk (Triple/Quad) for MS228 was greater than that expected from the maternal age alone (1 in 480). At the time of the assay, the sample was accompanied by an amniotic fluid (AF) specimen due to prior pregnancy complications in sibling-related pregnancies. The AFAFP in this sample was normal (MOM = 1.47). The amniotic fluid cells of this mock patient were subjected to subsequent genotyping and indeed indicated the presence of a Trisomy-21. MS228 produced a negative screen for Trisomy-18.

The specimen MS229 displayed an unusual biomarker profile in its screen results. The MSAFP was normal (MOM = 0.98) and the uE3 level was low (MOM = 0.80), but the MShCG was elevated (2.50 MOM) while the DIA was normal (MOM = 1.30). In view of these results, most of the participating laboratories called MS229 a negative screen NTD, T21, and T18, but some of the labs suggested further actions. Interestingly, both the triple and the quad test for T21 resulted in positive screens in only 19% of the labs due to the mostly normal MS-analyte profile and elevated hCG. The presence of an elevated hCG in a somewhat normal maternal serum profile prompts one to consider conditions where hCG predicts poor outcomes. Adverse pregnancy outcomes relative to an elevated maternal serum hCG (MOM = 2.5) usually involve placental dysfunction and chromosomal anomalies accompanied by sonographic adverse findings and growth retardation (see below). Interestingly, the MS229 specimen was obtained from a 28 year old caucasian patient and adverse outcomes were not a part of her personal family history.

It has long been observed that elevated concentrations in maternal serum intact MShCG were predictive of fetal abnormalities or placental complications at mid trimester. Hence, increased surveillance is recommended in pregnancies where the MShCG in the second trimester is inexplicably elevated (1,2). Uterine arterial doppler and placental ultrasound have been employed to confirm predicted severe placental dysfunction and adverse outcomes by means of elevated MShCG in conjunction with MSAFP (3). Hence, isolated elevations of MShCG alone do not confer an increased risk of fetal congenital anomalies and chromosomal abnormalities. Elevated MShCG levels in combination with sonographic “soft markers” for aneuploidy have been found to be associated with a high incidence of chromosomal anomalies despite a normal triple serum screen (4). For example, fetuses with mosaic trisomy-13 may be associated with both elevated MShCG and MSAFP levels in the second trimester as well as cases of *de novo* interstitial deletion of chromosome 4q 12-21 as seen in Down syndrome (5, 6). Although MShCG in the second trimester are not always elevated in normotensive women who later produced a growth-retarded fetus, MShCG levels are significantly higher in women who develop cases of severe pre-eclampsia (7). Finally, MShCG concentrations have been reported to be significantly higher in cases of threatened miscarriage compared with gestational-age matched controls (8).

The MShCG references listed below refer to the text discussed in the preceding paragraph:

1. Blundell, G., J. P. Ashby, C. Martin, C. H. Shearing, B. Langdale-Brown, J. Keeling, P. M. Ellis, M. Shade, S. E. Chambers, and P. R. Wenham. (1999). Clinical follow-up of high mid-trimester maternal serum intact human chorionic gonadotrophin concentrations in singleton pregnancies. *Prenat Diagn.* 19:219-23.
2. Ganapathy, R., R. F. Lamont, and P. Bassett. (2007). Unexplained elevated maternal serum beta-HCG concentration and adverse pregnancy outcome. *Prenat Diagn.* 27:995-9.
3. Alkazaleh, F., V. Chaddha, S. Viero, A. Malik, C. Anastasiades, H. Sroka, D. Chitayat, A. Toi, R. C. Windrim, and J. C. Kingdom. (2006). Second-trimester prediction of severe placental complications in women with combined elevations in alpha-fetoprotein and human chorionic gonadotrophin. *Am J Obstet Gynecol.* 194:821-7.
4. Celentano, C., P. E. Guanciali-Franchi, M. Liberati, C. Palka, D. Fantasia, E. Morizio, G. Calabrese, L. Stuppia, and S. Rotmensch. (2005). Lack of correlation between elevated maternal serum hCG during second-trimester biochemical screening and fetal congenital anomaly. *Prenat Diagn.* 25:220-4.
5. Chen, C. P., S. R. Chern, S. J. Cheng, T. Y. Chang, L. F. Yeh, C. C. Lee, C. W. Pan, W. Wang, and C. Y. Tzen. (2004). Second-trimester diagnosis of complete trisomy 9 associated with abnormal maternal serum screen results, open sacral spina bifida and congenital diaphragmatic hernia, and review of the literature. *Prenat Diagn.* 24:455-62.
6. Hsu, T. Y., F. T. Kung, C. Y. Ou, P. Y. Hsiao, F. J. Huang, C. C. Changchien, and S. Y. Chang. (1998). Prenatal diagnosis of *de novo* interstitial deletion of proximal 4q by maternal serum screening for Down syndrome. *Prenat Diagn.* 18:1323-7.
7. Luckas, M. J., R. Sandland, J. Hawe, J. P. Neilson, I. R. McFadyen, and J. W. Meekins. (1998). Fetal growth retardation and second trimester maternal serum human chorionic gonadotrophin levels. *Placenta.* 19:143-7.
8. Johns, J., S. Muttukrishna, M. Lygnos, N. Groome, and E. Jauniaux. (2007). Maternal serum hormone concentrations for prediction of adverse outcome in threatened miscarriage. *Reprod Biomed Online.* 15:413-21.

