Fetal Defect Marker Proficiency Test Mailout
September 14, 2010

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from September, 2010 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. These will be mailed to you separately. Please review and sign your evaluation. Retain the signed evaluation 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 = 30	**Sample #**	**MS 256**	**MS 257**	**MS 258**	**MS 259**	**MS 260**
	Gestational Age (weeks)	17	20	15	18	19
Maternal Race	Ethnic Group	Hispanic	White	Black	White	White
Maternal Weight	Pounds (lbs)	150	160	140	145	155
Maternal Age	Years	32	30	23	28	25
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std.Dev.	35.31 ± 2.60	152.10 ± 12.00	25.51 ± 2.30	51.90 ± 4.71	26.40 ± 2.21
	MOM ± Std.Dev.	0.92 ± 0.08	2.72 ± 0.21	0.81 ± 0.10	1.15 ± 0.11	0.53 ± 0.04
Unconjugated Estriol (uE3)	Mean ng/ml ± Std.Dev.	0.96 ± 0.30	1.82 ± 0.74	1.10 ± 0.38	0.04 ± 0.03	0.80 ± 0.24
	MOM ± Std.Dev.	0.99 ± 0.18	0.99 ± 0.20	1.81 ± 0.43	0.06 ± 0.04	0.55 ± 0.13
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std.Dev.	25.07 ± 2.14	18.94 ± 1.53	35.19 ± 3.34	27.24 ± 2.95	41.26 ± 4.68
	MOM ± Std.Dev.	1.04 ± 0.10	1.15 ± 0.13	0.84 ± 0.11	1.30 ± 0.15	2.28 ± 0.28
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std.Dev.	143.17 ± 18.43	219.72 ± 26.16	131.03 ± 21.39	175.10 ± 23.10	387.91 ± 43.67
	MOM ± Std.Dev.	0.86 ± 0.14	1.19 ± 0.21	0.70 ± 0.16	1.02 ± 0.18	2.24 ± 0.36
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	(-) (100%)	(+) (97%)	(-) (100%)	(-) (100%)	(-) (100%)
	Further Action G,U,A	NFA	G = 80% U = 77% A = 83%	NFA	NFA	NFA
	NTD Risk 1 in	10,000	100	10,000	6,995	10,000
Trisomy-21 Screen (Positive, Negative) percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 86% U = 57% A = 79%
	Risk Est. 1 in	1,550	6,950	5,150	390	33
2. Quad Test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	G = 76% U = 72% A = 76%
	Risk Est. 1 in	3,310	20,000	10,000	831	11
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	20,000	20,000	20,000	823	5,000

*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 ± 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.

Sample #	Summary Comments (Mock specimens):
MS 256 Wk 17.0	This specimen was obtained from a 32 year old hispanic woman (Gravida = 1, Parity = 0) in her 17th week gestation with a body weight of 150 lbs. She had no personal history of pregnancy loss. Her specimen was negative for NTD (100% consensus). Her screen was also negative for both Trisomies with all labs in agreement. Thus, no recommendations for further action were submitted or required. This specimen had no amniotic fluid counterpart.
MS 257 Wk 20.0	This specimen was obtained from a 30 year old white woman (Gravida = 3, Parity = 1) in her 20th week gestation with a body weight of 160 lbs. She had a family history of pregnancy complications. Her specimen, a second pregnancy sample, was screen positive for NTD (97% consensus; MOM=2.72), but screen negative for both Trisomies, with all labs in agreement. Recommendations for further action from labs performing the NTD screen were: genetic counseling, 72%, ultrasound, 82% and amniocentesis, 76%. The MS257 specimen had an amniotic fluid paired sample which was also elevated (MoM=3.77). The all-lab median risk for NTD of MS257 was 1 in 100.
MS 258 Wk 15.0	This specimen was procured from a 23 year old black woman (Gravida = 3, parity = 2) in her 15th week gestation with a body weight of 140 lbs. She had no 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 (100% consensus) with a race correction indicated. The labs were also in agreement that both Trisomy consensus screens were negative. Specimen MS258 was not paired with an amniotic fluid sample.
MS 259 Wk 18.0	This specimen was obtained from a 28 year old white woman (Gravida = 1, parity = 0) in her 18th week gestation with a body weight of 145 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD. Her aneuploidy screens were negative for both Trisomy-18 and Trisomy-21. Her MS-uE3 sample was extremely low or absent (see below for further discussion). This sample was not paired to an amniotic fluid specimen.
MS 260 Wk 19.0	This specimen was obtained from a 25 year old white woman (Gravida = 2, parity = 1) in her 19th week gestation with a body weight of 155 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100%) on the basis of low AFP and uE3, and elevated hCG and Inhibin-A levels. Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 71%; ultrasound, 64%; and amniocentesis, 71%; while the triple tests were: genetic counseling, 75%; ultrasound, 69%, and amniocentesis, 69%. Specimen MS260 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to an amniotic fluid specimen which also had a low AFAFP level (MOM = 0.80).

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

Sample#	Values	Summary Comments:
AF 256 Wk 20.9	AFP= 6.20 ± 0.71 µg/ml MOM= 1.09 ± 0.14	The AF256 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF256 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 257 Wk 20.0	AFP= 24.10 ± 3.41 µg/ml MOM= 3.77 ± 0.49	The AF257 sample was targeted for a screen positive AFAFP value in the upper gestational age range. All labs reported this specimen as a screen positive AFAFP value. The AF257 specimen was paired with maternal serum sample MS257, which was also elevated (MOM= 2.72).
AF 258 Wk 17.0	AFP= 11.51 ± 1.30 µg/ml MOM= 0.98 ± 0.10	The AF258 sample was targeted for a negative NTD screen for AFAFP in the upper gestational age screening range. All labs categorized this as an NTD screen negative specimen. This sample was not matched to a maternal serum specimen.
AF 259 Wk 18.0	AFP= 9.91 ± 1.10 µg/ml MOM= 1.03 ± 0.10	The AF259 sample was targeted as an NTD negative screen in the routine gestational age screening range. All labs categorized AF259 as a negative NTD screen specimen. This specimen had no maternal serum counterpart.
AF 260 Wk 19.0	AFP= 6.20 ± 0.70 µg/ml MOM= 0.80 ± 0.08	The AF260 sample was targeted for a non-elevated AFAFP value in the upper gestational age range. Most labs called AF260 a normal low MOM AFAFP specimen. This AFAFP sample was matched to maternal serum specimen MS260, which also showed low levels of AFP (MS-MOM=0.53).

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 16	**Sample #**	**FT 256**	**FT 257**	**FT 258**	**FT 259**	**FT 260**
	Gestational Age (weeks)	11.0	10.9	11.9	11.4	13.0
Maternal Race	Ethnic Group	Hispanic	Black	White	Asian	White
Maternal Weight	Pounds (lbs)	140	155	150	130	135
Maternal Age	Years	28	32	25	28	21
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	42	41	53	47	67
	NT Thickness (mm)	1.10	1.10	2.90	1.20	1.50
	NT - MOM	1.01 ± 0.10	1.02 ± 0.10	2.18 ± 0.22	0.99 ± 0.10	0.93 ± 0.08
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	50.41 ± 5.05	53.86 ± 5.88	141.06 ± 25.32	53.98 ± 5.32	44.16 ± 4.40
	MOM ± Std. Dev.	0.59 ± 0.05	0.65 ± 0.04	1.91 ± 0.23	0.65 ± 0.07	0.65 ± 0.05
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean mIU/mL ± Std. Dev.	3.88 ± 3.04	6.43 ± 4.98	1.68 ± 1.42	7.67 ± 5.99	9.26 ± 7.28
	MOM ± Std. Dev.	3.32 ± 1.72	6.43 ± 3.66	1.11 ± 0.58	5.58 ± 2.55	3.79 ± 1.65
Trisomy-21 Screen (Positive/Negative) percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (88%)	(-) (100%)	(-) (100%)
	Recommended Action NFA**	NFA	NFA	G = 81% U = 43% A = 63% C = 44%	NFA	NFA
	Risk Estimate 1 in	20,000	20,000	47	20,000	20,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	10,000	10,000	2,120	10,000	10,000

*N=total numbers may vary since some labs do not test all analytes. (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

**This percentage is normalized to labs requesting further action.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

N = 16 all-lab Consensus Values.

Sample#	Summary Comments:
FT 256 Wk 11.0	This specimen was obtained from a 28 year old Hispanic woman of average body weight (140 lbs.). Her gestational age at the time of screening was 11.0 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. The FT256 risk estimate for Trisomy-21 was 1 in 20,000, while the all-lab Trisomy-18 risk was 1 in 10,000. All labs were in agreement that FT256 was a negative screen for both Trisomy-21 and Trisomy-18.
FT 257 Wk 10.9	This specimen was obtained from a 32 year old black woman of average body weight (155 lbs.). Her gestational age at the time of screening was 10.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative and all testing Labs were in agreement. The FT257 risk estimate for Trisomy-21 was 1 in 20,000, while the all-lab Trisomy-18 risk was 1 in 10,000. All labs were in agreement that FT257 was a negative screen for both Trisomy-21 and Trisomy-18.
FT 258 Wk 11.9	This specimen was procured from a 25 year old white woman of average body weight (150 lbs.). Her gestational age at the time of screening was 11.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen positive for Trisomy-21 and all testing Labs were in agreement (see Critique). The FT258 risk estimate for Trisomy-21 was 1 in 47, while the Trisomy-18 risk was 1 in 2,120.
FT 259 Wk 11.4	This specimen was obtained from a 28 year old Asian woman of average body weight (130 lbs). Her gestational age at the time of screening was 11.4 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative and all testing Labs were in agreement. The FT259 risk estimate for Trisomy-21 was 1 in 20,000, while the Trisomy-18 risk was 1 in 10,000.
FT 260 Wk 13.0	This specimen was procured from a 21 year old white woman with a body weight of 135 lbs. Her gestational age at the time of screening was 13.0 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT260 was 1 in 20,000, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results of the targeted values for the NTD and the Trisomy Screens were consistent with the goals of our projected target values, risks, and outcomes. As displayed in the tables, maternal serum sample MS257 was targeted as an elevated specimen for NTD (Figs. 1 and 3) and the AF257 sample was matched to that specimen. Hence, specimen MS257 was screen positive for NTD, but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS257 specimen, the NTD screen resulted in a 1 in 100 all-lab risk for open neural tube defects (ONTD), and it achieved a 97% NTD screen consensus. The NTD-related recommended action for MS257 was genetic counseling, 80%; ultrasound, 77%; and amniocentesis, 83%. Sample MS260, a T21 screen positive specimen, was obtained from a white woman with a prior (sibling) history of pregnancy complications. The T21 MOM results for specimen MS260 (MSAFP-MOM = 0.53, MS-uE3-MOM = 0.55, MS-hCG-MOM = 2.28, DIA-MOM = 2.24) were all consistent with a T21 positive screen; thus, all labs (100%) classified this specimen as a T21 screen positive and most recommended further action. The MS260 sample was from a 25 year old woman and produced a risk from the quad test of 1 in 11 and a triple test risk of 1 in 33, both of which were greater than that expected from the maternal age alone (1 in 1000). The T21-related recommended action for MS260 was genetic counseling, 76%; ultrasound, 72%; and amniocentesis, 76%. Finally, samples MS256 and MS258, and MS259 produced negative screens for NTD, T21, and T18; corrections for body weight were not indicated for any of these samples.

Specimen MS259 produced an interesting case for DS risk assessment using the triple versus the quad testing platforms. This sample, demonstrating reduced or absent uE3, triggered a Down syndrome (DS) risk in two of the 14 laboratories that reported risks from the triple test platform, while none of the quad test users produced a DS risk (total labs

N=29). While less than 4% of the labs in this PT program employ the triple test instead of the quad test, this sample represents a call for caution for the triple test only users. However, most labs in this PT program use the quad test in clinical practice, but report the results of both quad and triple test platforms in the NYS PT survey. The ACOG Practice Bulletins of May 2001 (Clinical Management Guidelines for Obstetricians and Gynecologist, #27) and of January 2007 (#77) recommend the use of the quad over that of the triple test platform.

Specimen MS259 displayed an interesting situation in the second trimester screening results in that unconjugated estriol levels (uE3) were virtually absent or notably reduced (MOM < 0.06). In contrast, all other quad bio-markers were normal as follows: AFP MOM = 1.15; hCG MOM = 1.30; Inhibin MOM = 1.02. In cases of trisomy screen positive samples for Down syndrome (T21) and Edward's syndrome T18, uE3 levels are significantly lowered to MOMs of 50% or less than that of non-affected cases. Since uE3 contributes a role as an analyte member of both the triple and quad risk algorithm, the calculated trisomy risk factors would be highly influenced by absent or reduced levels of uE3. However, in the triple test there are only two other markers to compensate for abnormally low uE3 levels in cases where low uE3 levels are from causes other than a trisomy.

Physiological levels of uE3 normally rise throughout pregnancy; thus, low levels of uE3 (<0.3 MOM) would require a prenatal consultation, as this analyte pattern is usually associated with genetic disorders (Gagnon, 2008). In T21 pregnancies, for example, the median maternal uE3 levels average 0.55 MOM and the mass levels of uE3 in amniotic fluid can be even lower (30). Decreased levels of maternal uE3 (below the fifth centile) have also been frequently encountered (57%) in second trimester oligohydramios with both Down syndrome and neural tube defects as compared to 8% in the reference population (36). Low maternal serum uE3 levels have further been reported in affected pregnancies of Smith-Lemli-Opitz (SLO) syndrome, an autosomal recessive disorder comprising facial abnormalities, growth retardation, and multiple congenital anomalies, including abnormal genitalia, second and third toe syndactyly, and cleft palate (37). This condition is also associated with hypocholesterolemia together with elevated levels of 7-dehydrocholesterol resulting from lowered activity of the enzyme 7-dehydrocholesterol reductase. Even in the face of the congenital and enzyme defects, many infants with low or absent MS-uE3 exhibit a normal 46XX or 46XY karyotype.

Absent or low uE3 is not only observed in Smith-Lemli-Opitz syndrome (1 in 60,000), but is also seen in placental steroid sulfatase (STS) deficiency and in undetected intrauterine fetal death (39). Thus, very low or absent MS-uE3 has been correlated with STS deficiency in fetuses with normal karyotype but exhibiting a deletion of the STS gene (40; 41). In Down's screening, a finding of MS-uE3 levels lower than 0.1 MOM has also aided in the diagnosis of X-linked ichthyosis in second trimester 16 weeks triple test screening programs. Ichthyosis is an X-linked congenital disorder of skin keratination and scaling in the granular layer of the epidermis (42). This genetic disorder can affect skin scaling of the neck and trunk regions; moreover, it can produce eye cataracts. The X-linked ichthyosis (XLI) is a relatively common genetic disorder that occurs in about 1 in 2,000 – 6,000 male births (43). Clinically, XLI is characterized by scaling of the skin, producing large, polygonal, dark brown scales, and is prominent on the exterior aspects of the limbs. It is suspected that undetectable MS-uE3, associated with STS deficiency may be the cause of XLI (43). In most cases, STS deficiency results from a complete or partial deletion of the STS gene on chromosome Xp22.3. In the report by Watanabe (50), the prenatal detection of a male fetus affected with STS deficiency was described. This detection was the result of undetectable MS-uE3 levels in the second trimester maternal serum screening assay. In this instance, microdeletion of the STS gene was confirmed by fluorescence in situ hybridization analysis of cultured amniotic fluid cells. In an earlier report using the triple test analyte values (50), it was determined that MSAFP at 16 weeks was 0.83 MOM, hCG was 0.42 MOM, and MS-uE3 was 0.01 MOM. The authors of this latter report had suggested that the extremely low levels of MS-uE3 should prompt investigation of family history, since the maternal grandfather had demonstrated XLI associated with placental sulfatase deficiency. Very low levels of MS-uE3 have also been associated with additional adverse pregnancy outcomes. Patients with unexplained low second trimester MS-uE3 were reported to have an increased risk of pregnancy-induced hypertension, gestational diabetes, premature rupture of membranes, and onset of labor (44). In a large study of second trimester pregnancies, women exhibiting MS-uE3 levels at or below the sensitivity of the assay were followed (45). It was found that women who had unexplained very low levels of MS-uE3 were determined an increased risk for fetal STS deficiency and stillborn delivery at term. Low MS-uE3 levels are further associated with the onset of spontaneous labor (46). The MS-uE3 levels were found to gradually decrease in correlation with prolonged duration of labor in multiple pregnancies. Undetectable MS-uE3 levels have also been identified in 13 of 10,000 pregnancies in a New England study (47). Correlations of absent MS-uE3 with clinical conditions in this study included fetal death, overestimated gestational age, non-pregnancy, and chromosome abnormalities. Male offspring were more frequent among cases of adverse outcomes, and cesarean sections occurred about twice as often among cases; however, no perinatal deaths were found. Earlier studies had reported that low levels of MS-uE3 were associated with increased risk of pregnancy loss, early intrauterine death, and fetal distress (48). As a final note, the probable cause of low MS-uE3 levels in triploidy cases (69, XXX) has been attributed to placental insufficiency, and uE3 is a known marker for this pregnancy disorder (49).

