Fetal Defect Marker Proficiency Test Mailout
May, 2010

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from May 11, 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. 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 = 28	**Sample #**	**MS 251**	**MS 252**	**MS 253**	**MS 254**	**MS 255**
	Gestational Age (weeks)	18	15	17	19	16
Maternal Race	Ethnic Group	White	Hispanic	White	Black	Asian
Maternal Weight	Pounds (lbs)	150	140	155	180	160
Maternal Age	Years	25	21	28	29	30
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std.Dev.	123.30 ± 10.11	26.00 ± 2.31	18.31 ± 1.70	49.31 ± 4.11	30.62 ± 2.31
	MOM ± Std.Dev.	2.79 ± 0.25	0.87 ± 0.08	0.49 ± 0.05	0.99 ± 0.09	0.96 ± 0.08
Unconjugated Estriol (uE3)	Mean ng/ml ± Std.Dev.	1.62 ± 0.84	2.46 ± 1.42	0.60 ± 0.25	1.88 ± 1.11	0.99 ± 0.47
	MOM ± Std.Dev.	0.86 ± 0.20	2.44 ± 0.56	0.41 ± 0.10	0.84 ± 0.08	0.84 ± 0.19
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std.Dev.	22.87 ± 1.91	32.28 ± 3.17	13.51 ± 0.98	21.43 ± 1.89	31.66 ± 3.01
	MOM ± Std.Dev.	1.10 ± 0.12	0.81 ± 0.10	0.57 ± 0.07	1.21 ± 0.18	1.11 ± 0.13
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std.Dev.	130.63 ± 17.32	125.93 ± 18.22	105.79 ± 16.06	184.40 ± 23.62	121.85 ± 16.46
	MOM ± Std.Dev.	0.78 ± 0.13	0.66 ± 0.11	0.65 ± 0.11	1.16 ± 0.18	0.72 ± 0.12
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Pos (+) (89%)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)
	Further Action G,U,A	G = 54 % U = 68% A = 54%	NFA	NFA	NFA	NFA
	NTD Risk 1 in	117	5,500	9,800	7,700	7,600
Trisomy-21 Screen (Positive, Negative) percent 1. Triple test	Pos. (+) or Neg. (-)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	5,200	5,950	2,250	1,590	1,400
2. Quad Test	Pos. (+) or Neg. (-)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100 %)	Neg (-) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	20,000	20,000	5,000	2,660	5,500
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg (-) (100%)	Neg (-) (100%)	Neg (-)(B) (59%)	Neg (-) (100%)	Neg (-) (100%)
	Recommended Action**	NFA	NFA	G = 33% U = 30% A = 33%	NFA	NFA
	Risk Est. 1 in	10,000	20,000	125	20,000	18,000

*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 = 28 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 251 Wk 18.0	This specimen was obtained from a 25 year old White woman (Gravida = 3, Parity = 2) in her 18th week gestation with a body weight of 150 lbs. She had a family history of pregnancy complications. Her specimen, a second pregnancy sample, was a positive screen for NTD (89% consensus; MOM = 2.79). 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, 54%, ultrasound, 68% and amniocentesis, 54%. The MS251 specimen had an amniotic fluid paired sample which was also elevated (MOM = 2.21). The all-lab median risk for NTD of MS251 was 1 in 117.
MS 252 Wk 15.0	This specimen was obtained from a 21 year old Hispanic woman (Gravida = 1, Parity = 0) in her 15th week gestation with a body weight of 140 lbs. She had no personal history of pregnancy loss. Her specimen, a second pregnancy sample, was negative for NTD (100% consensus); no body weight correction was indicated. Her screen was also negative for both Trisomies with all labs in agreement. However, 80% of the labs reported an elevated uE3 for this specimen (MOM = 2.44). No recommendations of further action were submitted for the trisomy screen of the MS252 sample. This specimen had no amniotic fluid counterpart.
MS 253 Wk 17.0	This specimen was obtained from a 28 year old White woman (Gravida = 2, parity = 1) in her 17th week gestation with a body weight of 155 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened positive for NTD. However, her aneuploidy screen was negative for Trisomy-21 (T21, 100%) and borderline positive for Trisomy-18 (T18, 41%). Her quad biomarker values were all extremely low. Recommendations of further action from labs reporting the positive T18 screen were: genetic counseling, 33%; ultrasound, 30%; and amniocentesis, 33%. This sample was paired to an amniotic fluid specimen which also had a low AFAFP level (MOM = 0.54).
MS 254 Wk 19.0	This specimen was procured from a 29 year old, Black woman (Gravida = 3, parity = 2) in her 19th week gestation with a body weight of 180 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 body weight correction indicated. The labs were also in agreement that both trisomy screens were negative. Specimen MS254 was not paired with an amniotic fluid sample.
MS 255 Wk 16.0	This specimen was obtained from a 30 year old Asian woman (Gravida = 2, parity = 1) in her 16th week gestation with a body weight of 160 lbs. She had no family history of pregnancy complications or adverse outcomes. Her NTD and her aneuploidy screen were negative for both T21 and for T18. A race correction was indicated. No recommendation of further action for the NTD and T21 screen was reported from the participating labs. This specimen was not paired with an amniotic fluid specimen.