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in a bar-graph format (Figures 7-10) for each of the five MS samples. As shown in the MSAFP graph, AFP mass measurements among the individual kits largely agreed, although Siemens ADVIA-Centaur was slightly higher, and Siemens Immulite and Beckman Access or Unidel were marginally lower for some samples. For uE3, the mean/all kit median for Diagnostic Systems Lab hovered around 1.0 (see Fig. 8); however, labs employing Siemens Immulite or Immulite 2000 yielded values 1.2 to 2.1 times higher than the mean/all kit median (see dotted line). In contrast, Beckman Access measured uE3 values 35 to 45% lower than the mean/all kit median of 1.0. Regarding the hCG kits (see Fig. 9), all methods gave essentially the same result with less than 5% difference between the different methods. In order to enhance uniformity among the various kits employed to measure hCG, we incorporate intact recombinant (total) hCG analyte into our PT specimens. Finally, the method comparison of Inhibin-A is displayed in Fig. 10 for the Beckman Access/Unidel versus the Diagnostic Systems Lab (DSL) assay platforms. Consistently, Beckman was marginally higher than DSL. Labs lacking peer group companions and in-house assays will be deemed non-gradable (NG) for individual analyte groups as the situation dictates.

The bar graph in Figure 11 is provided to display kit performances among the amniotic fluid (AF-AFP) test samples. While Siemens ADVIA-Centaur/ACS-180 and Abbott-AxSYM kits were 15-20% higher (except for sample AF230), Beckman Unidel/and Beckman Access/2 were about 15% - 20% lower than the 1.0 mean/all-kit median. Finally, please be advised that these specimens are derived from actual AF samples, and therefore these results are directly relevant to patient screening. Please note: the samples AF226 (Abbott AxSYM) and AF230 (Siemens/ACS) exhibited higher means due to the presence of outliers in a small sample size (N=4).

C) Screening Software Utilized:

For informational purposes, it was deemed of interest to provide the software package usage by our participating laboratories. The alpha and Benetech software packages were each used by 22% of the labs respectively; Robert Maciel (RMA) software was employed by 28%; while in-house software comprised 16% and 12% of labs used programs classified as "other" which are proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples are now and will be included in all future mailouts in order to survey New York State licensed laboratories concerning participation and assay capabilities in first trimester Down syndrome screening. All laboratories that are **validation-approved** and presently performing first trimester Down syndrome screening are **REQUIRED** to test and report screen results; however, the laboratory results will **not** be graded at this time. Those laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT measurements in millimeters), last menstrual period (LMP), and draw date. Crown-rump length (CRL) measurements, race, and maternal body weight have been included in the case histories to better evaluate all-lab participant NT information requirements.

In the FT226 hispanic sample, the gestational age all-lab mean was reported as 13.0 weeks. Assay measurements for FT226 resulted in an all-lab total hCG mass measurement of 67.90 ± 8.40 based on two present methods, Beckman Access/Unidel and Siemens Immulite or 2000, while the all-lab PAPP-A mass assessments were 5.54 ± 2.71 obtained from the Diagnostic Systems Labs and Siemens Immulite kits (see Figs. 12 and 13). The first trimester all-lab trisomy-21 consensus for FT226 was screen negative, with a risk of 1 in 10,000. As observed in the FT table 2 above (see Section - B), the all-lab measurement for FT226 of total hCG resulted in a MOM value of 1.09. The all-lab MoM mean for PAPP-A was 2.90 ± 1.34 . However, it should be noted that the two methods employed for PAPP-A measurements, the Diagnostic Systems kit (N=10) and the Siemens Immulite kit (N=5) (see Fig. 13 for mean/all kit medians) measure PAPP-A quite differently. For example, the Siemens Immulite PAPP-A assay mass mean for FT226 was 9.56mIU/ml compared to Diagnostic Systems Lab 3.98 mIU/ml. Regardless of this, all the labs agreed that the FT226 sample was screen negative for Trisomy 21 (See Fig. 14 point distribution). Since PAPP-A measurement for first trimester Down syndrome is associated with low MOM values, higher PAPP-A MOMs together with normal hCG levels were consistent with the screen negative outcome. The all-lab T21 risk assessment for FT226 was 1 in 10,000. The FT226 specimen also resulted in a negative screen for Trisomy-18.

As shown in the above First Trimester table 2 (Section-B) for the FT227 Afro-American specimen, the gestational age all-lab mean was reported as 10.9 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of 89.80 ± 8.30 IU/ml based on the two methods above, while the all-lab PAPP-A mass assessment was 3.40 ± 1.69 mIU/ml. The first trimester all-lab Trisomy-21 screen consensus for FT227 was negative. The all-lab FT trisomy-21 risk assessment was 1 in 10,000, while the Trisomy-18 risk was also 1:10,000. As observed in the FT table above (Table 2, Section - B) the all lab measurement of total hCG for sample FT227 achieved a MOM value of 1.09 ± 0.12 . In comparison, the all-lab MoM for PAPP-A was 3.78 ± 2.12 , a normal value. Despite the assay differences in the PAPP-A Siemens mass mean values compared to the Diagnostic Systems Lab means, all labs agreed that the FT227 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). PAPP-A measurements for first trimester Down syndrome are associated with low MOM values, hence, high PAPP-A MOMs and normal hCG levels and NT measurement would be consistent with a screen negative outcome. The FT227 specimen also resulted in a negative screen for Trisomy-18.