In summary, it can be seen from the preceding paragraphs that the Down syndrome positive screen result produced by specimen MS259 may have been a false positive screen in the few laboratories that used the triple test. An actual triple marker DS screen outcome of low uE3 and normal AFP and hCG was published by Weintrob and Drovin;

however, in their case it was not related to Down syndrome (33). Rather, the low MS-uE3 levels in the Weintrob-Drovin case were determined to be an adrenocorticotropic hormone deficiency. This deficiency was caused by a new mutation in the TPIT gene, which is a cell-restricted transcription factor that is important for terminal differentiation of pituitary corticotrophic cells. By comparison with their published triple test result, it was evident that the MoM profiles were similar to those of the present NYS sample MS259; AFP MoM= 1.15 (NYS), AFP MoM= 1.0; uE3 MoM= 0.06 (NYS); uE3 MoM= 0.09; hCG MoM= 1.3 (NYS), hCG MoM= 1.0. Similar to our intended mock specimen MS259, the Weintrob-Drovin patient case was negative for Down syndrome following karyotyping. The NYS mock patient was intended to mimic a true low or absent MS-uE3 as reported in the case histories discussed above (see refs 35 & 36). Overall, fetal death and/or distress should be considered when a screen result of very low or absent MS-uE3 is encountered. Unexpectedly in the present PT-mailout, the triple screen results from MS259 produced a triple screen DS risk of 1 in 175 (N=2) and a negative quad screen of 1 in 831 (N=29) demonstrating the influence of Inhibin A to affect risk outcome.

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 (Figs. 7-10) for each of the five MS samples. As shown in Figures 7 and 8, AFP and uE3 mass measurements in serum among the individual kits largely agreed, although Siemens DPC Immulite was about 10% lower for AFP, and 10% higher for uE3. In contrast, when the kit MOM medians were compared, there was a somewhat larger Beckman Access2 and Unicel on the one hand and the difference (~40%) between the Siemens/DPC methods on the other hand (Fig. 8B). Regarding the hCG kits (see Fig. 9), the two Beckman instruments (Access2 and UNICEL DXL) yielded similar mean hCG values that were in between those from the Siemens/Bayer ADVIA-Centaur and ACS-180 instruments (+10%) and the Siemens DPC Immulite or Immulite 2000 instruments (-10%). In order to enhance uniformity among the various kits employed to measure hCG, we incorporate intact recombinant (total) hCG into our PT specimens. Finally, the method comparison of Inhibin-A displayed in Fig. 10 shows that the results from the Beckman Access/2 or Unicel were 25-30% higher than those from the Diagnostic Systems Lab (DSL) assay platform.

Interestingly, when the AFP measurements in amniotic fluid were compared, the differences among the various methods seemed somewhat bigger than in serum (Fig. 7B). In particular, results from the Beckman Unicel DXL instrument were about 40% lower than those from the Abbott AxSym, with the results from the other instruments somewhere in between. Since these specimens are derived from actual AF samples these results are directly relevant to patient screening**.**

C) Second Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 24% of the labs; Robert Maciel (RMA) software was employed by 31%; and in-house software comprised 14% and 7% of labs used programs classified as “other” which are presumably proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples have been provided. All laboratories that are **validation-approved** and presently perform 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), crown-rump length (CRL) measurements, race, maternal body weight, and draw date.

As demonstrated in the FT table 2 above (Section – II), the all lab measurement of the 11.0 week Hispanic FT256 specimen for total hCG resulted in a mass mean of 50.41 ± 5.05, with a MOM of 0.59 ± 0.05. Furthermore, the all-lab mass mean for PAPP-A was 3.88 ± 3.04 mIU/ml with a MOM of 3.32 ± 1.72. The all-lab T21 risk assessment was 1 in 20,000 for the FT256 specimen. The risk cut-off level for Hispanics ranges from 200 to 270 among the participating labs. Thus, the FT256 sample resulted in a 100% all lab T21 negative screen assessment. No further action was indicated. Finally, the FT256 specimen also screened negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Figures 13, 14).

As shown in Table 2 for the FT257 Black specimen, the gestational age all-lab mean was reported as 10.9 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 53.86 ± 5.88 IU/ml (MOM = 0.65 ± 4.98) and an all-lab PAPP-A mass measurement of 6.43 ± 4.98 mIU/ml (MOM = 6.43 ± 3.66). The all-lab T21 screen consensus for FT257 was negative with a risk assessment of 1 in 20,000. Similarly, the risk assessment for T18 was 1 in 10,000.

The all lab measurement of the 11.9 week Caucasian FT258 specimen for total hCG resulted in a mass mean of 141.06 IU/ml ± 25.32, with an elevated MOM of 1.91± 0.23 (Table 2). Furthermore, the all-lab mass mean for PAPP-A was 2.45 ± 1.68 mIU/ml ± 1.42 with a MOM of 1.11 ± 0.58. This resulted in an all-lab T21 risk assessment of 1 in 47 for the FT258 specimen. Since analyte MOM measurements for the first trimester Down syndrome screen detection are associated with raised NT, low PAPP-A, and high hCG MOMs, the FT258 results (Fig. 13) were consistent with a T21 positive screen. Even though the PAPP-A was only 1.11 MOM, the elevated NT and hCG were sufficient to produce a positive screen. Thus, the FT258 sample resulted in an 88% all lab T21 positive screen assessment. Further actions by the labs included genetic counseling, 81%; ultrasound, 43%; and amniocentesis/CVS = 63/43%. Finally, the FT258 specimen screened negative for T18 (1 in 2,120) using a risk cutoff of 1 in 100.

In the FT259 Asian sample, the gestational age all-lab mean was reported as 11.4 weeks. Assay measurements for FT259 resulted in an all-lab total hCG mass measurement of 53.98 ± 5.32 (MOM = 0.65 ± 0.07), while the all-lab PAPP-A mass assessment was 7.67 ± 5.99 (MOM = 5.58 ± 2.55). All labs agreed that the FT259 sample was screen negative for T21 (Fig. 13). The all-lab T18 risk assessment for FT259 was 1 in 10,000; hence, the FT259 specimen resulted in a negative screen for T18.

For the Caucasian FT260 specimen, the gestational age all-lab mean was reported as 13.0 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 44.16 ± 4.40 IU/ml (MOM = 0.65 ± 0.05) while the all-lab PAPP-A mass assessment was 9.26 ± 7.28 mIU/ml (MOM = 3.79 ± 1.65). The all-lab FT T21 risk assessment was 1 in 20,000 and all labs agreed that the FT260 sample was screen negative for T21 (Fig. 13). The FT260 specimen also resulted in a negative screen for T18 with an all-lab risk assessment of 1 in 10,000.

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 (Figs. 11, 12) for each of the five FT samples. As shown in Fig. 11, hCG measurement between the two kits differed somewhat, with the Beckman Unicel/Access instruments measuring approximately 30% above the Siemens/Immulite results. Furthermore, the results from the two PAPP-A kits varied widely with the mean/all kit median values from Diagnostic Systems Lab (DSL) being less than half of those obtained with Siemens/DPC Immulite or Immulite 2000 instruments. When the PAPP-A kit MOM's were compared, DPC Immulite was more than double that from DSL and Beckman.

E) First Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 27% and 20% of the labs, respectively; Robert Maciel (RMA) software was employed by 33%; and in-house software comprised 20%. None of the labs used programs classified as "other" which are proprietary software packages.

G.J. Mizejewski, Ph.D.

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Abstracts

A). Screening Abstract “Picks-of-the-Month”:

(1) Title: Screening for open neural tube defects.

Source: Clin Lab Med. 2010; 30(3): 721-5.

Authors: Krantz, D. A., T. W. Hallahan, et al.

Abstract: Maternal serum screening for congenital anomalies began over 30 years ago with the advent of alpha-fetoprotein (AFP) screening for open neural tube defects. It was from these screening programs that the more complex multiple marker Down syndrome screening programs developed. However, today open neural tube defect screening remains a relatively simple approach. In recent times, questions arise about the validity of the risk assessment associated with neural tube defect screening because of the impact of folate acid enrichment in diets and lack of outcome ascertainment. However, it still remains true that those with elevated AFP levels are at higher risk for having a pregnancy affected with open neural tube defect.

(2) Title: Second trimester serum predictors of preterm birth in a population-based sample of low-risk pregnancies.

Source: Prenat Diagn. 2010; 30(8): 727-33.

Authors: Jelliffe-Pawlowski, L. L., R. J. Baer, et al.

Abstract: OBJECTIVE: To examine the relationship between typically collected second trimester maternal serum biomarkers and preterm birth among pregnancies without intrauterine-growth-retardation or other specific risk factors. METHODS: Included were 102 861 singleton pregnancies without specific risks that resulted in the live birth of an infant of normal birth weight for gestational age without aneuploidy or a neural tube defect. Logistic binomial regression analyses were used to estimate the relative risk (RR) of giving birth preterm among pregnancies with an abnormal level of alpha-fetoprotein (AFP), human chorionic gonatotropin (hCG), and/or unconjugated estriol (uE3) compared to pregnancies with normal

biomarker levels. RESULTS: When compared to pregnancies with normal levels of AFP, hCG, and uE3, pregnancies with elevated levels of any biomarker [multiple of the median (MoM) >or= 2.0] were at an increased risk for preterm birth regardless of preterm grouping (RRs 1.3-5.4). Risks for preterm birth tended to increase substantially when at least two biomarkers were elevated (RRs 2.2-18.7). CONCLUSION: The results suggest that second trimester maternal serum biomarkers may help identify pregnancies at increased risk for preterm birth when no other identified risks are present. Data indicates that biomarkers may be particularly predictive of early preterm birth.

(3) Title: Down syndrome maternal serum screening in patients with renal disease.

Source: Am J Obstet Gynecol. 2010; 203(1): 60 e1-4.

Authors: Benachi, A., S. Dreux, et al.

Abstract: OBJECTIVE: The objective of the study was to determine the value of maternal serum Down syndrome screening in patients affected by renal disease. STUDY DESIGN: A study group of 54 pregnant women with renal diseases defined before pregnancy, was compared with a control group of 108 patients matched for maternal age, maternal weight, smoking status, and gestational age. Maternal serum markers (free beta-human chorionic gonadotropin [hCG], total hCG, alpha-fetoprotein) expressed in multiple of median and maternal renal function markers (creatinine, beta2-microglobulin, alpha1-microglobulin) were assayed. RESULTS: The percentage of patients in the Down syndrome at-risk group (>1:250) using free beta-hCG was significantly higher ($P < .02$) in the renal disease group (48%) than in the control group (12%). No significant difference was observed for total hCG (25% vs 15%). CONCLUSION: Down syndrome screening using free beta-hCG is not applicable in patients with renal disease whatever the maternal serum creatinine and can be used with caution when total hCG is used.

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

(1) Title: Prenatal diagnosis of severe epignathus in a twin: case report and review of the literature.

Source: Cleft Palate Craniofac J. 2010: 47(4): 421-5.

Authors: Tonni, G., G. Centini, et al.

Abstract: A prenatal ultrasound diagnosis of epignathus in a dichorionic-diamniotic twin pregnancy is reported. A complex mass protruding from the fetal face was seen at week 19. Amniocentesis resulted in a 46,XX fetus with elevated alpha-fetoprotein (alpha-FP). An increase in tumor size and severe polyhydramnios ensued. Selective feticide performed at 22 weeks led to untreatable uterine contractions with iatrogenic abortion and early neonatal mortality of the healthy cotwin. Without development of polyhydramnios and tumor growth, weekly scan and transvaginal cervical assessment would have been carried out and cesarean section planned at around 32 weeks. Necroscopy and histology aided the ultrasound-based prenatal diagnosis.

(2) Title: Congenital juvenile granulosa cell tumor of the testis in newborns.

Source: Anticancer Res. 2010; 30(5): 1731-4.

Authors: Zugor, V., A. P. Labanaris, et al.

Abstract: BACKGROUND: Granulosa cell tumor of the testis is a rare intermediate stromal cell tumor that can be distinguished in the adult and juvenile type. The juvenile type is the most common reason for scrotal swelling in newborns under the age of six months. Less than fifty cases of this disease entity have been reported in the literature. PATIENTS AND METHODS: In the following article, two newborn patients with scrotal swelling and a histological confirmation of juvenile granulosa cell tumor of the testis will be presented. RESULTS: Case 1: A newborn patient presented with massive scrotal swelling. Sonography of the testicle exhibited a multiple septic and cystic enlargement of the testicle without distinction of the testicular parenchyma being possible. The laboratory findings demonstrated normal testosterone levels,

beta-HCG and inhibin-B levels as well as an increased alpha-fetoprotein level of 35.350 ng/dl. Due to clinical and sonographic findings, an inguinal exploration and later, due to the impossibility of distinction of the testicular parenchyma, an inguinal orchiectomy of the right testicle was performed. Case 2: The clinical and sonographic examination of a newborn patient demonstrated a suspicious process of the left testicle. Sonography exhibited an enlarged testicle with cystic formations with the distinction of the testicular parenchyma not being possible. The laboratory findings demonstrated normal testosterone levels, beta-HCG and inhibin-B levels as well as an increased alpha-fetoprotein level of 9.038 ng/dl and LDH of 768 U/I. An inguinal orchiectomy of the left testicle was performed. In both cases, a histological diagnosis of juvenile granulosa cell tumor of the testis was made. CONCLUSION: These two aforementioned cases demonstrate that juvenile granulosa cell tumor of the testis is a benign disease encountered in newborns, which exhibits an excellent prognosis. Inguinal orchiectomy is the therapy of choice. After surgical removal of the involved testicle is performed no further management is required.

(3) Title: First- and second-trimester maternal serum markers for aneuploidy and adverse obstetric outcomes."

Source: Obstet Gynecol. 2010; 115(5): 1052-61.

Authors: Dugoff, L.

Abstract: Maternal serum levels of the first- and second-trimester markers for aneuploidy have been shown to be associated with adverse obstetric outcomes in the absence of aneuploidy or neural-tube defects. The likelihood of an adverse obstetric outcome increases as the values of the marker become more extreme, and as the number of abnormal markers increases. Although many of the associations between maternal serum markers for aneuploidy and adverse obstetric outcomes are statistically significant, the sensitivity and positive predictive values for the individual outcomes are too low for them to be clinically useful as screening tests. Currently in the United States there is not a uniformly accepted practice for the care of women with abnormal maternal serum markers regarding risk of future obstetric complications. There are no randomized trials assessing any type of intervention or treatment for patients with abnormal serum markers. Various strategies to manage patients with unexplained abnormal serum markers have been proposed. This article reviews the relationships between these markers and adverse obstetric outcomes. In addition, potential management strategies and future areas of research are discussed.

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

(1) Title: ADAM12 is an effective marker in the second trimester of pregnancy for prenatal screening of Down syndrome.

Source: Prenat Diagn. 2010; 30(6): 561-4.

Authors: Wang, M., S. Lu, et al.