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 251 Wk 18.0	AFP= 21.31 ± 2.60 µg/ml MOM= 2.21 ± 0.18	The AF251 sample was targeted for a screen positive AFAFP value in the routine gestational age range. Seventy-nine percent of the labs reported this specimen as a screen positive AFAFP value. The AF251 specimen was paired with maternal serum sample MS251 (MOM = 2.79), which was also elevated.
AF 252 Wk 16.0	AFP= 9.20 ± 1.11 µg/ml MOM= 0.64 ± 0.06	The AF252 sample was targeted for a normal AFAFP value in the lower gestational age range. All labs called AF252 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 253 Wk 17.0	AFP= 6.30 ± 0.92 µg/ml MOM= 0.54 ± 0.05	The AF253 sample was targeted as an NTD negative screen in the routine gestational age screening range. All labs categorized AF253 as a negative NTD screen specimen. This specimen had a maternal serum counterpart, MS253 (MOM = 0.49), which showed low levels of AFP.
AF 254 Wk 20.0	AFP= 4.21 ± 0.50 µg/ml MOM= 0.65 ± 0.07	The AF254 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 non-elevated specimen. This sample was not linked to a maternal serum specimen.
AF 255 Wk 19.0	AFP= 4.60 ± 0.60 µg/ml MOM= 0.58 ± 0.08	The AF255 sample was targeted for a non-elevated AFAFP value in the routine gestational age range. Most labs called AF255 a normal MOM AFAFP specimen. This AFAFP sample was not matched to a maternal serum specimen.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 16	**Sample #**	**FT 251**	**FT 252**	**FT 253**	**FT 254**	**FT 255**
	Gestational Age (weeks)	11.2	12.4	11.9	11.4	13.0
Maternal Race	Ethnic Group	White	Asian	Hispanic	White	Hispanic
Maternal Weight	Pounds (lbs)	150	140	155	145	155
Maternal Age	Years	20	28	22	25	23
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	45	60	52	47	68
	NT Thickness (mm)	1.1	1.4	1.2	2.5	1.6
	NT – MOM	0.98 ± 0.09	0.97 ± 0.10	0.93 ± 0.10	2.11 ± 0.22	0.99 ± 0.09
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	84.03 ± 13.10	65.42 ± 8.49	74.27 ± 12.19	172.25 ± 33.37	49.01 ± 6.99
	MOM ± Std. Dev.	1.10 ± 0.14	0.96 ± 0.10	1.06 ± 0.15	2.25 ± 0.42	0.80 ± 0.10
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean mIU/mL ± Std. Dev.	3.63 ± 2.09	4.49 ± 2.33	4.30 ± 2.62	2.03 ± 1.16	5.19 ± 2.78
	MOM ± Std. Dev.	3.22 ± 1.34	2.44 ± 0.75	3.08 ± 1.32	1.58 ± 0.68	2.61 ± 0.81
Trisomy-21 Screen (Positive/Negative) percent	Pos (+) or Neg. (-)	Neg (-) (100%)	Neg (-) (100%)	Neg (-) (100%)	Pos (+) (64%)	Neg (-) (100%)
	Recommended Action NFA or G = % C = % U = % A = %	NFA	NFA	NFA	G = 64% U = 43% A = 36% C = 29%	NFA
	Risk Estimate 1 in	14,500	14,000	15,000	195	15,000
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	10,000	5,845	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

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

N = 16 all-lab Consensus Values.