As demonstrated in the FT table 2 (Section – B) above, the all lab measurement of the 11.9 week Caucasian FT228 specimen for total hCG resulted in a mass mean of 158.51 IU/ml \pm 17.90, with an elevated MOM = 2.16. Furthermore, the all-lab mass mean for PAPP-A was 1.98 \pm 0.95 mIU/ml with a MOM = 1.48 \pm 0.74. The all-lab T21 risk assessment was 1 in 45 for the FT228 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT228 sample was screen positive (See Figure 14 risk distribution) demonstrating a high hCG MOM = 2.16, a mid-normal PAPP-A MOM = 1.48, and an increased NT MOM = 2.36. The risk cut-off level for Caucasians ranges from 200 to 270 among the participating labs. Since PAPP-A measurements for first trimester Down syndrome are associated with increased NT MOM values, low to normal PAPP-A, together with high hCG MOMs, these results would be consistent with a screen positive outcome. Thus, the FT228 sample resulted in a 99% all lab T21 positive screen assessment. Further actions by the labs included genetic counseling, 78%; ultrasound, 57%; and amniocentesis/CVS = 71%. Finally, the FT228 specimen screened negative for T18 (1 in 2,800) using a cutoff of 1 in 100.

As shown in the FT table 2 (Section-B) above for the Asian FT229 specimen, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of 81.70 \pm 9.71 IU/ml based on the two methods, while the all-lab PAPP-A mass assessment was 4.11 \pm 2.23 mIU/ml. The first trimester all-lab trisomy-21 screen consensus for the FT229 specimen was negative (100%). The all-lab FT Trisomy-21 risk assessment was 1 in 10,000. As observed in the table (Table 2, Section – B), the all lab measurement of total hCG MOM for FT229 achieved a value of 0.99 \pm 0.15. In comparison, the all-lab MOM mean for PAPP-A was 3.21 \pm 1.76, a relatively high value. Even in the face of assay differences in the PAPP-A Siemens kit mass mean compared to Diagnostic Systems Labs, all labs agreed that the FT229 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). Because PAPP-A measurements for first trimester Down syndrome are associated with low MOM values as observed, normal hCG MOM together with normal NT values were consistent with a screen negative outcome. The FT229 specimen also resulted in a negative screen for Trisomy-18.

As shown in the FT table 2 (Section-B) above for the Caucasian FT230 specimen, the gestational age all-lab mean was reported as 12.4 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of 71.61 \pm 6.60 IU/ml based on the two methods, while the all-lab PAPP-A mass assessment was 4.74 \pm 2.30 mIU/ml. The first trimester all-lab trisomy-21 screen consensus for FT230 was negative. The all-lab FT Trisomy-21 risk assessment was 1 in 10,000. As observed in the table (Table 2, Section – B), the all lab measurement of the total hCG MOM for FT230 achieved a value of 1.01 \pm 0.13. In comparison, the all-lab MOM mean for PAPP-A was 2.67, a relatively high value. Despite the difference in the PAPP-A Siemens assay mass mean compared to Diagnostic Systems Labs, all labs agreed that the FT230 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). Since PAPP-A measurements for first trimester Down syndrome are associated with low MOM values as observed, normal hCG MOM together with normal NT values were consistent with a screen negative outcome. The FT230 specimen also resulted in a negative screen for Trisomy-18.

D. 1.) First Trimester Assay kit Performance:

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in a bar-graph format (Figures 12, 13) for each of the five FT samples. As shown in the total FT hCG kit graph, hCG measurement between the two kits differed only slightly, with Beckman Unicel/Access kit measuring a little higher than the Siemens kits. In contrast, results from the two PAPP-A kits varied widely with the values from Diagnostic Systems Lab (DSL) being less than half of those obtained with Siemens Immulite or Immulite 2000 kit. Even though the MoMs between Siemens Immulite and DSL kit users differed by an average factor of 2.4, the final risk outcomes were the same. (see MoM table below).

<u>Specimen</u>	FT226	FT227	FT228	FT229	FT230
FT PAPP-A MoM Siemens Immulite or 2000 (DPB or D/DP5) Mean:					
Mean	4.73	6.39	2.47	5.58	4.38
SD	0.49	1.50	0.39	0.87	0.75
FT PAPP-A MoM Diagnostic System Labs (DS1) Mean:					
Mean	2.09	2.53	1.01	2.18	1.91
SD	0.42	0.55	0.24	0.52	0.44

G.J. Mizejewski, Ph.D.

New References (Suggested reading):

1. Dugoff, L., H. S. Cuckle, J. C. Hobbins, F. D. Malone, M. A. Belfort, D. A. Nyberg, C. H. Comstock, G. R. Saade, K. A. Eddleman, P. Dar, S. D. Craigo, I. E. Timor-Tritsch, S. R. Carr, H. M. Wolfe, and M. E. D'Alton. (2008). Prediction of patient-specific risk for fetal loss using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers. *Am J Obstet Gynecol.* 199:290.e1-6.
2. Kang, J. H., A. Farina, J. H. Park, S. H. Kim, J. Y. Kim, N. Rizzo, A. Elmakky, H. S. Jun, W. B. Hahn, and D. H. Cha. (2008). Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: correlation with the week of onset and the severity. *Prenat Diagn.* 28:704-9.