Abstract: OBJECTIVE: To estimate the use of maternal serum ADAM12 as a second-trimester Down syndrome serum marker. METHODS: Samples from a total of 46 Down syndrome pregnancies and 184 unaffected singleton pregnancies matched for gestational age and maternal weight were retrieved from storage and measured for ADAM12; 35 false-positive pregnancies were included among the controls to assess reductions in false-positive rates by inclusion of ADAM12 in the risk calculation of an algorithm that used alpha-fetoprotein (AFP) and human chorionic gonadotrophin (hCG) (double screen). RESULTS: ADAM12 was measured and expressed as multiple of the gestation-specific median (MoM) and corrected for maternal weight. The median ADAM12 level in the affected pregnancies was 1.26 MoM compared with 1.0 MoM in the unaffected control pregnancies ($p < 0.05$). In unaffected pregnancies, there was a significant correlation between ADAM12 and AFP ($r = 0.314$) but not hCG ($r = 0.018$). Statistical modeling predicted that ADAM12 as a second serum marker could increase the detection rate from 48 to 85%, while reducing the false-negative and false-positive rates. CONCLUSION: ADAM12 can be used as an effective second-trimester serum marker for prenatal screening of Down syndrome.

(2) Title: [Biochemical prenatal tests and uterine artery Doppler examination in prediction of PIH and IUGR in the third trimester of pregnancy].

Source: Ginekol Pol. 2010; 81(5): 352-7.

Authors: Slowakiewicz, K., M. Perenc, et al.

Abstract: OBJECTIVES: PIH and IUGR are serious complications in the third trimester of pregnancy. Many publications claim a connection between false positive prenatal tests and subsequent occurrence of PIH and IUGR. DESIGN: The aim of the study was to estimate the usefulness of the biochemical markers of fetal defects and uterine Doppler examination in predicting PIH and IUGR in the third trimester of pregnancy. METHODS: We examined 156 pregnant patients in The Department of the Fetal Medicine and Gynecology Medical University of Lodz, between 2006-2009. In case of each pregnant woman we estimated biochemical markers in the first (PAPP-A + beta-hCG) and second trimester (AFP, beta-hCG, uE3 - triple test). Each patient underwent three ultrasonographic examinations in the first, second and third trimester (between 11-13, 15-20, and 22-27 weeks gestation, respectively) with uterine artery Doppler examination. We monitored these pregnancies for PIH and IUGR and divided them into three groups: 28 patients with PIH (study group 1), 14 patients with IUGR (study group 2), and 114 patients with uncomplicated pregnancies (controls). RESULTS: In both study groups we observed: higher concentration of beta-hCG, higher percentage of the positive biochemical prenatal tests and abnormal uterine artery Doppler waveform. Positive triple test was the strongest predictor of PIH and IUGR (PPV=60.87% for PIH and PPV = 30.77% for IUGR). CONCLUSIONS: Biochemical markers and abnormal uterine artery Doppler waveform are associated with PIH and IUGR. These parameters can be the base for the test identifying pregnant patients with high risk of PIH and IUGR.

(3) Title: Amniotic fluid alpha-fetoprotein microheterogeneity in the prenatal diagnosis of congenital disorders of glycosylation type Ia.

Source: Clin Chem Lab Med. 2010

Authors: Marklova, E. and Z. Albahri

Abstract: BACKGROUND: Congenital disorders of glycosylation are a group of clinically and biochemically diverse defects. The current screening method (based on analysis of transferrin), which is used postnatally for the most frequent types, is however not suitable for prenatal diagnosis. The aim of the study was to investigate whether alterations in the microheterogeneity of alpha-fetoprotein would provide more reliable results. METHODS: During the 14th-19th weeks of gestation, 140 amniotic fluid samples were obtained by amniocentesis and tested for fetal developmental abnormalities. alpha-Fetoprotein was analyzed using isoelectric focusing on Immobiline DryPlate pH 4-7, rehydrated in urea (8 mol/L), and molecular forms of the glycoprotein were detected by immunofixation and silver staining. Results: A difference in the relative proportion of individual alpha-fetoprotein bands (particularly increase of band II density) was found in a case where a congenital disorder of glycosylation was diagnosed postnatally, and in two other samples from pregnancies which resulted in termination, without further examination. CONCLUSIONS: Our potential for further testing is limited; thus far, no other congenital disorders of glycosylation-positive samples have been available. Verification of our results in another laboratory with the exclusion of several potentially pertinent variables is advisable.

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

(1) Title: A sensitive amperometric immunosensor for alpha-fetoprotein based on carbon nanotube/DNA/Thi/nano-Au modified glassy carbon electrode.

Source: Colloids Surf B Biointerfaces. 2010; 79(2): 421-6.

Authors: Ran, X. Q., R. Yuan, et al.

Abstract: A novel amperometric immunosensor for the determination of alpha-fetoprotein (AFP) was constructed using films of multi-wall carbon nanotubes/DNA/thionine/gold nanoparticles (nano-Au). Firstly, multiwall carbon nanotubes (MWCNT) dispersed in poly(diallydimethlammonium chloride) (PDDA) were immobilized on the nano-Au film which was electrochemically deposited on the surface of glassy carbon electrode. Then a negatively charged DNA film was absorbed on the positively charged PDDA. Subsequently, thionine was attached to the electrode via the electrostatic interaction between thionine and the DNA. Finally, the nano-Au was retained on the thionine film for immobilization of AFP antibody (anti-AFP). The modification process was characterized by cyclic voltammetry (CV) and scanning

electron microscope (SEM). The factors possibly influenced the performance of the proposed immunosensors were studied in detail. Under optimal conditions, the proposed immunosensor exhibited good electrochemical behavior to AFP in a two concentration ranges: 0.01-10.0 and 10.0-200.0 ng/mL with a relatively low detection limit of 0.04 ng/mL at three times the background noise. Moreover, the selectivity, repeatability and stability of the proposed immunosensor were acceptable.

(2) Title: Conductive carbon nanoparticles-based electrochemical immunosensor with enhanced sensitivity for alpha-fetoprotein using irregular-shaped gold nanoparticles-labeled enzyme-linked antibodies as signal improvement.

Source: Biosens Bioelectron. 2010; 25(12): 2657-62.

Authors: Tang, J., B. Su, et al.

Abstract: A new electrochemical immunoassay protocol for sensitive detection of alpha-fetoprotein (AFP, as a model) is designed using carbon nanoparticles (CNPs)-functionalized biomimetic interface as immunosensing probe and irregular-shaped gold nanoparticles (ISNGs)-labeled horseradish peroxidase-anti-AFP conjugates (HRP-anti-AFP-ISNG) as trace label. The low-toxic and high-conductive CNPs provided a high capacity nanoparticulate immobilization surface and a facile pathway for electron transfer. In comparison with conventional label methods, i.e. spherical gold nanoparticles-labeled HRP-anti-AFP and HRP-labeled anti-AFP, the electrochemical immunosensor using HRP-anti-AFP-ISNGs as trace labels exhibited high bioelectrocatalytic response toward enzyme substrate and a wide dynamic range from 0.02 to 4.0 ng/mL with a low detection limit of 10 pg/mL toward AFP (at 3sigma). The developed immunoassay method showed good selectivity and acceptable reproducibility. Clinical serum samples with various AFP concentrations were evaluated by using the electrochemical immunosensor and the referenced enzyme-linked immunosorbent assay (ELISA), respectively, and received in good accordance with results obtained from these two methods.

(3) Title: Anal Chim Acta. 2010; 665(1): 63-8.

Source: Multiplex immunodetection of tumor markers with a suspension array built upon core-shell structured functional fluorescence-encoded microspheres.

Authors: Long, Y., Z. Zhang, et al.

Abstract: A new suspension array built upon laboratory-prepared functional fluorescence-encoded polystyrene beads (FFPBs) was developed for multiplex immunodetection of tumor markers. The FFPBs were synthesized by copolymerizing rhodamine 6G (R6G) and carboxyl function groups on the surface of the seed beads forming a core-shell structure. The fabrication process was facile and the encoding fluorescence intensity of the beads can be precisely controlled by adjusting the quantity of R6G. In present work, we demonstrated that the quantity variation of impregnated R6G had negligible effect on the coupling efficiency of biomolecules onto the surface of the FFPBs. The R6G encoding fluorescence remained good monodispersity upon capture probe coupling and immunocomplex formation. No fluorescence resonance energy transfer was observed between the R6G doped in the bead shell and fluorophore used for antibody labeling. Under the optimal conditions, the proposed suspension array allowed simultaneous detection of alpha-fetoprotein, carcinoembryonic antigen, and prostate specific antigen in the ranges of 0.07-500 ng mL(-1), 1-2000 ng mL(-1), and 0.5-500 ng mL(-1), respectively, with detection limits of 0.0626 ng mL(-1), 0.554 ng mL(-1), and 0.250 ng mL(-1). Test on clinical serum samples demonstrated that the results obtained with suspension array were in good agreement with those of the reference electrochemiluminescence immunoassay method. We conclude that the laboratory-made FFPBs are sufficient as the microcarrier for the construction of suspension array in clinical diagnosis.

E). Special Abstract Selection:

(1) Title: Cross-trimester repeated measures testing for Down's syndrome screening: an assessment.

Source: Health Technol Assess. 2010; 14(33): 1-80.

Authors: Wright, D., I. Bradbury, et al.

Abstract: OBJECTIVES: To provide estimates and confidence intervals for the performance (detection and false-positive rates) of screening for Down's syndrome using repeated measures of biochemical markers from first and second trimester maternal serum samples taken from the same woman. DESIGN: Stored serum on Down's syndrome cases and controls was used to provide independent test data for the assessment of screening performance of published risk algorithms and for the development and testing of new risk assessment algorithms. SETTING: 15 screening centres across the USA, and at the North York General Hospital, Toronto, Canada. PARTICIPANTS: 78 women with pregnancy affected by Down's syndrome and 390 matched unaffected controls, with maternal blood samples obtained at 11-13 and 15-18 weeks' gestation, and women who received integrated prenatal screening at North York General Hospital at two time intervals: between 1 December 1999 and 31 October 2003, and between 1 October 2006 and 23 November 2007. INTERVENTIONS: Repeated measurements (first and second trimester) of maternal serum levels of human chorionic gonadotrophin (hCG), unconjugated estriol (uE3) and pregnancy-associated plasma protein A (PAPP-A) together with alpha-fetoprotein (AFP) in the second trimester. MAIN OUTCOME MEASURES: Detection and false-positive rates for screening with a threshold risk of 1 in 200 at term, and the detection rate achieved for a false-positive rate of 2%. RESULTS: Published distributional models for Down's syndrome were inconsistent with the test data. When these test data were classified using these models, screening performance deteriorated substantially through the addition of repeated measures. This contradicts the very optimistic results obtained from predictive modelling of performance. Simplified distributional assumptions showed some evidence of benefit from the use of repeated measures of PAPP-A but not for repeated measures of uE3 or hCG. Each of the two test data sets was used to create new parameter estimates against which screening test performance was assessed using the other data set. The results were equivocal but there was evidence suggesting improvement in screening performance through the use of repeated measures of PAPP-A when the first trimester sample was collected before 13 weeks' gestation. A Bayesian analysis of the combined data from the two test data sets showed that adding a second trimester repeated measurement of PAPP-A to the base test increased detection rates and reduced false-positive rates. The benefit decreased with increasing gestational age at the time of the first sample. There was no evidence of any benefit from repeated measures of hCG or uE3. CONCLUSIONS: If realised, a reduction of 1% in false-positive rate with no loss in detection rate would give important benefits in terms of health service provision and the large number of invasive tests avoided. The Bayesian analysis, which shows evidence of benefit, is based on strong distributional assumptions and should not be regarded as confirmatory. The evidence of potential benefit suggests the need for a prospective study of repeated measurements of PAPP-A with samples from early in the first trimester. A formal clinical effectiveness and cost-effectiveness analysis should be undertaken. This study has shown that the established modelling methodology for assessing screening performance may be optimistically biased and should be interpreted with caution.

(2) Title: Alpha-fetoprotein producing tumor cells in children with Wilms' tumor.

Source: Fetal Pediatr Pathol. 2010; 29(3): 127-32.

Authors: Kesik, V., A. Ozcan, et al.

Abstract: Alpha fetoprotein (AFP) is generally used as a marker in diagnosis and follow-up of germ cell tumors and hepatoblastomas. However, serum AFP levels were elevated in our three patients with Wilms tumor. The elevated levels could only be decreased completely by surgery and not by chemotherapy. Histopathologically, the tumors consisted of blastemal, stromal, and epithelial cells. Chemotherapy was only effective on stromal and epithelial components of the tumors. In AFP staining, the source of AFP production was identified as blastemal tumor cells. Because the increased AFP levels were decreased after surgery, AFP levels may be used in the follow-up of the patients with Wilms tumor. Herein, we report three patients with Wilms tumor whose serum AFP levels were elevated and who had diffuse WT-1 and focal AFP expression in all tumors, immunohistochemically.

(3) Title: Second-trimester maternal serum quadruple test for Down syndrome screening: a Taiwanese population-based study.

Source: Taiwan J Obstet Gynecol. 2010; 49(1): 30-4.

Authors: Shaw, S. W., S. Y. Lin, et al.

Abstract: OBJECTIVE: To assess the usefulness of quadruple test screening for Down syndrome in Taiwan. MATERIALS AND METHODS: Maternal serum concentrations of alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol, and inhibin A were measured in 21,481 pregnant women from 15 to 20 weeks of gestation. RESULTS: Of the 21,481 women, 977 returned values greater than the high-risk cut-off value (1 in 270). Most of these women (86.2%) decided to have an invasive procedure for genetic diagnosis. Nine cases of Down syndrome and 19 cases of other chromosomal anomalies were detected prenatally. Two children with Down syndrome were diagnosed after delivery even though a low estimated risk was determined following the quadruple test. The detection rate was 81.8% (nine out of 11 cases), with a 4.4% false-positive rate. The median multiple of the median value for a-fetoprotein, human chorionic gonadotropin, unconjugated estriol and inhibin A were 0.87, 2.34, 0.77 and 2.16, respectively, in affected cases. CONCLUSION: This is the first study of the quadruple test for Down syndrome in a Chinese population. Our findings suggested that the second-trimester quadruple test provides an effective screening tool for Down syndrome in Taiwan.

(4) Title: Early oestrogens in shaping reproductive networks: evidence for a potential organisational role of oestradiol in female brain development.

Source: J Neuroendocrinol. 2010; 22(7): 728-35.

Authors: Bakker, J. and O. Brock

Abstract: A central tenet of contemporary theories on mammalian brain and behavioural sexual differentiation is that an organisational action of testosterone, secreted by the male's testes, controls male-typical aspects of brain and behavioural development, whereas no active perinatal sex hormone signalling is required for female-typical sexual differentiation. Furthermore, the available evidence suggests that many, although not all, of the perinatal organisational actions of testosterone on the development of the male brain result from the cellular effects of oestradiol formed via neural aromatisation of testosterone. However, a default developmental programme for the female brain has been criticised. Indeed, we review new results obtained in aromatase knockout mice indicating that oestradiol actively contributes to the differentiation of female-typical aspects of brain and behavioural sexual differentiation. Furthermore, we propose that male-typical neural and behavioural differentiation occurs prenatally in genetic males under the influence of oestradiol, which is avoided in foetal genetic females by the neuroprotective actions of alpha-fetoprotein, whereas female-typical neural and behavioural differentiation normally occurs postnatally in genetic females under the influence of oestradiol that is presumably produced by the ovaries.

(5) Title: Comparison of second-trimester maternal serum free-beta-human chorionic gonadotropin and alpha-fetoprotein between normal singleton and twin pregnancies: a population-based study.

Source: Chin Med J (Engl). 2010; 123(5): 555-8.

Authors: Zheng, M. M., Y. L. Hu, et al.