Sample#	Summary Comments:
FT 251 Wk 11.2	This specimen was obtained from a 20 year old Caucasian woman of average body weight (150 lbs.). Her gestational age at time of screening was 11.2 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 FT251 risk estimate for T21 was 1 in 14,500, while the all-lab T18 risk was also 1 in 10,000 (negative screen). All labs were in agreement that FT251 was a negative screen for both T21 and T18.
FT 252 Wk 12.4	This specimen was obtained from a 28 year old Asian woman of medium body weight (140 lbs). Her gestational age at time of screening was 12.4 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 FT252 risk estimate for T21 was 1 in 14,000 while the T18 risk was also 1 in 10,000.
FT 253 Wk 11.9	This specimen was obtained from a 22 year old Hispanic woman of average body weight (155 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 negative and all testing Labs were in agreement. The FT253 risk estimate for T21 was 1 in 15,000, while the all-lab T18 risk was also1 in 10,000 (negative screen). All labs were in agreement that FT253 was a negative screen for T21 and T18.
FT 254 Wk 11.4	This specimen was procured from a 25 year old Caucasian woman of average body weight (145 lbs.). Her gestational age at time of screening was 11.4 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen positive for T21 and two-thirds of the testing Labs (64%) were in agreement (see Critique). The FT254 risk estimate for T21 was 1 in 195, while the T18 risk was 1 in 5,845.
FT 255 Wk 13.0	This specimen was procured from a 23 year old Hispanic woman with a body weight of 155 lbs. Her gestational age at time of screening was 13.1 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for T21 and T18. The risk estimate for FT255 was 1 in 10,000, and the T18 risk was 1 in 10,000. All labs were in agreement with both aneuploid screen assessments.

III. Critique and Commentary:

A) 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 were consistent with the goals of our projected target values, risks, and outcomes. Overall, MS251 resulted in a positive screen for NTD; MS252 demonstrated an elevated uE3 level; and MS 253 was borderline positive for T18. Samples MS252, MS254, and MS255 produced negative screens for NTD, T21 and T18. The results for samples 252 and 253 are further discussed below.

Specimen **MS252** produced an interesting case in that unconjugated estriol (uE3) was notably elevated (MOM = 2.44). In contrast, all other quad biomarkers were normal or low (AFP MOM = 0.87; hCG MOM = 0.81; inhibin MOM = 0.66). In cases of Down syndrome (T21) and Edward's syndrome (T18), uE3 levels are significantly lowered, often times to MOMs of 50% or less of normal. Although uE3 contributes a role as a member of the triple and quad platforms, elevated uE3 is rarely encountered by the second trimester screening laboratory. Even though maternal serum levels of uE3 normally increase throughout pregnancy, they acutely rise between 35 and 37 weeks. This increase is due to an acceleration of fetal adrenal steroidogenesis in the third trimester of pregnancy (27). These increases in uE3 have been found to correlate predictably with gestational age and are useful for term pregnancy dating, prediction of labor complications, and signaling the onset of labor.

Elevated uE3 in the pregnancy clinic is not as common as low or absent levels and its usefulness appears to be more limited. Serum uE3 levels have been correlated with urinary levels such that the more convenient and rapid commercial serum RIAs and ELISA assay platforms may be employed (27). Also, the levels of uE3 have been found to correlate with the levels of estetrol (E4), a natural human steroid estrogen produced exclusively by the fetal liver during pregnancy. Thus, E4 is the fetal steroid equivalent of E3 and reaches the maternal blood circulation via the placenta, peaking at term after a continual rise during pregnancy (30). In the fetal placental unit, prolactin interacts with hCG in the control of estrogen (estriol) and progesterone secretions. In pregnant women who had been treated with bromocriptine prior to and during early pregnancy for pituitary adenomas, prolactin, estrodiol, and progesterone levels were found to be subnormal, while uE3 levels were elevated (29). These bromocriptine studies showed that the drug affected reproductive functions long after withdrawal, especially prolactin activity.