3. Xie, Z., S. Lu, Y. Zhu, Y. Sun, and Y. Jin. (2008). Second-trimester maternal serum free-beta-human chorionic gonadotropin and alpha-fetoprotein levels in normal twin and singleton pregnancies: a report of local Chinese population. *Prenat Diagn.* 28:735-8.
4. Dreux, S., C. Olivier, J. M. Dupont, N. Leporrier, G. Study, J. F. Oury, and F. Muller. (2008). Maternal serum screening in cases of mosaic and translocation Down syndrome. *Prenat Diagn.* 28:699-703.
5. Urato, A. C., R. Quinn, A. Pulkkinen, S. D. Craigo, and W. Allan. (2008). Maternal smoking during pregnancy and false positive maternal serum alpha-fetoprotein (MSAFP) screening for open neural tube defects. *Prenat Diagn.* 28:778-80.
6. Mitsuhashi, N., S. Kobayashi, T. Doki, F. Kimura, H. Shimizu, H. Yoshidome, M. Ohtsuka, A. Kato, H. Yoshitomi, S. Nozawa, K. Furukawa, D. Takeuchi, K. Suda, S. Miura, and M. Miyazaki. (2008). Clinical significance of alpha-fetoprotein: involvement in proliferation, angiogenesis, and apoptosis of hepatocellular carcinoma. *J Gastroenterol Hepatol.* 23:e189-97.
7. Le Meaux, J. P., V. Tsatsaris, T. Schmitz, Y. Fulla, O. Launay, F. Goffinet, and E. Azria. (2008). Maternal biochemical serum screening for Down syndrome in pregnancy with human immunodeficiency virus infection. *Obstet Gynecol.* 112:223-30.
8. Thornburg, L. L., K. M. Knight, C. J. Peterson, K. B. McCall, R. A. Mooney, and E. K. Pressman. (2008). Maternal serum alpha-fetoprotein values in type 1 and type 2 diabetic patients. *Am J Obstet Gynecol.* 199:135.e1-5.
9. Wang, P., H. Zhang, W. Li, Y. Zhao, and W. An. (2008). Promoter-defined isolation and identification of hepatic progenitor cells from the human fetal liver. *Histochem Cell Biol.* 130:375-85.
10. Chen, H. W., H. L. Chen, Y. H. Ni, N. C. Lee, Y. H. Chien, W. L. Hwu, Y. T. Huang, P. C. Chiu, and M. H. Chang. (2008). Chubby face and the biochemical parameters for the early diagnosis of neonatal intrahepatic cholestasis caused by citrin deficiency. *J Pediatr Gastroenterol Nutr.* 47:187-92.
11. Manganaro, R., L. Marseglia, C. Mami, G. Saitta, R. Gargano, and M. Gemelli. (2008). Serum alpha-fetoprotein (AFP) levels in breastfed infants with prolonged indirect hyperbilirubinemia. *Early Hum Dev.* 84:487-90.
12. Sifakis, S., N. Mantas, A. Konstantinidou, O. Koukoura, E. Avgoustinakis, and E. Koumantakis. (2008). A stillborn fetus with amniotic band syndrome and elevated levels of alpha-fetoprotein plus beta-human chorionic gonadotropin: a case report. *Fetal Diagn Ther* 24:111-4.
13. Echevarria, M. E., J. Fangusaro, and S. Goldman. (2008). Pediatric central nervous system germ cell tumors: a review. *Oncologist.* 13:690-9.
14. Shaw, S. W., J. J. Hsu, C. N. Lee, C. H. Hsiao, C. P. Chen, T. T. Hsieh, and P. J. Cheng. (2008). First- and second-trimester Down syndrome screening: current strategies and clinical guidelines. *Taiwan J Obstet Gynecol.* 47:157-62.
15. Erden, G., A. O. Barazi, G. Tezcan, and M. M. Yildirimkaya. (2008). Biological variation and reference change values of CA 19-9, CEA, AFP in serum of healthy individuals. *Scand J Clin Lab Invest* 68:212-8.
16. Costa, S., M. P. De Carolis, I. Savarese, S. Lacerenza, and C. Romagnoli. (2008). Hepatic hematoma in a neonate with a high level of alpha-fetoprotein. *Eur J Pediatr.* 167:591-3.
17. Crocoli, A., S. Madafferi, A. Jenkner, A. Zaccara, and A. Inserra. (2008). Elevated serum alpha-fetoprotein in Wilms tumor may follow the same pattern of other fetal neoplasms after treatment: evidence from three cases. *Pediatr Surg Int.* 24:499-502.
18. Sullivan, I., J. Faulds, and C. Ralph. (2008). Contamination of salvaged maternal blood by amniotic fluid and fetal red cells during elective Caesarean section. *Br J Anaesth.* 101:225-9.
19. Ranganath, L., W. Taylor, L. John, and Z. Alfirevic. (2008). Biochemical diagnosis of placental infarction/damage: acutely rising alkaline phosphatase. *Ann Clin Biochem.* 45:335-8.
20. Sell, S. (2008). Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 29:161-80.