Abstract: BACKGROUND: The second-trimester maternal serum screening in twin pregnancy is still controversial, as the serum marker levels in twins are not as clear as those in singletons. This study aimed to evaluate the relationship between the levels of the second-trimester maternal serum free beta-human chorionic gonadotropin (free beta-HCG) and alpha-fetoprotein (AFP) in normal twin and singleton pregnancies and to estimate feasible analysis methods for utilizing these markers in second trimester screening for twin pregnancy. METHODS: On the basis of a prospective population-based study of second-trimester maternal serum screening, the concentrations of maternal serum AFP and free beta-HCG of 195 normal twin pregnancy and 26,512 singleton controls at gestational weeks 15 to 20 were measured by time-resolved fluoroimmunoassay in one laboratory. The levels of markers were compared between the twins and singletons using weight-correction and gestational age-specific model. RESULTS: According to the research protocol, 95 communities were randomly sampled, which covered the whole Jiangsu province, the east of China. A total of 26 803 pregnant women (98%), from the target population accepted prenatal screening for maternal serum AFP, beta-HCG detection, and all babies were followed up for at least six months. There were 197 (0.73%) twin pregnancies, of which one case had fetal trisomy 18, and one case with fetal anencephaly. The others were normal twin pregnancy. From a total enrollment of 26 803 women participants, 26 512 women with normal singleton pregnancies were selected as the model controls. The other 291 pregnancies, including trisomy 21, neural tube defect

(NTD), trisomy 18, and other fetal abnormalities, were excluded. No significant differences were found in the medians of gestational age-specific maternal serum free beta-hCG and AFP in normal twin pregnancy comparing with twice those in model controls with the exception of the medians for free beta-hCG during the 16th gestational week (P = 0.012). CONCLUSION: The weight-correction and gestational age-specific levels of Chinese Han population maternal serum free beta-hCG and AFP in normal twins were twice the levels as those in the singleton controls during the 17-19 gestational weeks.

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) headtotoe.apta.org/topic/medtest/hw1663/results.htm
4) www.pregnancy-info.net/slpha_feto_protein.html
5) www.healthopedia.com/alpha-fetoprotein