In further case histories, elevated uE3 has been detected in cases of Klinefelter's syndrome in adults that later developed systemic lupus erythematosus (SLE) (26). Thus, adult males with SLE were found to exhibit Klinefelter's disorder

traits manifesting with small genitalia, gynecomastia, lack of secondary sexual characteristics, and abnormalities of estrogen metabolism including elevated uE3. The authors proposed that chronic estrogenic stimulation may be significant for the future development of SLE in adults. In further case histories, elevated estriol was noted in women who had experienced a spontaneous abortion following second trimester amniocentesis (31). The elevated uE3 was accompanied by increased prolactin and decreased hCG levels in both maternal serum and amniotic fluid. It was suggested that these hormonal differences may relate to the fetal adrenal stress response in the subsequent abortion, which developed into a situation aggravated by the amniocentesis procedure. Finally, a different case-control study followed by a retrospective cohort study revealed that significantly higher pregnancy uE3 levels were found in mothers of infants who eventually needed orchiopexy as compared to infants that did not need this surgical procedure (32). Thus, high maternal uE3 levels in pregnancy can serve as a predictor of a potentially necessary surgical intervention for undescended testis.

An unexplained elevated uE3 in the second trimester should receive normal antenatal care, as it is not commonly associated with adverse antenatal outcomes (33). Furthermore, levels of uE3 in early second trimester are not significantly altered in preeclampsia, but show slightly higher levels in the mothers of normal female as compared to normal male fetuses in the second trimester (27). Furthermore, alterations in maternal serum uE3 levels have also been associated with oligohydramnios, fetal triploidy, and vacuolization of the trophoblast cells (28).

Specimen **MS253** is of special interest in that the sample was a borderline positive T18 screen with 41% of labs reporting an elevated risk. This patient had a prior history of pregnancy complications; thus, a paired amniotic fluid sample had been obtained for analysis at time of specimen collection. Low levels of AFP and uE3 by themselves are not indicative of a Down syndrome; however, the combination of the three analytes in the triple test resulted in a borderline risk for T18 in this particular situation. Subsequent Stage-II ultrasound and amniocentesis karyotyping were found to confirm a T18 condition in this patient. Labs that found an elevated risk recommended further actions as follows: G=33%, U=30%, and A=33%. It is well-known that all three low biomarker levels have been associated with T18 risk when using the triple test platform.

T18 is known also as Edward's syndrome. The T18 condition is a lethal disorder for those fetuses that survive to a live birth. There is a preponderance of females approaching a 4:1 ratio at birth, with females surviving to about 1000 days, and males to 100 days. Statistically, 30% of the liveborns die within one month, 60% die within two months, and only 10% live longer than one year. However, the T18 survivors display profound physical and mental disabilities, poor somatic growth problems, skeletal dysmorphisms, major infection problems, and multiple visceral malformations.

The anatomical malformations of T18 can include the following: craniofacial dysmorphisms of the mouth (small narrow palate), jaws (micrognathia), and skull (occipital protuberance). The neck (short flaccid skin), sternum (short reduced ossification), abdomen (defective herniated muscles), and a narrow pelvis are also included in these structural anomalies. In addition, the limb abnormalities can involve extended hands, clinched fists, hypoplastic fingernails, malformed legs, rocker bottom feet, and digital syndactyly. Concerning the genitalia, cryptorchidism is prevalent in the males and genital hypotrophy in the females. Renal malformations can be common displaying ectopic or horseshoe kidney, hydronephosis, megalo-ureter, and double ureter. Less common malformations can include: harelip or cleft palate, atresia of the choana, trachea-esophageal fistula, bifid uterus, ovarian hypoplasia, phocomelia, lobster-claw deformity, and atresia of the external auditory canal. Thus, the anatomical malformations are many and varied and hardly compatible with a normal lifestyle.

In general, the frequency of T18 is 1 in 8,000 livebirths, born to mothers averaging 33 years of age with a paternal age of 35 years of age. The mean birthweight is 2,240 grams which is paradoxically low since postmaturity (42 weeks) is observed in most births. Growth retardation is severe, accompanied by mental retardation, low fetal activity, oligohydraminos, small placenta, and hypoplasia of the skeletal muscles. Infant nutrition is poor due to a weak suckling response, and premature death is common to this disorder. In general, the prognosis for newborns afflicted with T18 is dismal at best.