21. Dodd, J. M., K. Sahi, A. McLeod, R. C. Windrim, and J. P. Kingdom. (2008). Heparin therapy for complications of placental dysfunction: a systematic review of the literature. *Acta Obstet Gynecol Scand* 87:804-11.
22. Uversky, V. N., C. J. Oldfield, and A. K. Dunker. (2008). Intrinsically disordered proteins in human diseases: introducing the D2 concept. *Annu Rev Biophys* 37:215-46.
23. Joo, J. G., A. Beke, C. Papp, Z. Szigeti, A. Csaba, and Z. Papp. (2008). Major diagnostic and pathological features of iniencephaly based on twenty-four cases. *Fetal Diagn Ther* 24:1-6.
24. Wang, X., Q. Y. Zhang, Z. J. Li, X. T. Ying, and J. M. Lin. (2008). Development of high-performance magnetic chemiluminescence enzyme immunoassay for alpha-fetoprotein (AFP) in human serum. *Clin Chim Acta*. 393:90-4.
25. Tang, H., X. Y. Tang, M. Liu, and X. Li. (2008). Targeting alpha-fetoprotein represses the proliferation of hepatoma cells via regulation of the cell cycle. *Clin Chim Acta*. 394:81-8.
26. Anheim, M., M. C. Fleury, J. Franques, M. C. Moreira, J. P. Delaunoy, D. Stoppa-Lyonnet, M. Koenig, and C. Tranchant. (2008). Clinical and molecular findings of ataxia with oculomotor apraxia type 2 in 4 families. *Arch Neurol*. 65:958-62.
27. Costa, S., M. P. De Carolis, I. Savarese, S. Lacerenza, and C. Romagnoli. (2008). Author reply: Alpha-fetoprotein levels in the neonatal period. *Eur J Pediatr*. 167:963.
28. Inanc, B., A. E. Elcin, and Y. M. Elcin. (2008). Human Embryonic Stem Cell Differentiation on Tissue Engineering Scaffolds: Effects of NGF and Retinoic Acid Induction. *Tissue Eng Part A* 7:7.
29. Jung, Y. J., K. H. Ryu, K. A. Cho, S. Y. Woo, J. Y. Seoh, S. J. Cho, S. Y. Joo, K. Yoo, and H. Ho-Seoung. (2008). In vitro hepatic differentiation of human umbilical cord blood and bone marrow cells. *Pediatr Hematol Oncol*. 25:481-91.
30. Donalson, K., S. Turner, H. Wastell, and H. Cuckle. (2008). Second trimester maternal serum ADAM12 levels in Down's syndrome pregnancies. *Prenat Diagn* 10:10.
31. Kim, S., Y. H. Kim, and W. K. Min. (2007). [Prenatal serum marker screening in Korea: survey results]. *Korean J Lab Med*. 27:28-33.

Abstracts

A). Screening Abstract "Picks-of-the-Month":

(1) Title: Mid-trimester maternal serum AFP levels in predicting adverse pregnancy outcome.

Source: *Clin Exp Obstet Gynecol*. 2008;35(3):208-10.

Authors: Gkogkos, P., Androutopoulos, G., Vassilakos, P., Panayiotakis, G., Kourounis, G., Decavalas, G.

Abstract: OBJECTIVE: In this prospective study, we investigated the association between mid-trimester maternal serum alpha-fetoprotein AFP (MSAFP) levels and adverse pregnancy outcome in a South-Western Greek population. MATERIALS AND METHODS: 110 healthy Greek women with spontaneous pregnancies, investigated for MSAFP levels between the 13th and 24th week of gestation and followed for adverse pregnancy outcome. AFP levels > 2.0 multiples of the median value for gestation were considered abnormal. Statistical analysis was performed by Pearson's chi-square test. RESULTS: Elevated MSAFP levels were detected in a total of 27 of the 110 women studied (24.5%). Among them, only four women (14.8%) developed pregnancy complications. CONCLUSION: Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology and MSAFP screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications.

(2) Title: Prediction of patient-specific risk for fetal loss using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers

Source: *Am J Obstet Gynecol*. 2008;199(3):290.e1-6.

Authors: Dugoff, L., Cuckle, H. S., Hobbins, J. C., Malone, F. D., Belfort, M. A., Nyberg, D. A., Comstock, C. H., Saade, G. R., Eddleman, K. A., Dar, P., Craigo, S. D., Timor-Tritsch, I. E., Carr, S. R., Wolfe, H. M., D'Alton, M. E.

Abstract: OBJECTIVE: To develop and evaluate a method of estimating patient-specific risk for fetal loss by combining maternal characteristics with serum markers. STUDY DESIGN: Data were obtained on 36,014 women from the FaSTER trial. Separate likelihood ratios were estimated for significant maternal characteristics and serum markers. Patient-specific risk was calculated by multiplying the incidence of fetal loss by the likelihood ratios for each maternal characteristic and for different serum marker combinations. RESULTS: Three hundred eighteen women had fetal loss < 24 weeks (early) and 103 > 24 weeks (late). Clinical characteristics evaluated included maternal age, body mass index, race, parity, threatened abortion, previous preterm delivery, and previous early loss. Serum markers studied as possible predictors of early loss included first-trimester pregnancy-associated plasma protein A and second-trimester alpha-fetoprotein, and unconjugated estriol. A risk assessment for early loss based on all of these factors yielded a 46% detection rate, for a fixed 10% false-positive rate, 39% for 5% and 28% for 1%. The only significant marker for late loss was inhibin A. The detection rate was 27% for a fixed 10% false-positive rate and only increased slightly when clinical characteristics were added to the model. CONCLUSION: Patient-specific risk assessment for early fetal loss using serum markers, with or without maternal characteristics, has a moderately high detection. Patient-specific risk assessment for late fetal loss has low detection rates.