Figure 1

Maternal Sera AFP MoM

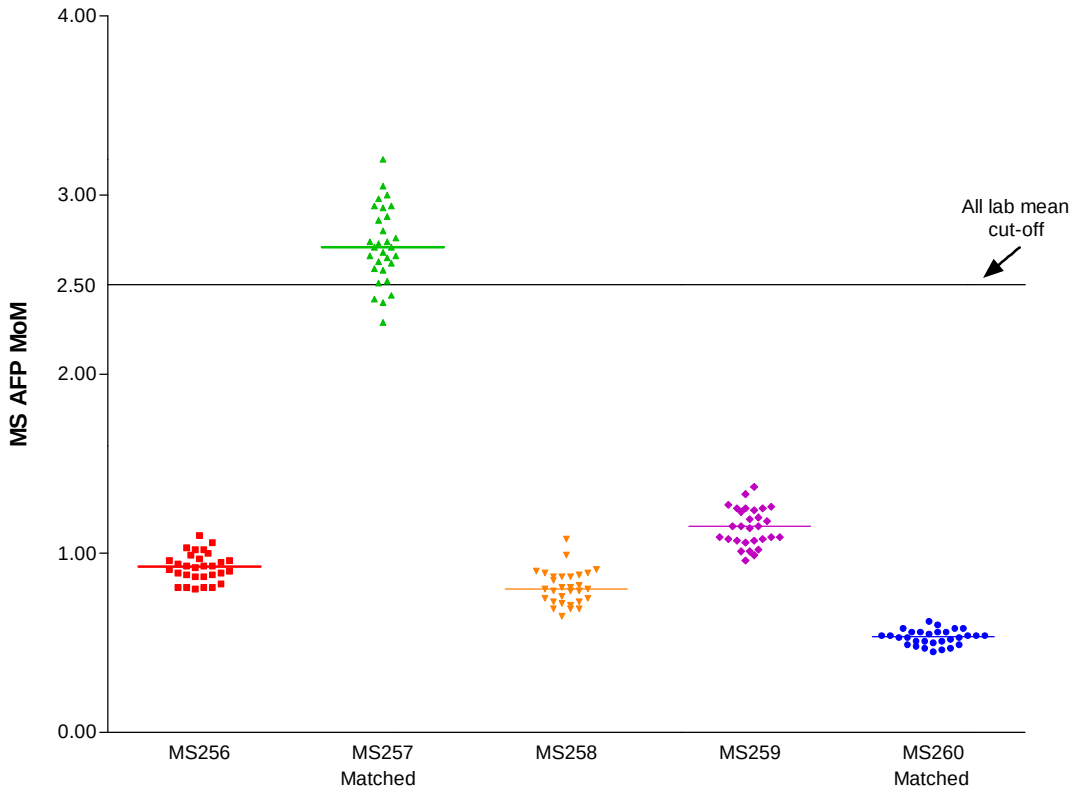


Figure 2

Amniotic Fluid AFP MoM

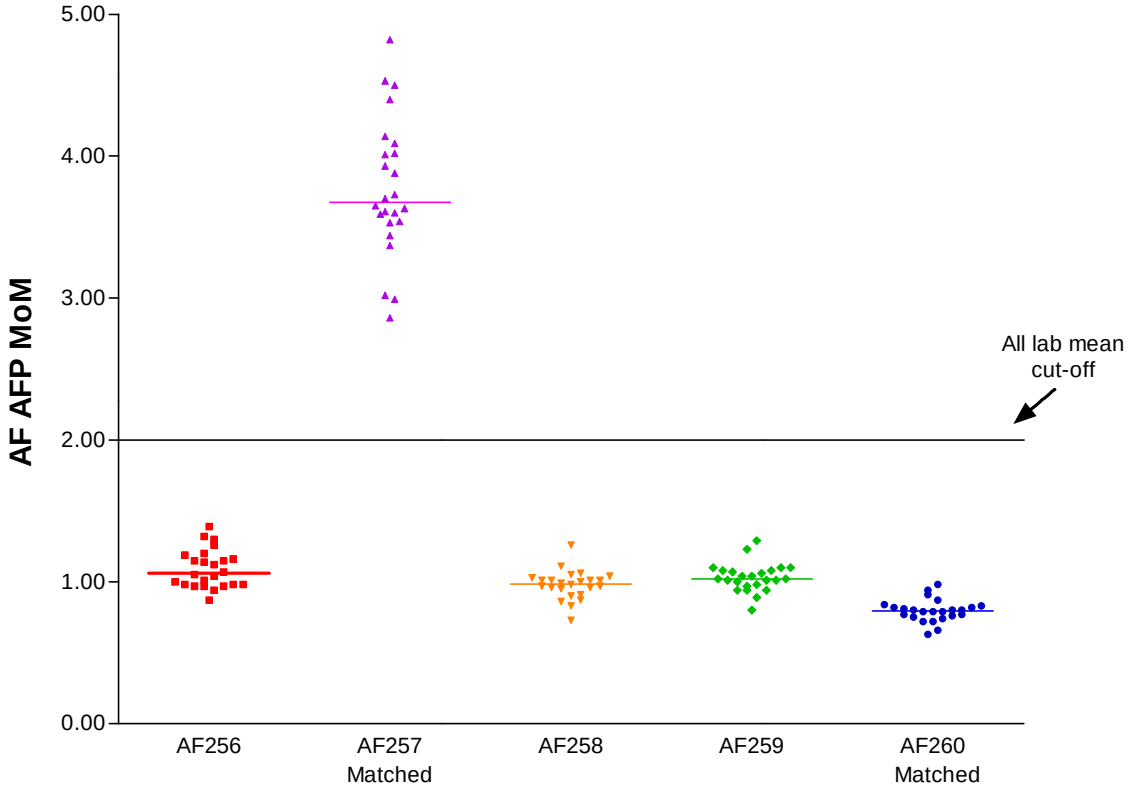


Figure 3

Graphic Distribution of Second Trimester
Neural Tube Defect Risk Estimates

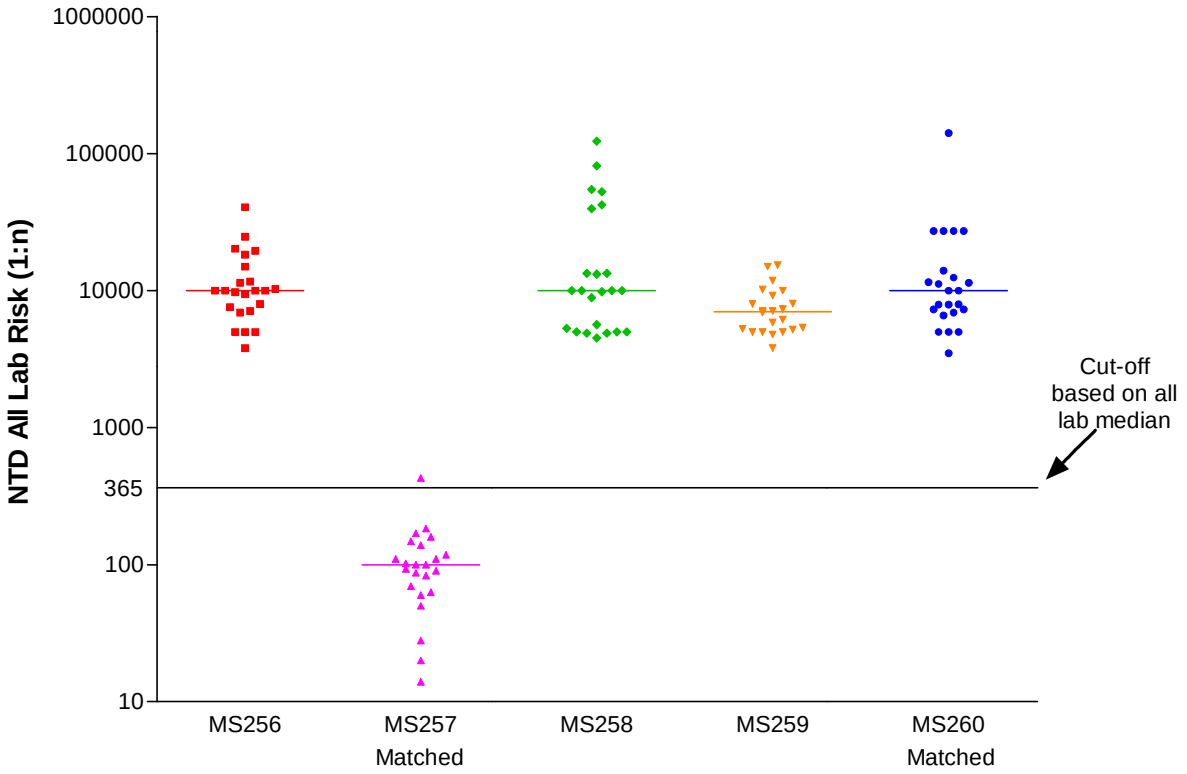


Figure 4

Graphic Distribution of Second Trimester
Trisomy 18 Risk Estimates

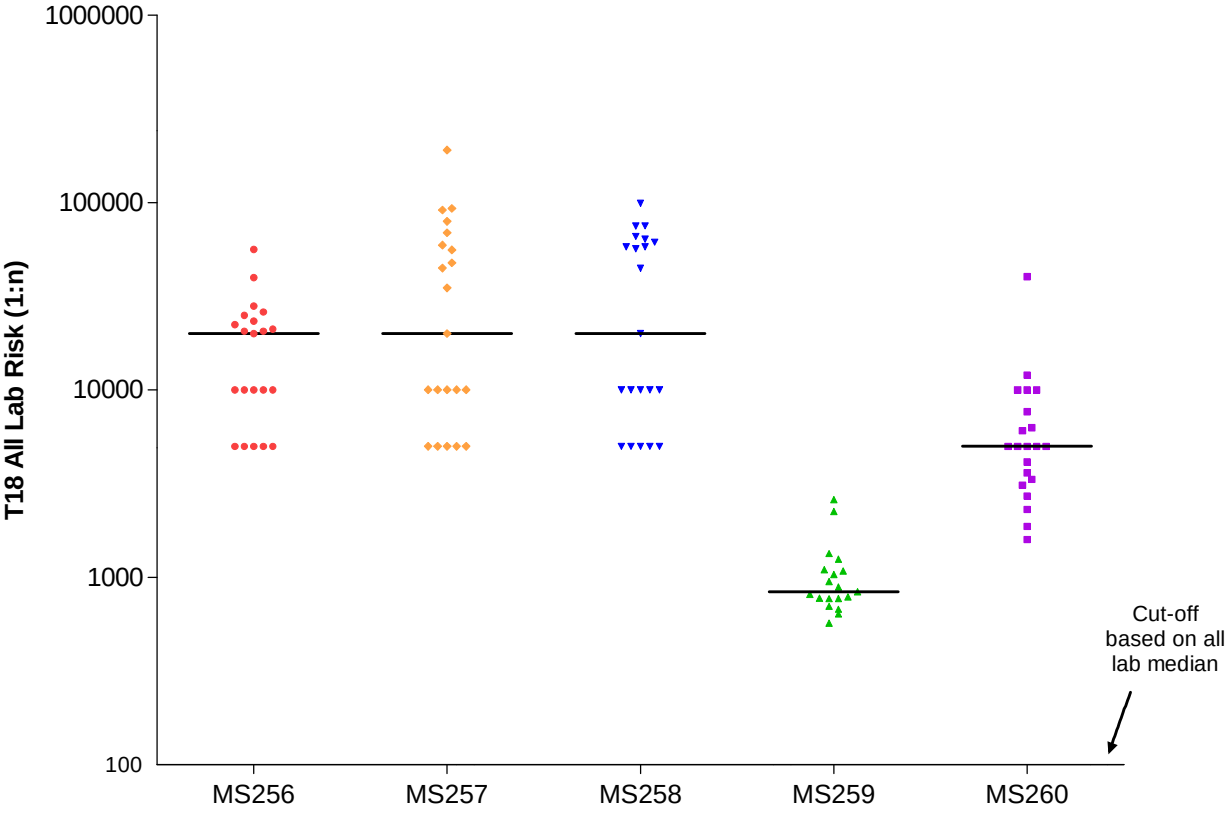


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

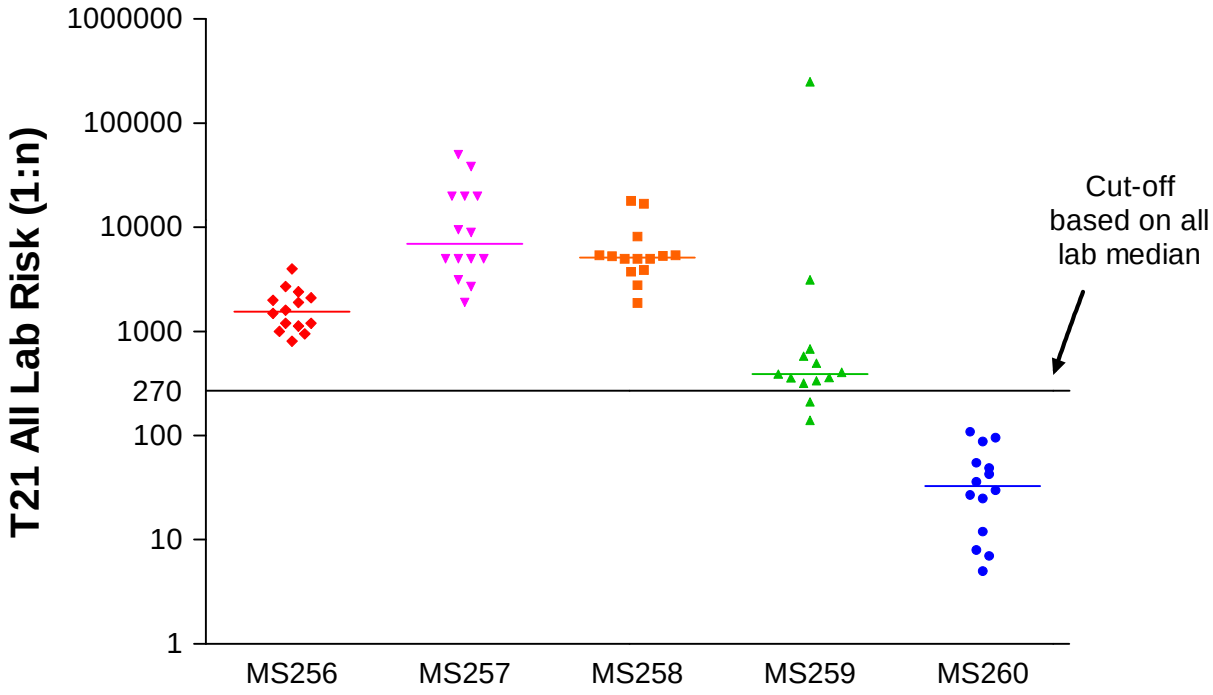
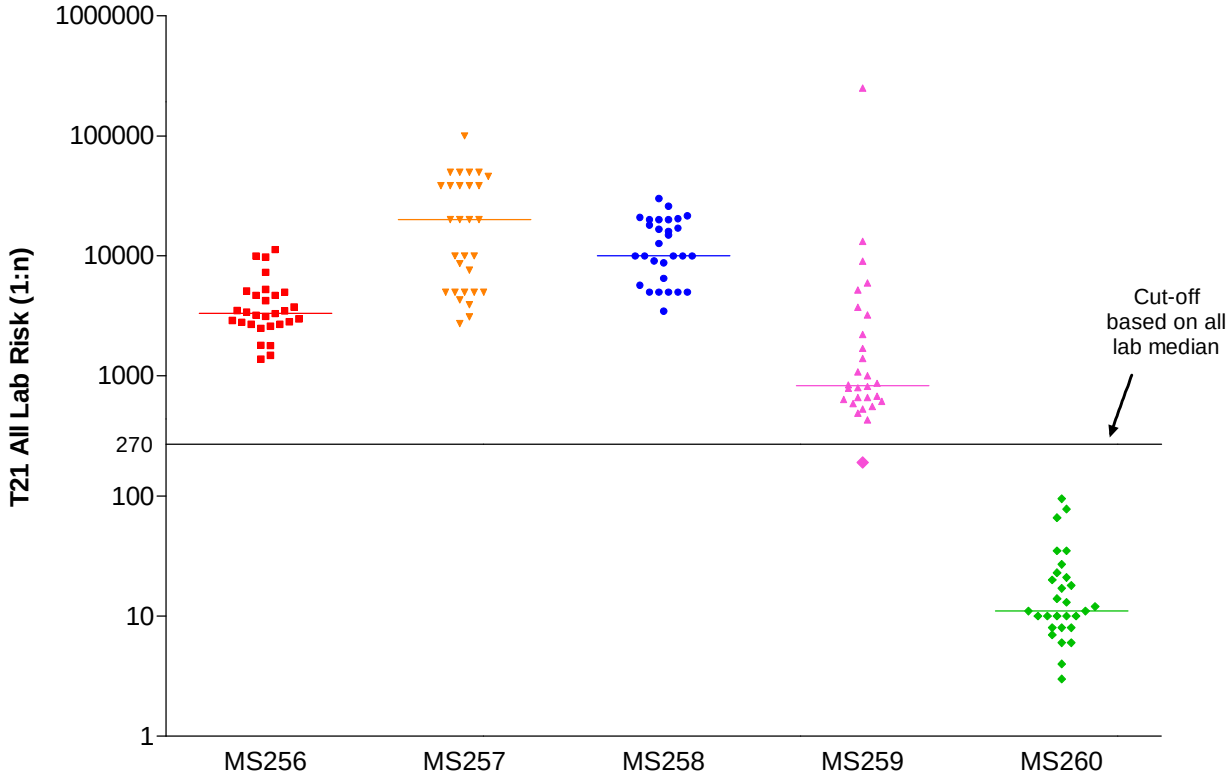


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates



◆The cut-off (1:270) is based on all lab median but this lab's risk of 1:190 uses a cut-off of 1:150.