B) <u>Second Trimester Assay Kit Performance:</u>

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 Fig. 7, AFP mass measurements among the individual kits largely agreed, although Siemens/Bayer ADVIA-Centaur was slightly higher, and DPC Immulite was 10 to 20% lower than the median for some samples. For uE3, the mean/all kit median for Beckman UNICEL and Access-2 was around 1.0 (Fig. 8); however, labs employing DPC Immulite 2000 or Immulite 2500 yielded values that were 2.2 to 2.7 times higher than the median (see dotted line). It is noteworthy that the results from the "New Generation" DPC uE3 Immulite 2000 or 2500 kits were near the median value, in contrast to the results from the older DPC Immulite 2000 or 2500 kits (Fig. 8A). Interestingly, however, the New Generation DPC uE3 kit yielded MOM values that were about 1.2 times above the median, while the older DPC Immulite kits showed MOM values 5 to 20% lower than the medians (Fig. 8B). Regarding the hCG kits (Fig. 9), the Siemens/Bayer ADVIA-Centaur /ACS-180 results were slightly above the median, the Beckman Access-2 and UNICEL DXL yielded mean hCG values around the median, and the DPC Immulite 2500 or Immulite 2000 kits demonstrated slightly lower values (Fig. 9). 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/2 or Unicel versus the Diagnostic Systems Lab (DSL) assay platforms. Results from the Beckman kits were 15 to 20% higher than those from DSL (Fig. 10). Labs lacking peer group companions and in-house assays were deemed non-gradable (NG) for individual analyte groups as the situation dictated.

The bar graph in Figure 11 is provided to display kit performances among the amniotic fluid (AF-AFP) test samples. Overall, Siemens/Bayer ADVIA-Centaur/ACS-180 and Abbott AS XYM were 10% higher, while Beckman Unicel and Beckman Access/2 were about 15% - 25% lower than the median. DPC Immulite results remained constant around the median. Please be

advised that these specimens are derived from actual AF samples, and therefore these results are directly relevant to patient screening**.**

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 24% of the labs; Robert Maciel (RMA) software was employed by 31%, while in-house software comprised 14%, and 7% of labs used programs classified as "other", which are proprietary software packages.

C) First Trimester Screen:

Five first trimester maternal serum mock samples have been provided and will be included in all future mailouts in order to survey and assess New York State licensed laboratories concerning participation and assay capabilities in first trimester Down syndrome screening. 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), and draw date. Crown-rump length (CRL) measurements, race, and maternal body weight have also been included in the case histories to better evaluate all-lab participant NT information requirements.

As demonstrated in the FT table 2 (Section – II) above, the all lab measurement of the 11.2 week Caucasian **FT251** specimen for total hCG resulted in a mass mean of 84.03 ± 13.10, with a non-elevated MOM = 1.10. Furthermore, the all-lab mass mean for PAPP-A was 3.63 ± 2.09 mIU/ml with a MOM = 3.22. The all-lab T21 risk assessment was 1 in 14,000 for the FT251 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT251 sample was screen negative (see Fig. 14). The risk cut-off level for Caucasians ranges from 200 to 270 among the participating labs. Thus, the FT251 sample resulted in a 100% negative T21 screen assessment. No further action was indicated. Finally, the FT251 specimen also screened negative for T18 (1 in 10,000) using a cutoff of 1 in 100.

As shown in the FT table 2 (Section-II) for the **FT252** Asian specimen, the gestational age all-lab mean was reported as 12.4 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of 65.42 ± 8.49 IU/ml, while the all-lab PAPP-A mass assessment was 4.49 ± 2.33 mIU/ml. The first trimester all-lab T21 screen consensus for FT252 was negative. The all-lab FT T21 risk assessment was 1 in 10,000. The all lab measurement of total hCG for sample FT252 achieved a MOM value of 0.96; in comparison, the all-lab MOM for PAPP-A was 4.49. All labs agreed that the FT252 sample was screen negative for T21 (Fig. 14). The FT252 specimen also resulted in a negative screen for T18 with a risk assessment of 1 in 10,000.