(3) Title: Maternal serum alpha-fetoprotein values in type 1 and type 2 diabetic patients

Source: Am J Obstet Gynecol. 199(2):E1, 2008.

Authors: Thornburg, L. L., Knight, K. M., Peterson, C. J., McCall, K. B., Mooney, R. A., Pressman, E. K.

Abstract: OBJECTIVE: Maternal serum alpha-fetoprotein (MSAFP) values are reported to be lower in type 1 diabetic patients, and a correction factor is often applied. We sought to determine whether type 2 diabetic patients require the same MSAFP adjustments as type 1 diabetic patients. STUDY DESIGN: We performed a retrospective review of MSAFP levels from a university laboratory in type 1 and type 2 diabetic patients between July 2000 and August 2006, matched 1:2 with controls. Groups were compared using analysis of variance and Student t testing. RESULTS: Seventy-seven type 1 and 75 type 2 diabetic patients were compared with 304 controls. Type 1 and type 2 diabetic patients differed significantly from each other and controls before corrections. Diabetic patients were similar to each other, but significantly lower than controls, after weight corrections. These differences were eliminated by a 10% correction factor. CONCLUSION: Type 1 and type 2 diabetic patients require both weight and diabetes corrections to adjust MSAFP values to nondiabetic levels.

B). Case History Screening "picks-of-the-month":

(1) Title: A stillborn fetus with amniotic band syndrome and elevated levels of alpha-fetoprotein plus beta-human chorionic gonadotropin: a case report

Source: Fetal Diagn Ther. 24(2):111-4, 2008.

Authors: Sifakis, S., Mantas, N., Konstantinidou, A., Koukoura, O., Avgoustinakis, E., Koumantakis, E.

Abstract: Amniotic band syndrome is an uncommon, congenital fetal abnormality with multiple disfiguring and disabling manifestations. A wide spectrum of clinical deformities are encountered and range from simple ring constrictions to major craniofacial and visceral defects. We report a case of constriction amniotic bands involving upper extremities and intrauterine fetal death due to strangulation of umbilical cord. Abnormally elevated levels of alpha-fetoprotein and beta-chorionic gonadotropin were detected at 17 weeks' gestation. They were probably caused by the loss of cutaneous integrity of the fetus (alpha-fetoprotein), and by the placental attempt to counteract the fetal growth restriction and hypoxia, due to the strangulation of umbilical cord by the amniotic bands (beta-chorionic gonadotropin).

(2) Title: Chubby face and the biochemical parameters for the early diagnosis of neonatal intrahepatic cholestasis caused by citrin deficiency

Source: J Pediatr Gastroenterol Nutr. 47(2):187-192, 2008.

Authors: Chen, H. W., Chen, H. L., Ni, Y. H., Lee, N. C., Chien, Y. H., Hwu, W. L., Huang, Y., T., Chiu, P. C., Chang, M. H.

Abstract: OBJECTIVES: To identify facial and biochemical characteristics as early clinical features of neonatal intrahepatic cholestasis due to citrin deficiency (NICCD). PATIENTS AND METHODS: Ten patients with diagnoses of NICCD by SLC25A13 mutation analysis in Taiwan were recruited. A "Chubby Index" was developed for objective measurement of their facial characteristics. Liver function profiles were analyzed and compared with data on neonatal hepatitis and biliary atresia. RESULTS: Chubby face was observed in early infancy in all 5 patients whose serial photographs were taken. A significant difference in the Chubby Index was seen between NICCD infants and healthy infants (1.331 +/- 0.07 vs 1.068 +/- 0.059; P < 0.05). NICCD is characterized by an aspartate aminotransferase-to-alanine aminotransferase ratio of 2 or greater, a direct bilirubin-to-total bilirubin ratio under 0.67, and a standard deviation score for alpha-fetoprotein of 4 or greater, with respect to neonatal hepatitis and biliary atresia. Although chubby face, abnormal liver function profiles, and multiple amino acidemia gradually disappeared after age 1 year, an increase in hepatic echogenicity was observed in most patients in long-term follow-up. CONCLUSIONS: Our Chubby Index is an informative measurement of the facial characteristics of infants with NICCD. The chubby face features, along with an aspartate aminotransferase-to-alanine aminotransferase ratio of 2 or greater, a direct bilirubin-to-total bilirubin ratio under 0.67, and a standard deviation score for alpha-fetoprotein of 4 or greater, may serve as useful clinical indicators for diagnosing NICCD early in infancy.

(3) Title: Hepatic hematoma in a neonate with a high level of alpha-fetoprotein

Source: Eur J Pediatr. 167(5):591-3, 2008.

Authors: Costa, S., De Carolis, M. P., Savarese, I., Lacerenza, S., Romagnoli, C.