Figure 7A **MS AFP FEDM PT 9/10 Method Comparison**

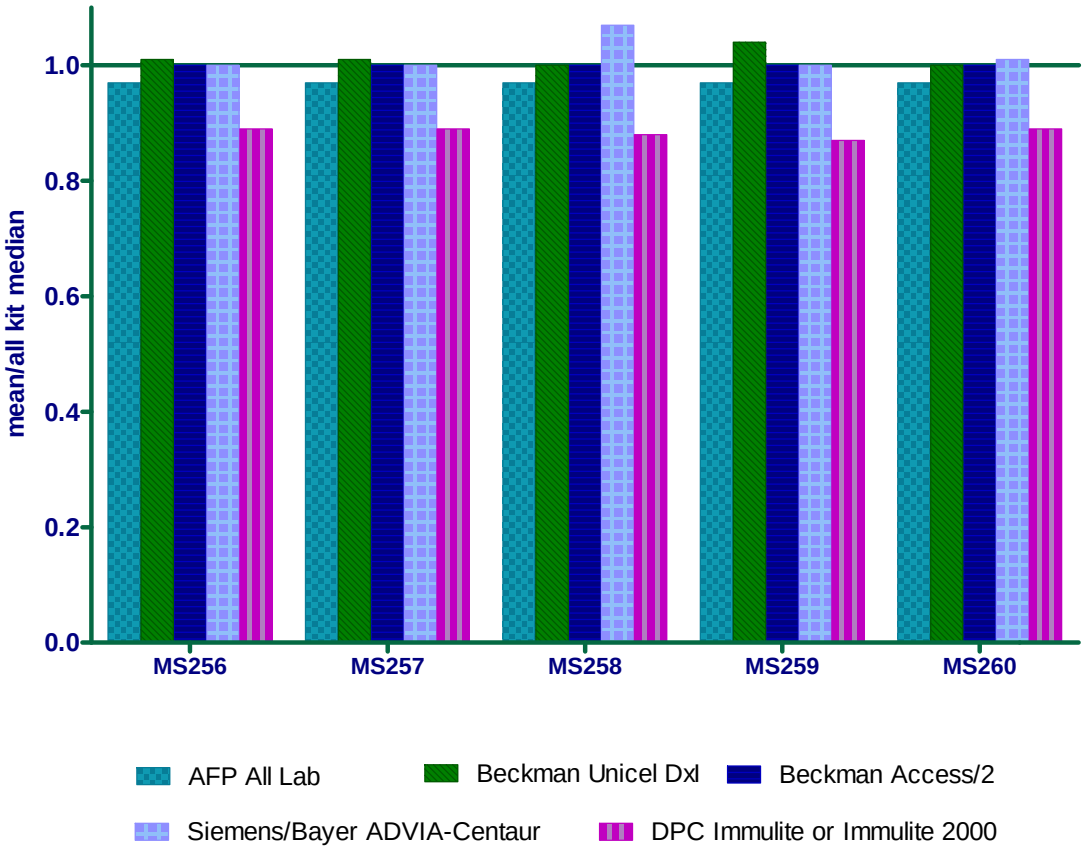


Figure 7B **AF AFP FEDM PT 9/10 Method Comparison**

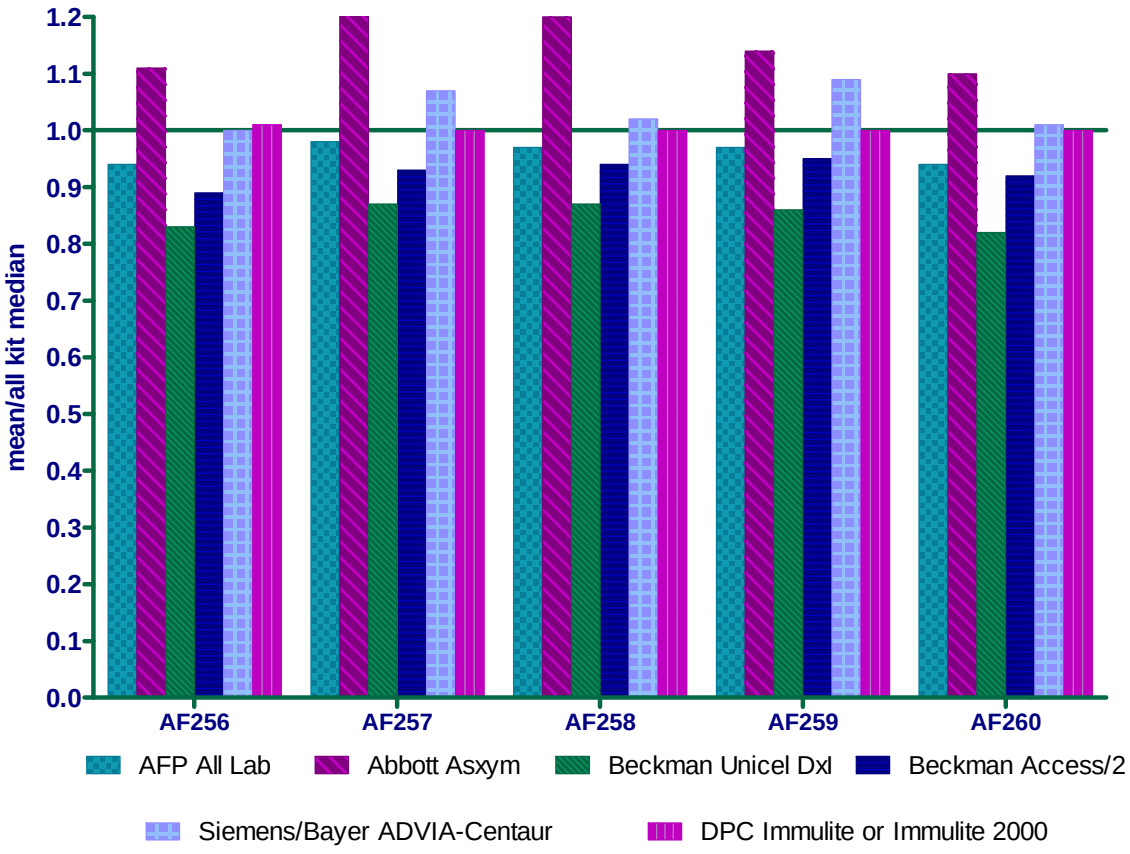


Figure 8A

MS uE3 FEDM PT 9/10 Method Comparison

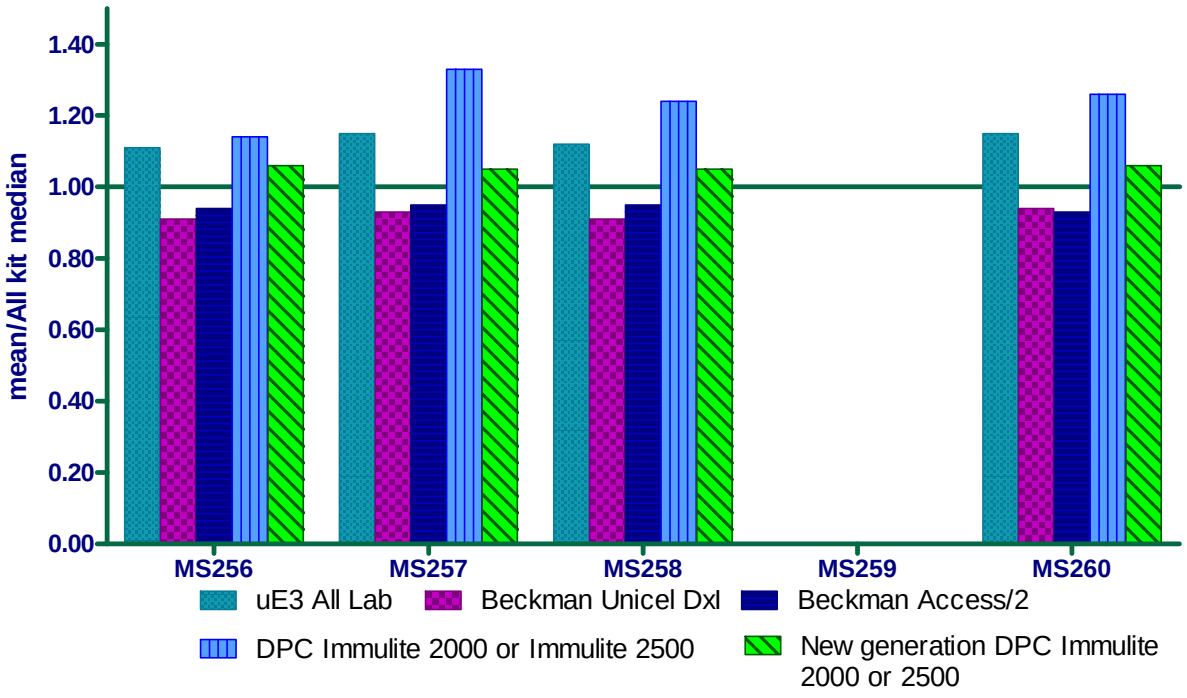


Figure 8 B

MS uE3 MOM FEDM PT 9/10 Method Comparison

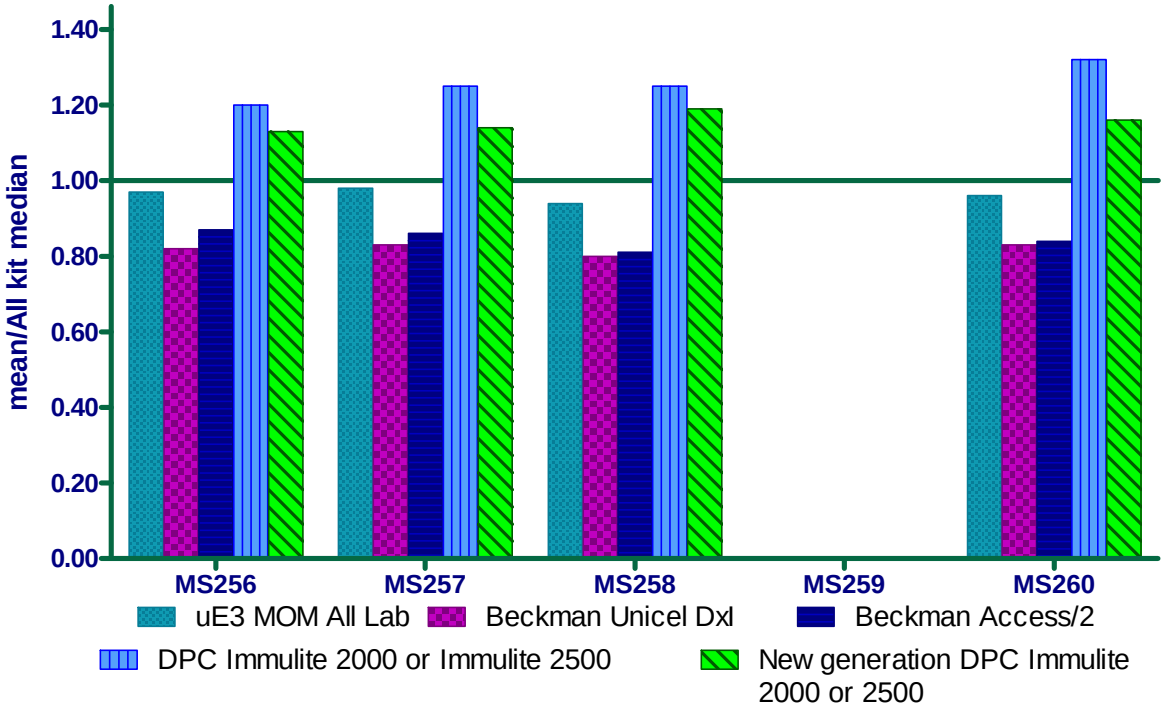


Figure 9

MS hCG FEDM PT 9/10 Method Comparison

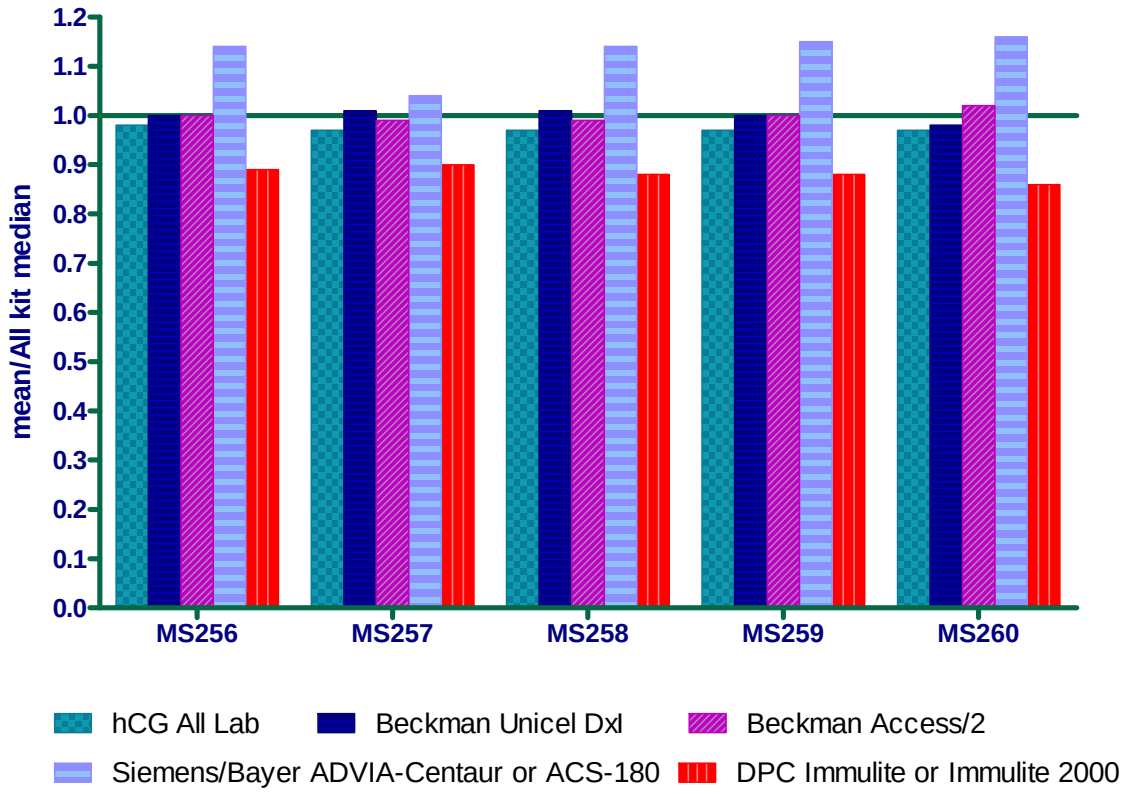


Figure 10

MS Inhibin A FEDM PT 9/10 Method Comparison

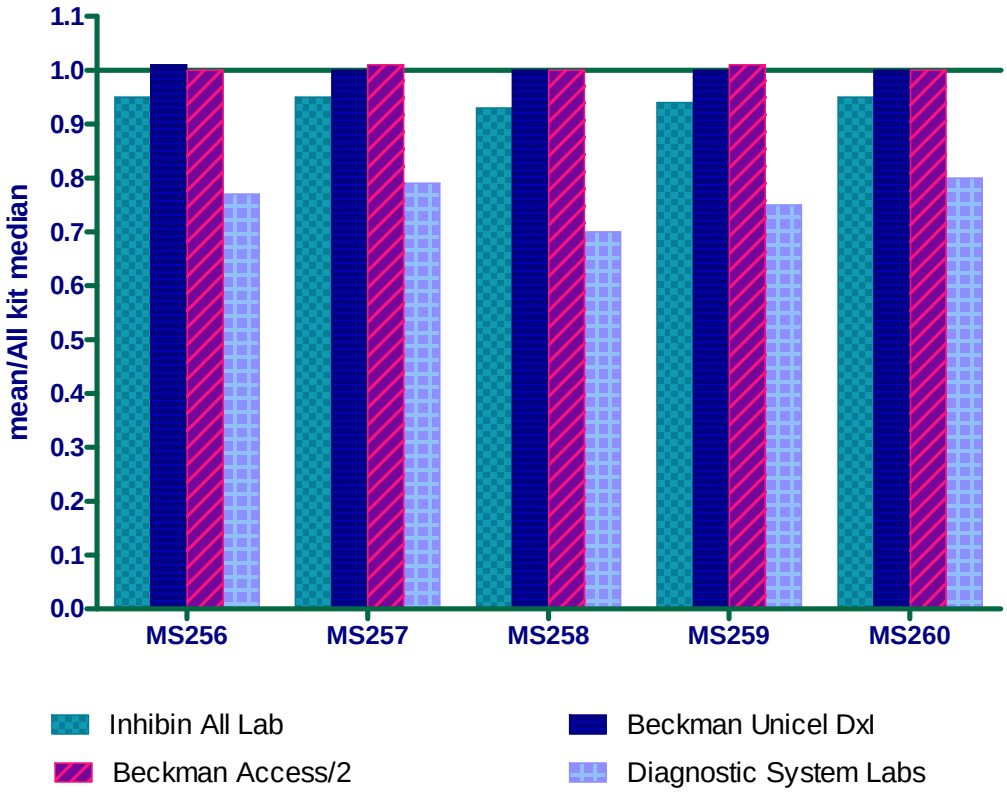


Figure 11

FT hCG FEDM PT 9/10 Method Comparison

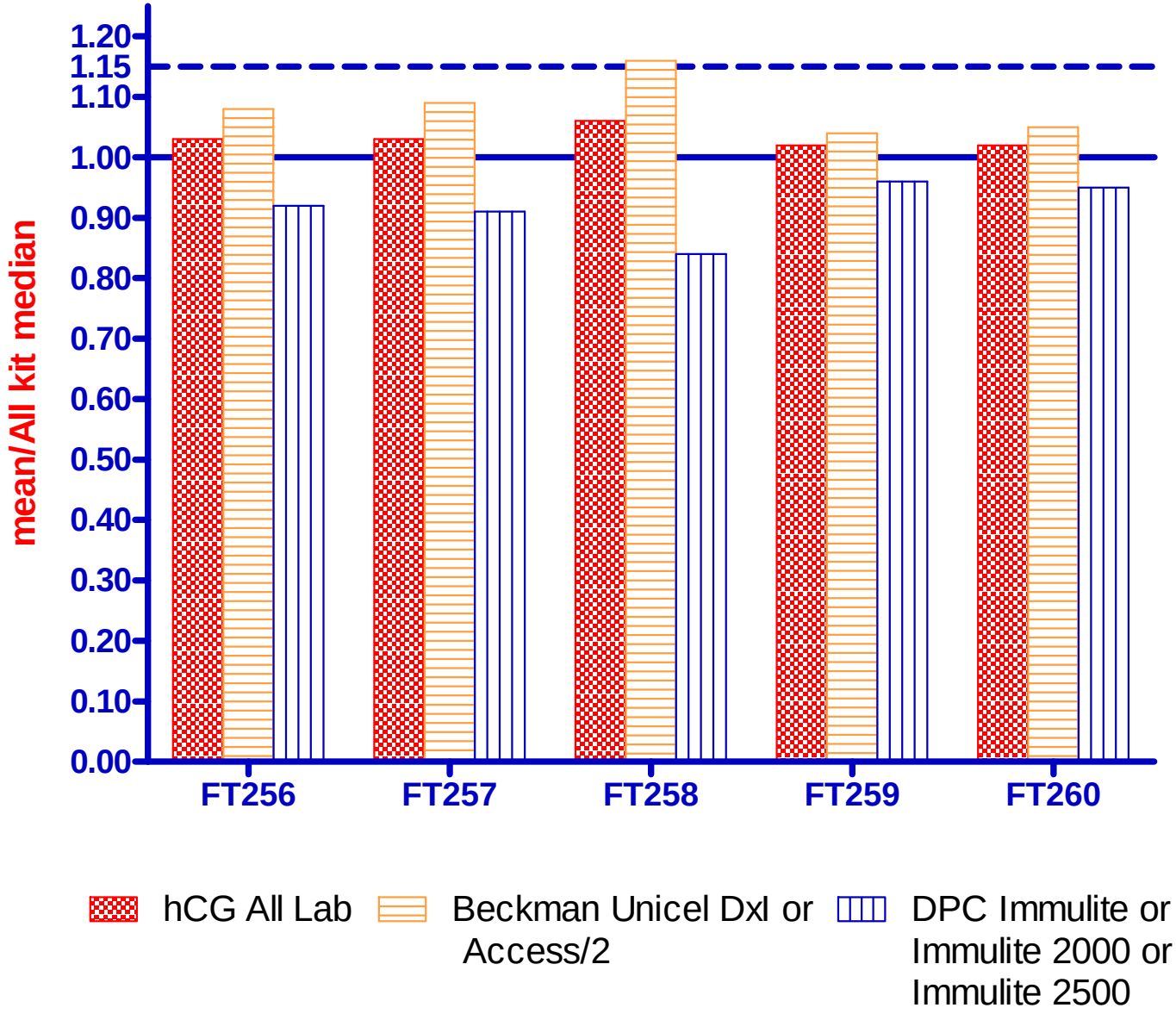
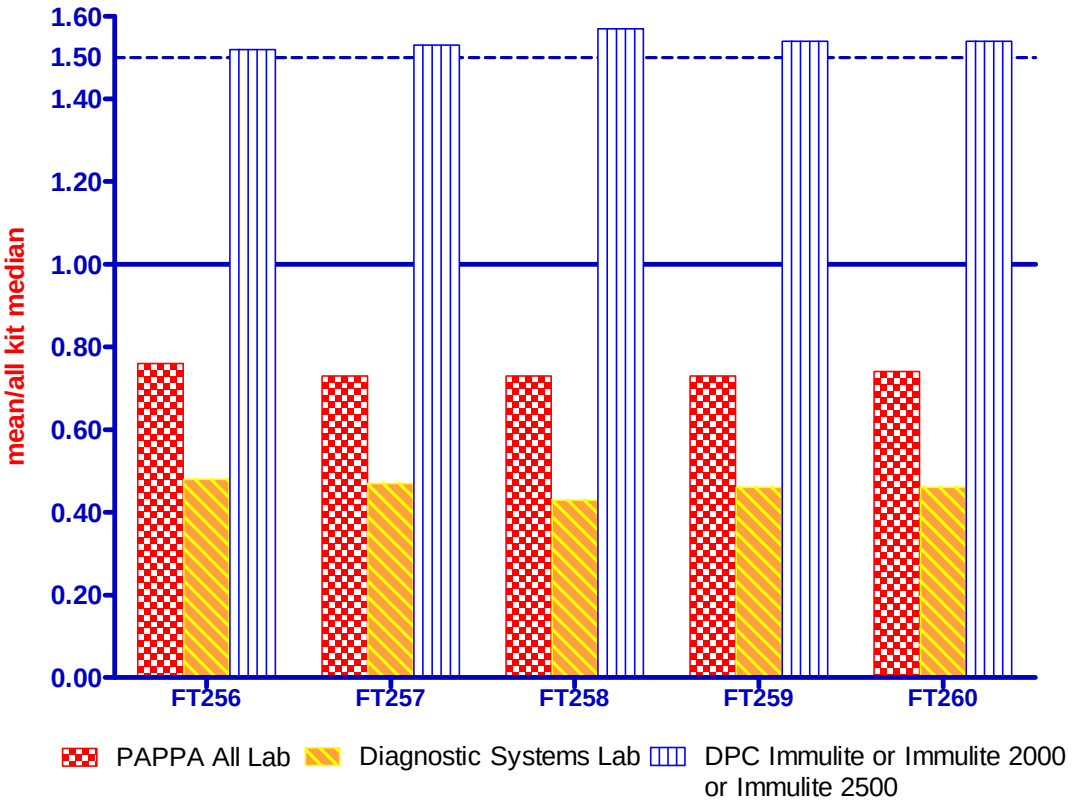


Figure 12A

FT PAPP- A FEDM PT 9/10 Method Comparison



*Please note Beckman is not included due to the difference in mass units used (ng/ml vs mIU/ml)