In the **FT253** Hispanic sample, the gestational age all-lab mean was reported as 11.9 weeks. Assay measurements for FT253 resulted in an all-lab total hCG mass measurement of 74.27 ± 12.19, while the all-lab PAPP-A mass assessment was 4.30 ± 2.62 (Figs. 12 and 13). The first trimester all-lab T21 consensus for FT253 was screen negative, with a risk of 1 in 10,000. The all-lab measurement for FT253 for total hCG resulted in a MOM value of 1.06 and the all-lab MOM mean for PAPP-A was 3.08. All labs agreed that the FT253 sample was screen negative for T21 (Fig. 14). The all-lab T18 risk assessment for FT253 was 1 in 10,000, hence, the FT253 specimen resulted in a negative screen for T18.

The all lab measurement of the 11.4 week Caucasian **FT254** specimen for total hCG resulted in a mass mean of 172.25 IU/ml ± 33.37, with an elevated MOM of 2.25. Furthermore, the all-lab mass mean for PAPP-A was 2.03 ± 1.16 mIU/ml with a MOM of 1.58 ± 0.68. The all-lab T21 risk assessment was 1 in 195 for the FT254 specimen. Since the first trimester Down syndrome screen risk is associated with raised NT, low PAPP-A, and high hCG MOMs, the FT254 the results (Fig. 14B) were indeed consistent with a T21 positive screen. Even though the PAPP-A was only 1.58 MOM, the elevated NT and hCG MOMs were sufficient to produce a positive screen. Thus, the FT254 sample resulted in 64% of the labs reporting it as a T21 positive screen assessment. Further actions submitted by the labs included G = 64%; U = 43%; and A/CVS = 36/29%. Finally, the FT254 specimen screened negative for T18 (1 in 297) using a cutoff of 1 in 100 with a calculated risk of 1 in 10,000.

For the Hispanic **FT255** specimen, the gestational age all-lab mean was reported as 13.0 weeks. Assay measurements from participating laboratories resulted in an all-lab total hCG mass measurement of 49.01 ± 6.99 IU/ml, while the all-lab PAPP-A mass assessment was 5.19 ± 2.78 mIU/ml. The first trimester all-lab T21 screen consensus for the FT255 specimen was negative (100%). The all-lab FT T21 risk assessment was 1 in 10,000. The all lab measurement of total hCG for FT255 produced a MOM value of 0.80; in comparison, the all-lab MOM for PAPP-A was 2.61. All labs agreed that the FT255 sample was screen negative for T21 (Fig. 14). The FT255 specimen also resulted in a negative screen for T18 with an all-lab risk assessment of 1 in 10,000.

D) 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. 12, 13) for each of the five FT samples. As shown in Fig. 12, hCG measurement between the two kits differed somewhat, with Beckman Unicel/Access kit measuring 10-15% above the kit median and DPC being about (10-15%) lower. 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 DPC Immulite kits. When the PAPP-A kit MOM's were compared, DPC Immulite was 1.6 to 2.0 times higher than DSL and Beckman (Fig. 13B).

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 27%, Robert Maciel (RMA) software was employed by 30%, while in-house software comprised 16% of the labs. None of the labs used First Trimester screening programs classified as "other" (proprietary software packages).

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New and Related References (suggested reading):

1. Yin ZF, Wang CH (2003) [Research advances on alpha-fetoprotein physiological function and clinical potential]. Ai Zheng 22:108-111.
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Abstracts

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

(1) Title: Prenatal testing for Down syndrome: comparison of screening practices in the UK and USA

Source: J Genet Couns 2009, 19:112-130.

Authors: Tapon, D.

Abstract: Prenatal testing for Down Syndrome is a topic covered in every genetic counselor's training as it constitutes the main workload of genetic counselors in prenatal settings. Most Western countries nowadays offer some type of testing for Down Syndrome. However, practices vary according to country with regards to what tests are offered, insurance coverage and the legal situation concerning the option of terminating an affected pregnancy. In view of the growing interest in international genetic counseling issues, this article aims to compare prenatal testing practices in two English-speaking countries: the United Kingdom and the United States of America. A case will be presented to highlight some of the differences in practice. The topic underlines important implications for genetic counseling practice, such as patients' understanding of testing practices, risk perception, counseling provision and impact of prenatal testing results.