Abstract: Hepatic hematomas in neonates are uncommon lesions. When they are large or subcapsular in location, they can rupture with clinical signs of hemoperitoneum. We report a case of subcapsular hepatic hematoma (SHH) associated with a high level of alpha-fetoprotein (AFP), for which diagnosis was made with conservative management, following up with the reduction in size at ultrasound examination and the reduction of the level of AFP.

C). News of Notes: Abstract of New Markers:

(1) Title: Second trimester maternal serum ADAM12 levels in Down's syndrome pregnancies

Source: Prenat Diagn. 28: 904-907, 2008.

Authors: Donalson, K., Turner, S., Wastell, H., Cuckle, H.

Abstract: OBJECTIVE: To estimate the utility of maternal serum ADAM12 as a Down's syndrome marker. METHODS: Samples from 71 Down's syndrome affected pregnancies were retrieved from -20 degrees C storage together with 710 controls matched for gestation and storage time. ADAM12 was measured prior to identification of the affected pregnancies, and expressed in multiples of the gestation-specific median (MoM). RESULTS: The median ADAM12 level in the affected pregnancies was 1.36 MoM with a 10th-90th centile range of 0.90-1.94 MoM compared with 1.01 and 0.65-1.52 MoM in the unaffected control pregnancies (P = < 0.0001, two-side Wilcoxon Rank Sum Test). The Mahalanobis distance between the medians was 0.96 compared with 0.92, 1.18, 1.07 and 1.24 for alpha-fetoprotein, intact human chorionic gonadotrophin (hCG), unconjugated estriol and inhibin-A respectively in the same samples. In unaffected pregnancies there were highly statistically significant correlations between ADAM12 and each of the other markers; in the affected pregnancies the only significant correlations were with hCG (P <= 0.0001) and inhibin-A (P <= 0.05). Statistical modelling predicted that ADAM12 as a fifth marker could increase the detection rate by 2-3% or reduce the false-positive rate by 0.9-1.7%. CONCLUSIONS: ADAM12 is a second trimester marker of Down's syndrome, with discriminatory power similar to existing markers. It could be considered in multi-marker combinations.

(2) Title: Biochemical diagnosis of placental infarction/damage: acutely rising alkaline phosphatase

Source: Ann Clin Biochem. 45(Pt 3):335-8, 2008.

Authors: Ranganath, L., Taylor, W., John, L., Alfirevic, Z.

Abstract: There are currently no simple tests in clinical use to detect acute placental damage. A case is described to demonstrate that a routinely used measurement such as alkaline phosphatase (ALP) can be employed to detect acute damage to the placenta. Seventeen serial blood samples, three pre-delivery, were collected from a 22-

year-old primigravida who delivered a stillborn baby. Retrospectively, blood samples were analysed for total and heat-stable ALP as well as human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) as a measure of placental function when an unusual pattern of change in ALP was noticed. Histological examination of the placenta revealed new and old placental infarcts. Total and heat-stable ALPs as well as AFP peaked by more than eight-, 19- and two-fold, respectively over 16 h. Plasma hCG fell sharply even before delivery of placenta by five-fold over 16 h before further falling slowly to baseline. The fall in hCG is also consistent with the placental damage being acute and critical. As far as we are aware this is the first description of changes in circulating proteins reflecting placental damage.

(3) Title: Serum alpha-fetoprotein (AFP) levels in breastfed infants with prolonged indirect hyperbilirubinemia

Source: Early Hum Dev. 84(7):487-90, 2008.

Authors: Manganaro, R., Marseglia, L., Mami, C., Saitta, G., Gargano, R., Gemelli, M.

Abstract: The aim of this prospective study was to verify normal serum AFP (alpha-fetoprotein) levels in jaundiced breastfed infants with indirect hyperbilirubinemia. **METHODS:** The study was conducted in clinically jaundiced breastfed infants, 20, or more, days old, referred to our outpatient ambulatory. Inclusion criteria were: birth at term after a physiologic pregnancy, with an Apgar score >7 at 1 and 5 min, no evidence of congenital anomalies or diseases, direct bilirubin <1 mg/dl, normal values of alpha-1-antitrypsin, glucose-6-phosphate dehydrogenase, thyroid stimulating hormone, triiodothyronine, tyroxine, and normal growth. 30 non-jaundiced breastfed infants age-weight-matched, were used as control group. **RESULTS:** 98 jaundiced breastfed infants satisfied inclusion criteria. Their mean serum concentration of AFP was significantly higher than control infants (3548 vs 1095 ng/ml, $p < 0.001$). Serum AFP levels of jaundiced infants were directly associated with serum indirect bilirubin and gamma-glutamyltranspeptidase concentrations. **CONCLUSIONS:** The most probable explanation of elevated AFP in jaundiced breastfed infants may be the presence in human milk of one or more factors which affect hepatocyte growth and/or function. Based on our finding we demonstrated that in jaundiced breastfed infants normal range of serum AFP levels are higher than previously published data for healthy infants. Our data can be useful for a right interpretation of AFP levels in breastfed infants with prolonged jaundiced and may be used to avoid unnecessary investigations.

D). News of Notes: Abstracts of New Testing Agents/Methods:

1) Title: Maternal biochemical serum screening for Down syndrome in pregnancy with human immunodeficiency virus infection

Source: Obstet Gynecol. 112(2 Pt 1):223-30, 2008.