Figure 12B

FT PAPP- A MOM FEDM PT 9/10 Method Comparison

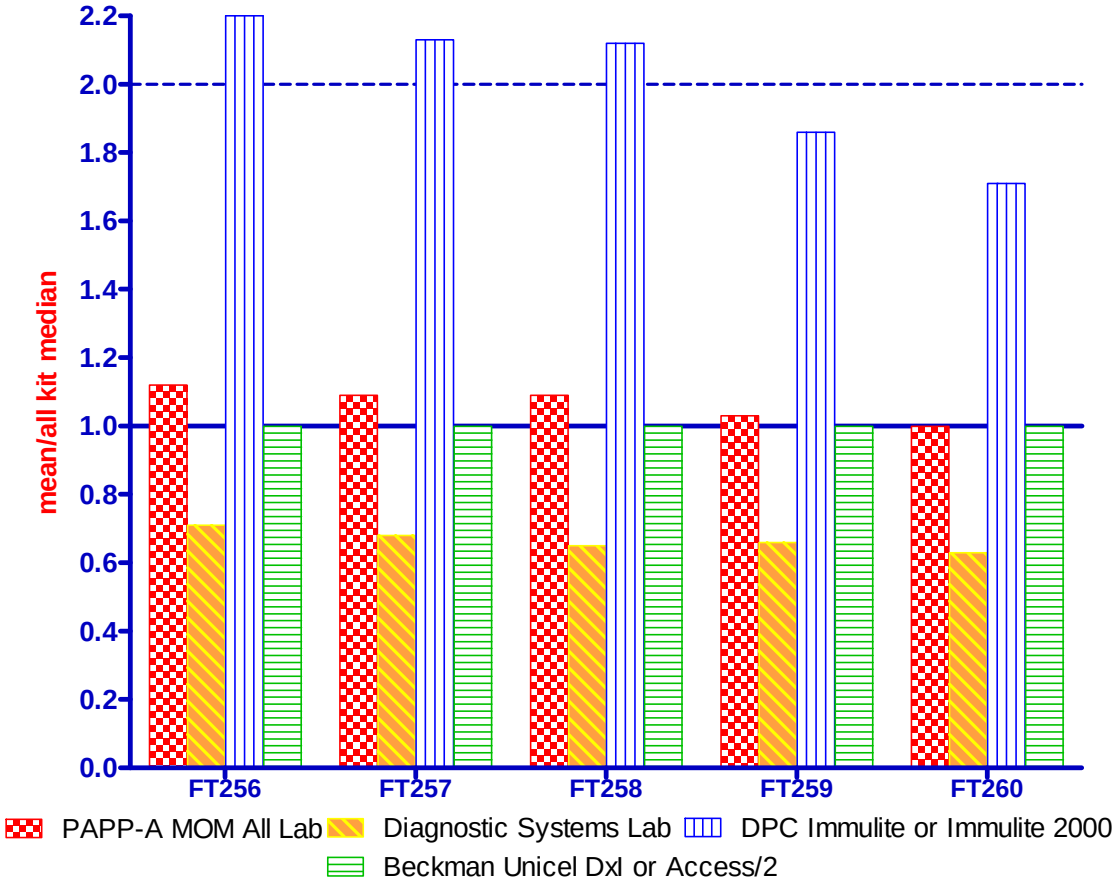


Figure 13

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

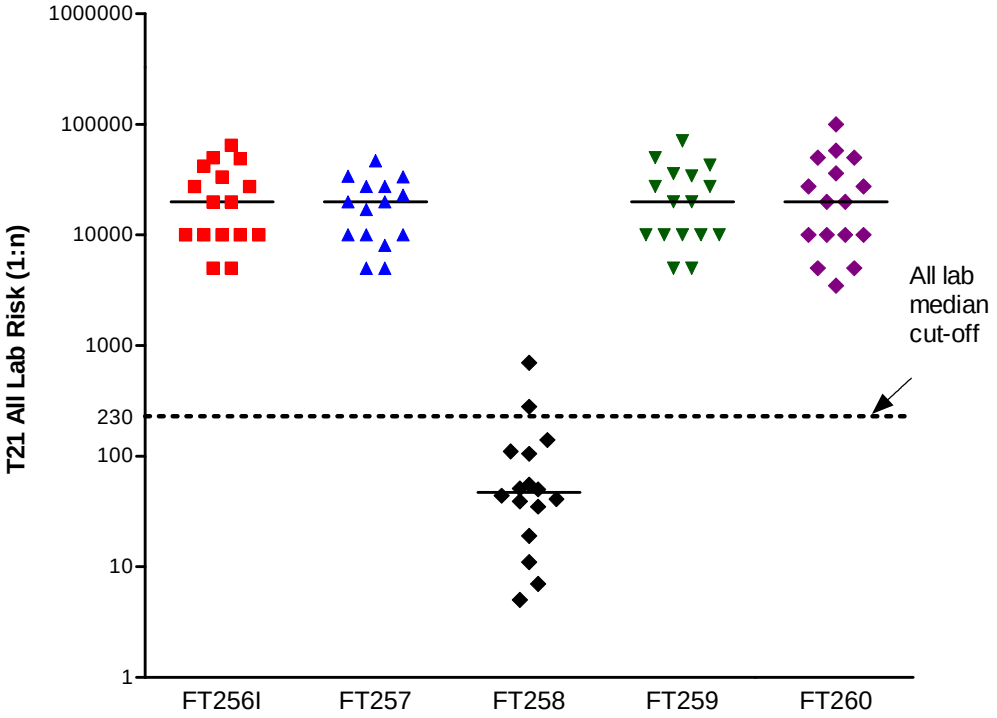
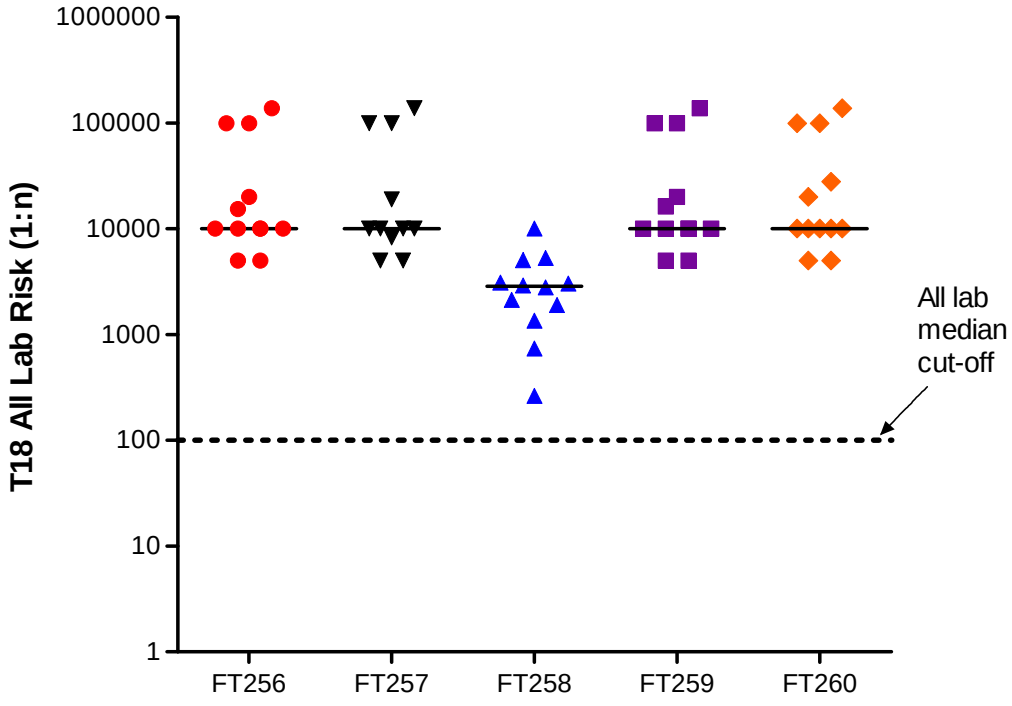


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates



New York State Fetal Defect Markers Proficiency Test,
September 2010
Summary of Results

	MS 256	MS 257	MS 258	MS 259	MS 260
Gestational Age All Lab Mean:					
Mean	17.0	20.0	15.0	18.0	19.0
SD	0.00	0.00	0.00	0.00	0.00
%CV	0.0%	0.0%	0.0%	0.0%	0.0%
X+3*SD	17.0	20.0	15.0	18.0	19.0
X-3*SD	17.0	20.0	15.0	18.0	19.0
N	30	30	30	30	30

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP All Lab Mean:					
mean	35.3	152.1	27.5	51.9	26.4
SD	2.6	12.0	2.3	4.7	2.2
%CV	7.4%	7.9%	8.3%	9.0%	8.2%
mean+3SD	43.2	188.2	34.4	65.9	33.0
mean-3SD	27.5	115.9	20.6	37.9	19.9
N	30	30	30	29	30
median	35.2	152.0	27.7	52.7	26.5
mean/all kit median	0.97	0.97	0.97	0.97	0.97

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	36.6	158.0	28.3	56.0	27.4
SD	2.0	10.0	1.9	3.2	1.5
%CV	5.3%	6.3%	6.7%	5.7%	5.6%
mean + 3SD	42.4	187.9	34.0	65.6	32.0
mean - 3SD	30.7	128.1	22.7	46.5	22.9
N	8	8	8	7	8
Median	36.4	157.1	27.8	56.6	26.8
mean/All kit median	1.01	1.01	1.00	1.04	1.00

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP Beckman Access (BCX/BC1) mean:					
mean	36.5	156.7	28.5	53.7	27.3
SD	2.0	9.3	2.0	3.7	1.8
%CV	5.4%	5.9%	6.9%	6.9%	6.4%
mean+3SD	42.4	184.6	34.5	64.8	32.6
mean-3SD	30.5	128.7	22.6	42.6	22.0
N	9	9	9	9	9
median	36.3	153.4	28.4	52.8	27.9
mean/all kit median	1.00	1.00	1.00	1.00	1.00

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP Siemens/Bayer ADVIA-Centaur(COB/BA1) mean:					
mean	36.2	156.2	30.4	53.6	27.6
N	2	2	2	2	2
mean/all kit median	1.00	1.00	1.07	1.00	1.01

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP DPC Immulite or 2000 (DPB or DPD/DP5) mean:					
mean	32.5	138.6	25.1	46.4	24.3
SD	1.5	5.4	1.1	1.4	2.2
%CV	4.7%	3.9%	4.3%	3.0%	8.9%
mean+3SD	37.1	154.8	28.4	50.6	30.8
mean-3SD	27.9	122.3	21.9	42.3	17.8
N	9	9	9	9	9
median	32.3	139.0	25.0	46.1	23.7
mean/all kit median	0.89	0.89	0.88	0.87	0.89

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP kit average:					
mean	35.4	152.3	28.1	52.4	26.7
SD	2.0	9.2	2.2	4.2	1.5
all kit median	36.3	156.4	28.4	53.7	27.4

	MS 256	MS 257	MS 258	MS 259	MS 260
MS AFP MoMs All Lab Mean:					
mean	0.92	2.72	0.81	1.15	0.53
SD	0.08	0.21	0.10	0.11	0.04
%CV	8.6%	7.7%	11.8%	9.3%	7.9%
mean+3SD	1.16	3.35	1.10	1.46	0.66
mean-3SD	0.68	2.09	0.52	0.83	0.40
N	30	30	30	29	30

New York State Fetal Defect Markers Proficiency Test,
September 2010
Summary of Results

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 All Lab Mean:					
mean	0.96	1.82	1.10	0.04	0.80
SD	0.30	0.74	0.38	0.03	0.24
%CV	30.9%	40.7%	34.0%	87.6%	30.7%
mean+3SD	1.85	4.05	2.23	0.13	1.53
mean-3SD	0.07	-0.40	-0.02	-0.06	0.06
N	29	29	29	17	29
mean/all kit median	1.11	1.15	1.12	1.90	1.15

MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	0.84	1.53	0.94	0.01	0.70
SD	0.06	0.10	0.05	0.01	0.06
%CV	6.9%	6.7%	5.8%	43.3%	8.0%
mean+3SD	1.01	1.84	1.10	0.03	0.86
mean-3SD	0.67	1.22	0.77	0.00	0.53
N	8	8	8	3	8
Median	0.82	1.52	0.94	0.01	0.68
mean/all kit median	0.91	0.93	0.91	0.28	0.94

MS uE3 BeckmanAccess (BCX/BC1) mean:					
mean	0.87	1.58	0.98	0.02	0.69
SD	0.06	0.12	0.09	0.01	0.05
%CV	7.5%	7.6%	8.7%	41.4%	7.6%
mean+3SD	1.06	1.94	1.24	0.04	0.85
mean-3SD	0.67	1.22	0.73	0.00	0.53
N	9	9	9	9	9
median	0.88	1.59	0.99	0.02	0.71
mean/all kit median	0.94	0.95	0.95	0.40	0.93

MS uE3 DPC Immulite 2000 or 2500(DPD or F/DP5) mean:					
Mean	1.06	2.20	1.28	0.08	0.94
SD	0.38	1.10	0.45	0.02	0.24
%CV	36.3%	50.0%	35.0%	27.2%	26.1%
mean+3SD	2.21	5.50	2.63	0.14	1.67
mean-3SD	-0.09	-1.10	-0.07	0.01	0.20
N	6	6	6	3	6
Median	0.93	1.75	1.13	0.07	0.89
mean/all kit median	1.14	1.33	1.24	1.60	1.26

MS uE3 New generation DPC Immulite 2000 or 2500(DPD or F/DP6) mean:					
Mean	0.98	1.74	1.08	0.09	0.79
SD	0.14	0.12	0.15	0.02	0.05
%CV	14.5%	6.8%	13.8%	25.0%	6.7%
mean+3SD	2.21	5.50	2.63	0.14	1.67
mean-3SD	-0.09	-1.10	-0.07	0.01	0.20
N	5	5	5	2	5
Median	0.98	1.69	1.04	0.09	0.76
mean/All Kit Median	1.06	1.05	1.05	1.78	1.06

MS UE3 kit average:					
mean	0.94	1.76	1.07	0.05	0.78
SD	0.10	0.30	0.15	0.04	0.11
all kit median	0.92	1.66	1.03	0.05	0.74

New York State Fetal Defect Markers Proficiency Test,
September 2010
Summary of Results

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 MoMs All Lab Mean:					
Mean	0.95	0.96	1.67	0.05	0.51
SD	0.22	0.21	0.42	0.04	0.13
%CV	23.5%	22.5%	25.1%	75.4%	25.1%
X+3SD	1.62	1.60	2.94	0.18	0.90
X-3SD	0.28	0.31	0.41	-0.07	0.13
N	29	29	28	20	28
mean/All Kit Median	0.97	0.98	0.94	0.85	0.96

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 MoM (DPD or F/DP5) Mean:					
Mean	1.18	1.22	2.23	0.09	0.71
SD	0.31	0.25	0.67	0.01	0.23
%CV	26.5%	20.2%	30.1%	15.7%	32.4%
X+3SD	2.12	1.95	4.24	0.13	1.39
X-3SD	0.24	0.48	0.22	0.05	0.02
N	6	6	6	5	6
mean/All Kit Median	1.20	1.25	1.25	1.41	1.32

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 MoMs (BCU/BC1) Mean:					
Mean	0.81	0.81	1.44	0.04	0.44
SD	0.03	0.07	0.11	0.05	0.02
%CV	3.8%	8.2%	7.8%	133.9%	4.8%
X+3SD	0.90	1.01	1.77	0.20	0.50
X-3SD	0.72	0.61	1.10	-0.12	0.38
N	8	8	8	4	8
mean/All Kit Median	0.82	0.83	0.80	0.63	0.83

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 MoM (DPD or F/DP6) Mean:					
Mean	1.11	1.11	2.13	0.09	0.62
SD	0.21	0.14	0.49	0.03	0.09
%CV	18.5%	12.9%	23.2%	28.6%	14.8%
X+3SD	1.73	1.54	3.62	0.16	0.90
X-3SD	0.49	0.68	0.65	0.01	0.34
N	5	5	5	4	5
mean/All Kit Median	1.13	1.14	1.19	1.37	1.16

	MS 256	MS 257	MS 258	MS 259	MS 260
MS uE3 MoMs (BCX/BC1) Mean:					
Mean	0.85	0.83	1.44	0.02	0.45
SD	0.07	0.08	0.16	0.01	0.04
%CV	8.2%	9.4%	10.8%	37.2%	9.3%
X+3SD	1.07	1.07	1.91	0.04	0.57
X-3SD	0.64	0.60	0.97	0.00	0.32
N	9	9	9	7	9
mean/All Kit Median	0.87	0.86	0.81	0.29	0.84

	MS 256	MS 257	MS 258	MS 259	MS 260
MS UE3 MoM kit average:					
mean	0.99	0.99	1.81	0.06	0.55
SD	0.18	0.20	0.43	0.04	0.13
all kit median	0.98	0.97	1.79	0.06	0.53

New York State Fetal Defect Markers Proficiency Test,
September 2010
Summary of Results

	MS 256	MS 257	MS 258	MS 259	MS 260
MS hCG All Lab Mean:					
mean	25.07	18.94	35.19	27.24	41.26
SD	2.14	1.53	3.34	2.95	4.68
%CV	8.5%	8.1%	9.5%	10.8%	11.3%
mean+3SD	31.5	23.5	45.2	36.1	55.3
mean-3SD	18.7	14.3	25.2	18.4	27.2
N	29	29	29	28	29
mean/all kit median	0.98	0.97	0.97	0.97	0.97

	MS 256	MS 257	MS 258	MS 259	MS 260
MS hCG Siemens/Bayer ADVIA-Centaur (COB/BA1) mean:					
mean	29.2	20.2	41.3	32.3	49.5
N	2	2	2	2	2
mean/all kit median	1.14	1.04	1.14	1.15	1.16

MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	25.61	19.66	36.40	27.99	41.65
SD	1.08	1.22	1.41	1.65	2.26
%CV	4.2%	6.2%	3.9%	5.9%	5.4%
mean+3SD	30.23	23.31	43.56	35.64	53.23
mean-3SD	21.32	15.29	28.17	20.45	34.08
N	8	8	8	7	8
median	25.95	19.55	36.85	27.80	42.10
mean/All kit median	1.00	1.01	1.01	1.00	0.98

MS hCG DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	22.8	17.6	31.9	24.6	36.5
SD	1.3	1.1	2.6	2.2	3.5
%CV	5.5%	6.5%	8.2%	8.8%	9.7%
mean+3SD	26.6	21.0	39.7	31.1	47.1
mean-3SD	19.0	14.2	24.1	18.1	25.8
N	9	9	9	9	9
median	23.1	17.8	32.6	25.4	35.1
mean/all kit median	0.89	0.90	0.88	0.88	0.86

MS hCG Beckman Access (BCX/BC1) mean:					
mean	25.8	19.3	35.9	28.0	43.7
SD	1.5	1.3	2.6	2.5	3.2
%CV	5.8%	6.9%	7.2%	9.0%	7.3%
mean+3SD	30.2	23.3	43.6	35.6	53.2
mean-3SD	21.3	15.3	28.2	20.4	34.1
N	9	9	9	9	9
median	25.8	19.4	35.8	27.6	42.3
mean/all kit median	1.00	0.99	0.99	1.00	1.02

MS hCG kit average:					
mean	25.9	19.2	36.4	28.2	42.8
SD	2.6	1.1	3.9	3.1	5.4
all kit median	25.7	19.5	36.1	28.0	42.7

	MS 256	MS 257	MS 258	MS 259	MS 260
MS hCG MoMs All Lab Mean:					
mean	1.04	1.15	0.84	1.30	2.28
SD	0.10	0.13	0.11	0.15	0.28
%CV	9.2%	11.7%	12.6%	11.4%	12.5%
mean+3SD	1.33	1.55	1.16	1.74	3.13
mean-3SD	0.75	0.74	0.52	0.85	1.43
N	28	29	29	28	29

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	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A all lab/DSL mean:					
Mean	143.17	219.72	131.03	175.10	387.91
SD	18.43	26.16	21.39	23.40	43.67
%CV	12.9%	11.9%	16.3%	13.4%	11.3%
mean + 3SD	198.5	298.2	195.2	245.3	518.9
mean- 3SD	87.9	141.2	66.9	104.9	256.9
N	29	29	29	28	29
All Lab Median	148.8	227.1	140.1	183.0	400.0
mean/all kit median	0.95	0.95	0.93	0.94	0.95