Authors: Le Meaux, J. P., Tsatsaris, V., Schmitz, T., Fulla, Y., Launay, O., Goffinet, F., Azria, E.

Abstract: **OBJECTIVE:** To estimate the influence of human immunodeficiency virus (HIV) infection and antiretroviral therapy on maternal serum markers levels and the false-positive rate with biochemical maternal serum screening for Down syndrome. **METHODS:** We performed a 1:1 matched case-control study comparing 132 HIV-infected women with single pregnancy to controls selected among non-HIV-infected women matched on geographical origin and fetal sex. **RESULTS:** Of HIV-infected women, 47.7% were receiving antiretroviral therapy. Groups did not differ in multiples of the median (MoM) levels of total human chorionic gonadotrophin. The MoM alpha fetoprotein level did not differ between total HIV-infected women and control women but was significantly lower for untreated HIV-positive women compared with control women (0.91 compared with 1.03 MoM, $P < .01$) and compared with treated HIV-positive women (0.91 compared with 1.18 MoM, $P < .01$). The false-positive rate of biochemical screening did not differ between groups. **CONCLUSION:** Untreated HIV infection is associated with lower maternal serum alpha fetoprotein levels. Nevertheless, the false-positive rate of double-marker second-trimester Down syndrome serum screening did not appear to be affected in our sample of HIV-infected women, whether women were receiving antiretroviral therapy at the time of the test or not.

(2) Title: Biological variation and reference change values of CA 19-9, CEA, AFP in serum of healthy individuals

Source: Scand J Clin Lab Invest. 68(3):212-8, 2008.

Authors: Erden, G., Barazi, A. O., Tezcan, G., Yildirimkaya, M. M.

Abstract: **OBJECTIVE:** The use of tumour markers in diagnosis and monitoring is very common. Tumour marker results vary - preanalytical sources of variation, total random analytical error (CV(a)), and within-subject

(intraindividual) normal biological variation. There are not so many studies evaluating the biological variations and reference change values (RCV) of these parameters. The aim of our study was to assess: (i) the average inherent intra- and inter-individual biological variation (CV(i) and CV(g)) for CA 19-9, CEA, AFP in a group of healthy individuals; (ii) the significance of changes in serial results of each marker; and (iii) the index of individuality. MATERIAL AND METHODS: The study group comprised 49 healthy volunteers ranging in age between 18 and 60 years (25 M and 24 F). Four blood samples were obtained from each subject; one at each 14-day interval. Each sample from one individual was assayed in duplicate. CA 19-9, CEA, AFP levels were measured by an immunoluminometric assay on a random-access analyser (Architect i2000; Abbott Diagnostics Division). The intra- (CV(i)) and inter-individual (CV(g)) biological variations were estimated from the data generated. Reference change value (RCV) was calculated. RESULTS: The intra-individual/inter-individual biological variations (CVs) for CA 19-9, CEA, AFP were 27.2/64.24 %, 30.87/37.14 % and 26.67/43.65 %, respectively. The critical differences (RCVs) of CA 19-9, CEA, AFP were 64.71 %, 72.57 % and 62.62 %, respectively ($Z = 1.65$ for unidirectional changes; $p < 0.05$). CONCLUSIONS: Intra-individual biological variation contributes to the variation in serial results and should therefore be included in the criteria for serum tumour marker assessment.

(3) Title: Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: correlation with the week of onset and the severity

Source: Prenat Diagn. 28(8):704-9, 2008.

Authors: Kang, J. H., Farina, A., Park, J. H., Kim, S. H., Kim, J. Y., Rizzo, N., Elmakky, A., Jun, H. S., Hahn, W. B., Cha, D. H.

Abstract: OBJECTIVES: To estimate the combined screening performance of first and early second trimester prenatal serum markers for Down syndrome, in screening for the development of preeclampsia, and analyze the correlation among marker levels, week of onset, and severity of the disease. METHODS: A retrospective cohort study was carried out on 32 women with preeclampsia and 3044 controls. Serum samples from these pregnancies were assayed for pregnancy-associated plasma protein-A (PAPP-A), alpha-fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotrophin (hCG), and inhibin-A. A likelihood ratio and the odds of being affected given a positive result (OAPR) of various combinations of markers were calculated and receiver operating characteristic (ROC) curves analysis was performed. RESULTS: In the pregnancies that subsequently developed preeclampsia, first trimester PAPP-A concentration was significantly lower and concentrations of early second trimester inhibin-A and hCG significantly elevated. Levels of early second trimester uE3 and AFP were not significantly altered. We also found that inhibin-A correlates with both onset of the disease and the severity. CONCLUSION: Down syndrome biochemical markers levels are altered in those patients who subsequently developed preeclampsia and may be a useful screening test for preeclampsia. Inhibin-A is the most predictive marker and correlates with the severity of subsequent preeclampsia and inversely with the week of occurrence of preeclampsia.

VI. Potentially helpful website connections/locations:

- 1) pregnancy.about.com/cs/afp/a/afptesting.htm
- 2) health.allrefer.com/health/alpha-fetoprotein-info.html
- 3) headtoe.apta.org/topic/medtest/hw1663/results.htm
- 4) www.pregnancy-info.net/slpha_feto_protein.html
- 5) www.healthopedia.com/alpha-fetoprotein