	MS 256	MS 257	MS 258	MS 259	MS 260
MS Inhibin A Beckman Access (BCX/BC1) mean:					
Mean	150.8	231.9	141.2	187.3	407.4
SD	9.3	16.4	8.9	8.2	19.2
%CV	6.2%	7.1%	6.3%	4.4%	4.7%
mean + 3SD	178.7	281.0	167.8	211.8	464.9
mean- 3SD	122.9	182.8	114.5	162.7	349.9
N	13	13	13	13	13
median	153.0	235.9	143.4	187.2	406.1
mean/All kit median	1.00	1.01	1.00	1.01	1.00

	MS 256	MS 257	MS 258	MS 259	MS 260
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	152.8	230.6	141.5	185.8	407.1
SD	13.9	13.8	8.1	10.5	29.2
%CV	9.1%	6.0%	5.7%	5.7%	7.2%
mean + 3SD	194.4	272.1	165.8	217.4	494.6
mean- 3SD	111.2	189.1	117.2	154.3	319.5
N	9	9	9	8	9
median	149.6	230.0	143.0	188.3	401.0
mean/all kit median	1.01	1.00	1.00	1.00	1.00

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A Diagnostic System Labs (DS1) Mean:					
Mean	116.6	183.1	98.8	140.2	327.0
SD	8.1	18.5	17.1	17.5	36.3
%CV	7.0%	10.1%	17.3%	12.5%	11.1%
mean + 3SD	141.0	238.5	150.0	192.9	435.9
mean- 3SD	92.2	127.8	47.6	87.6	218.0
N	7	7	7	7	7
median	119.0	181.4	102.1	149.4	331.5
mean/all kit median	0.77	0.79	0.70	0.75	0.80

	MS 256	MS 257	MS 258	MS 259	MS 260
MS Inhibin A kit average:					
mean	140.1	215.2	127.1	171.1	380.5
SD	20.4	27.8	24.5	26.8	46.4
all kit median	150.8	230.6	141.2	185.8	407.1

	MS 256	MS 257	MS 258	MS 259	MS 260
MS Inhibin A MoM All Lab Mean:					
mean	0.86	1.19	0.70	1.02	2.24
SD	0.14	0.21	0.16	0.18	0.36
%CV	16.8%	17.3%	22.4%	17.3%	15.9%
mean+3SD	1.29	1.81	1.17	1.55	3.31
mean-3SD	0.43	0.58	0.23	0.49	1.17
N	29	29	29	28	29

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	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP All Lab Mean :					
mean	6.2	24.1	11.5	9.9	6.2
SD	0.7	3.4	1.3	1.1	0.7
%CV	10.8%	14.2%	11.4%	10.7%	10.7%
mean+3SD	8.2	34.5	15.5	13.1	8.2
mean-3SD	4.2	13.8	7.6	6.7	4.2
N	24	24	24	24	24
mean/all kit median	0.94	0.98	0.97	0.97	0.94

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP Abbott Axsym (ABB/AB2) mear:					
mean	7.3	31.5	14.3	11.6	7.3
N	2	2	2	2	2
mean/all kit median	1.11	1.28	1.20	1.14	1.10

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	6.6	24.5	11.9	10.2	6.6
SD	0.3	3.0	0.8	0.7	0.2
%CV	4.8%	12.2%	7.0%	6.5%	3.4%
mean+3SD	7.6	33.5	14.4	12.2	7.3
mean-3SD	5.7	15.6	9.4	8.2	5.9
N	7	7	7	7	7
median	6.5	23.3	11.7	10	6.5
mean/all kit median	1.01	1.00	1.00	1.00	1.00

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP MoMs All Lab Mean:					
mean	1.09	3.77	0.98	1.03	0.80
SD	0.14	0.49	0.10	0.10	0.08
%CV	12.4%	13.0%	10.5%	9.8%	9.9%
mean+3SD	1.50	5.24	1.29	1.33	1.03
mean-3SD	0.69	2.31	0.67	0.73	0.56
N	24	24	24	24	24

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	5.5	21.4	10.3	8.7	5.5
SD	0.4	2.5	0.8	0.7	0.6
%CV	7.5%	11.8%	7.8%	8.3%	10.1%
X+3SD	7.2	27.1	14.0	11.6	7.2
X-3SD	4.5	18.7	8.3	7.7	5.0
N	6	6	6	6	6
median	5.3	20.7	10.2	8.9	5.6
mean/All kit median	0.83	0.87	0.87	0.86	0.82

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP Beckman Access (BCX/BC1) mean:					
mean	5.9	22.9	11.1	9.7	6.1
SD	0.4	1.4	1.0	0.7	0.4
%CV	7.6%	6.1%	8.6%	6.8%	6.2%
mean+3SD	7.2	27.1	14.0	11.6	7.2
mean-3SD	4.5	18.7	8.3	7.7	5.0
N	6	6	6	6	6
median	6.0	23.15	11.1	9.55	6.05
mean/all kit median	0.89	0.93	0.94	0.95	0.92

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP Siemens/Bayer ADVIA-Centaur(COB/BA1)mean:					
mean	6.6	26.4	12.2	11.2	6.7
N	2	2	2	2	2
mean/all kit median	1.00	1.07	1.02	1.09	1.01

	AF 256	AF 257	AF 258	AF 259	AF 260
AF AFP kit average:					
mean	6.4	25.3	12.0	10.3	6.4
SD	0.7	3.9	1.5	1.1	0.7
all kit median	6.6	24.5	11.9	10.2	6.6

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	FT256	FT257	FT258	FT259	FT260
FT Gestational Age All Lab Mean:					
Mean	11.0	10.9	11.9	11.4	13.0
SD	0.11	0.10	0.10	0.10	0.06
%CV	1.0%	0.9%	0.8%	0.9%	0.5%
X+3*SD	11.3	11.2	12.2	11.7	13.1
X-3*SD	10.6	10.6	11.6	11.1	12.8
N	17	17	17	17	17

	FT256	FT257	FT258	FT259	FT260
FT NT MoMs All Lab Mean:					
Mean	1.01	1.02	2.18	0.99	0.93
SD	0.10	0.10	0.22	0.10	0.08
%CV	10.3%	10.1%	10.0%	10.1%	9.1%
X+3SD	1.32	1.32	2.83	1.29	1.18
X- 3SD	0.70	0.71	1.52	0.69	0.67
N	16	15	16	16	16
All Median	0.99	1.01	2.12	0.98	0.90

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	FT256	FT257	FT258	FT259	FT260
FT hCG All Lab Mean:					
mean	50.41	53.86	141.06	53.98	44.16
SD	5.05	5.88	25.32	5.32	4.40
%CV	10.0%	10.9%	17.9%	9.9%	10.0%
X+3SD	65.6	71.5	217.0	69.9	57.4
X-3SD	35.3	36.2	65.1	38.0	31.0
N	16	16	16	16	16
mean/All kit median	1.03	1.03	1.06	1.02	1.02
FT hCG kit average:					
mean	48.9	52.0	133.1	53.1	43.3
SD	5.9	6.9	30.1	3.2	3.2
all kit median	48.9	52.0	133.1	53.1	43.3

	FT256	FT257	FT258	FT259	FT260
FT hCG MoMs All Lab Mean:					
Mean	0.59	0.65	1.91	0.65	0.65
SD	0.05	0.04	0.23	0.07	0.05
%CV	7.7%	5.9%	11.9%	11.5%	8.2%
X+3SD	0.73	0.77	2.59	0.88	0.81
X- 3SD	0.45	0.54	1.22	0.43	0.49
N	15	14	14	15	15
All Median	0.61	0.66	1.90	0.64	0.64

	FT256	FT257	FT258	FT259	FT260
FT hCG Beckman Unicel or Access (BCU or BCX/BC1) mean:					
mean	53.0	56.9	154.4	55.4	45.6
SD	3.4	3.0	16.2	5.0	4.3
%CV	6.3%	5.2%	10.5%	9.0%	9.4%
X+3SD	63.1	65.8	203.1	70.4	58.5
X-3SD	42.9	48.0	105.7	40.4	32.7
N	11	11	11	11	11
median	53.9	57.3	156.5	53.9	45.3
mean/All kit median	1.08	1.09	1.16	1.04	1.05
FT hCG DPC Immulite or 2000 or 2500(DPB or D or F/DP5) mean:					
mean	44.7	47.2	111.8	50.8	41.1
SD	2.9	5.1	13.5	5.1	3.0
%CV	6.6%	10.9%	12.1%	10.0%	7.3%
X+3SD	53.5	62.6	152.4	66.2	50.0
X-3SD	35.9	31.7	71.1	35.5	32.1
N	5	5	5	5	5
median	43.8	49.9	114.0	49.5	40.3
mean/All kit median	0.92	0.91	0.84	0.96	0.95

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	FT256	FT257	FT258	FT259	FT260
FT PAPP-A All Lab Mean: (does not include Beckman)					
Mean	3.88	6.43	1.68	7.67	9.26
SD	3.04	4.98	1.42	5.99	7.28
%CV	78.4%	77.4%	84.6%	78.2%	78.6%
mean + 3SD	13.00	21.37	5.95	25.65	31.09
mean- 3SD	-5.24	-8.51	-2.59	-10.32	-12.58
N	9	10	10	10	10
All Lab Median	2.30	4.11	1.00	4.77	5.74
mean/All kit median	0.76	0.73	0.73	0.73	0.74

	FT256	FT257	FT258	FT259	FT260
FT PAPP-A DPC Immullite or 2000 or 2500 (DPB or D or F/DP5) Mean:					
Mean	7.77	13.47	3.63	16.07	19.37
SD	1.33	1.40	0.89	2.51	3.66
%CV	17.1%	10.4%	24.6%	15.6%	18.9%
X + 3SD	11.76	17.68	6.31	23.60	30.35
X - 3SD	3.79	9.25	0.96	8.53	8.38
N	3	3	3	3	3
Kit Median	8.53	13.60	3.93	16.70	20.40
mean/All kit median	1.52	1.53	1.57	1.54	1.54

***Not included in all lab (unit in ng/ml)**

FT PAPP-A Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	1478.92	2880.00	704.36	3615.69	4629.08
SD	136.76	133.96	34.78	156.48	174.26
%CV	9.2%	4.7%	4.9%	4.3%	3.8%
X + 3SD	1889.21	3281.89	808.70	4085.12	5151.84
X - 3SD	1068.63	2478.11	600.02	3146.25	4106.31
N	9	8	9	8	8
Kit Median	1470.00	2888.10	700.00	3626.80	4669.70

	FT256	FT257	FT258	FT259	FT260
FT PAPP-A Diagnostic Systems Lab (DS1) Mean:					
Mean	2.45	4.14	1.00	4.85	5.78
SD	0.40	0.35	0.09	0.25	0.41
%CV	16.2%	8.4%	9.0%	5.1%	7.0%
X + 3SD	3.64	5.19	1.27	5.58	7.00
X - 3SD	1.26	3.10	0.73	4.11	4.56
N	3	4	4	4	4
Kit Median	2.30	4.11	1.00	4.77	5.74
mean/All kit median	0.48	0.47	0.43	0.46	0.46

FT PAPP-A kit average (does not include Beckman):					
mean	5.11	8.80	2.31	10.46	12.57
SD	0.66	0.75	0.57	1.60	2.30
all kit median	0.17	0.09	0.17	0.10	0.13

FT PAPP-A MoM All Lab Mean:					
Mean	3.32	6.43	1.11	5.58	3.79
SD	1.72	3.66	0.58	2.55	1.65
%CV	51.9%	56.9%	51.8%	45.6%	43.5%
mean + 3SD	8.49	17.40	2.84	13.22	8.73
mean- 3SD	-1.85	-4.54	-0.62	-2.06	-1.16
N	16	15	16	16	16
All Lab Median	2.60	5.34	0.99	4.96	3.70

October 2010

Teachings on Alpha-fetoprotein

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By: G. J. Mizejewski, Ph.D.

AFP and Proteomics:

The field of proteomics is defined as the study of the protein products of the genome, and of their interactions and functions. In a similar fashion, the proteins expressed at a given time in a given environment constitute a proteome (220). The protein under study, AFP, is just one protein out of from the entire proteome of cells that are undergoing development. Proteomics relies upon the determination of cellular function and regulation through large-scale (array) measurement of protein function and interaction (221, 222). Fortunately, studies on the detection, structure, function, and regulation of AFP have benefited from experiences of a 30-year headstart on the emerging field of proteomics. Although the in vivo functions of AFP still remain imprecisely understood, studies in the 1990s shed considerable light on the multiple physiological roles that AFP can play (11, 16, 18). In the realm of molecular structure research, cloning of the AFP gene and subsequent amino-acid sequencing constituted focal points from which a wealth of information that could then be probed from the gene bank. Concomitantly, investigations were reported in which the isoforms, epitopes, and conformational variants of AFP were being enumerated (17). Finally, regulation of the AFP gene has long served as an investigational tool of researchers who are seeking to uncover the mechanisms of gene suppression, or quenching (silencing) of protein expression in the juvenile-to-adult transition.

The study of proteomics presents researchers with a formidable challenge for a number of reasons. First, protein levels vary widely, according to both cell type and environment. For example, AFP in the fetal compartments is present in mg/ml concentrations, while maternal SAFP

concentrations differ by several log orders of magnitude, i.e, ng/ml. Second, unlike genomics, a field in which the researcher can amplify genes using the polymerase chain reaction (PCR), protein science has no comparable amplification method by which to aid the study of low-abundance proteins (223). In this respect, AFP has an advantage, namely, that it is naturally produced in high concentrations by the developing embryo/fetus. In addition, it can be synthesized in abundant quantities in recombinant systems such as E. coli, yeast, the insect bacculovirus system, and in transgenic rodents and livestock (i.e., goats). Third, proteomics is limited by the fact that the absolute quantity of protein that is synthesized is not a key feature because protein activities are tightly regulated post-translationally. Therefore, a protein can be abundant, yet possess minimal physiological activity (e.g., ALB). In contrast, HAFP synthesized in transgenic mouse models of systemic arthritis (224) and autoimmune myasthenia gravis (225) has been shown to have an ameliorative effect on these diseases, as does native AFP during human pregnancy. Finally, because proteins interact functionally in vivo, protein-protein and protein-small molecule interactions need to be evaluated in processes of biomedical interest [to researchers]. AFP has already been demonstrated to act synergistically with cytokines and peptidic hormones, and to interact with small molecules, such as estrogens, fatty acids, heavy metals, and drugs (11, 16).

From a technological standpoint, traditional proteomics involves separation of the proteins in a proteome, coupled to a means of identification. Until recently, the tools of choice were two-dimensional gel electrophoresis (2DGE) for separation, and mass spectroscopy for protein identification (226, 227). Both of these methods have been extensively used for the identification of AFP in saline tissue extracts, cell lysates, tissue brei, and biological fluids (i.e., sera, AF, urine). AFP extracted from cell culture media has been also been well utilized (228, 229). However, 2DGE often fails, for one of several reasons. First, 2DGE does not work well for the separation of membrane proteins,

which represent nearly 50% of the important molecular targets (230). Although the separation of membrane-bound AFP has been unsuccessful, the isolation of the cell-surface receptor for AFP has been achieved by isolation of cell membranes from human breast adenocarcinomas (95). Second, proteins of low abundance may be underrepresented in a 2DGE analysis, yet they often represent key players in sites of biological regulation. As ontogeny procedes, the gene for AFP is down-regulated, and partially masked, resulting in a serum concentration of AFP that is barely detectable at 5 ng/ml (231-233). Thus, the function and structure of the true AFP form in the adult will be extremely difficult to elucidate. Although 2DGE is powerful, researchers wishing to apply proteomics to the discovery of molecular targets must seek ways in which to measure both protein abundance and biological activity. AFP is one among a select group of proteins for which an activity-based growth bioassay (non-enzymatic) has been developed (165, 171, 234, 235). Therefore, emerging technologies employing proteins/peptides will require high-throughput automation and techniques linked to protein array methods, isotope encoding, two-hybrid systems, cyberspace information technology, and activity-based methods, in order to advance the utility of proteomics in biomedical research. In this fashion, the evolving proteomics of AFP will be gradually unveiled over the coming years. Due to the existence already of many years of study on its structure and function, AFP is a protein well suited to cross over the threshold, into the exciting field of proteomics.

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