(2) Title: The ability of the quadruple test to predict adverse perinatal outcomes in a high-risk obstetric population

Source: J Med Screen 2009, 16:55-59.

Authors: Lao MR, Calhoun BC, Bracero LA, Wang Y, Seybold DJ, Broce M, Hatjis CG

Abstract: OBJECTIVE: To determine the ability of the quadruple Down's syndrome screening test (quad screen) to predict other adverse perinatal outcomes (APO) in a high-risk obstetric population. SETTING: A tertiary medical centre in West Virginia. METHODS: We retrospectively reviewed 342 obstetric patients with quad screen data from a single clinic. The quad screen included maternal serum levels of alphafetoprotein (AFP), human chorionic gonadotrophin (hCG), uncongjugated oestriol (uE(3)), and inhibin A. The risk of APO was compared between patients with at least one abnormal marker versus no abnormal markers and >or=2 abnormal markers versus <2 abnormal markers. Abnormal markers were determined by cut-off values produced by Receiver Operator Characteristic (ROC) curves and the FASTER trial. Unadjusted and adjusted effects were estimated using logistic regression analysis. RESULTS: The risk of having an APO increased significantly for

patients with abnormal markers by about three-fold using ROC and two-fold using FASTER trial thresholds. CONCLUSIONS: The quad screen shows value in predicting risk of APO in high-risk patients.

(3) Title: A survey of the knowledge and attitudes of pregnant Thai women towards Down syndrome screening

Source: J Obstet Gynaecol Res. 2009, 35:876-881

Authors: Pruksanusak N, Suwanrath C, Kor-Anantakul O, Prasartwanakit V, Leetanaporn R, Suntharasaj T, Hanprasertpong T

Abstract: AIM: To determine the knowledge and attitudes of pregnant Thai women towards Down syndrome screening. METHODS: A total of 714 pregnant women were recruited attending antenatal clinics in Songklanagarind Hospital from February through June 2007. Their knowledge and attitudes concerning Down syndrome screening were evaluated through self-administered questionnaires. The data were analyzed using SPSS version 12.0. RESULTS: The mean age of the respondents was 29.9 +/- 6.4 years. Regarding their knowledge of Down syndrome and its screening test, the mean scores were 43.6% and 20.6%, respectively. Most pregnant women (77.6%) had a positive attitude to Down syndrome screening. In addition, 92.2% of cases would accept a Down syndrome screening test. Multivariate logistic regression analysis showed that levels of education and types of health insurance were factors associated with knowledge of Down syndrome screening. Maternal age was the only factor affecting attitudes. CONCLUSION: Most pregnant women had inadequate knowledge of Down syndrome and the screening test. However, they did have a positive attitude and were willing to accept the test. Providing knowledge on Down syndrome and the screening test for pregnant women should be implemented in our antenatal care services and community.

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

(1) Title: A case of intraocular yolk sac tumor in a child and its pathogenesis

Source: J Aapos. 2009, 13:613-615

Authors: Fujino T, Okamura A, Hatsukawa Y, Nakayama K, Inoue M, Nakayama M

Abstract: While yolk sac tumor is one of the most common malignant germ cell tumors occurring in young children, it is rarely found in extragonadal sites. We report a case of intraocular yolk sac tumor in a 4-year-old boy. The diagnosis was confirmed by histologic examination and by the rapid normalization of serum alpha-fetoprotein level following enucleation. We propose that yolk sac cells can potentially migrate into the eye at 22 days of embryonic life during neural tube formation, when the head and tail of the neuropore open contemporaneously and communicate with the amniotic cavity.

(2) Title: Transient Abnormal Myelopoiesis Associated with Down Syndrome Presenting as Severe Hydrops Fetalis: A Case Report.

Source: Fetal Diagn Ther 2010, 2010:16

Authors: Malin GL, Kilby MD, Velangi M

Abstract: We present a case of transient abnormal myelopoiesis (TAM) presenting as non-immune fetal hydrops (NIHF). Hydrops fetalis (HF) is a condition associated with very high perinatal mortality, especially when no treatable cause, such as fetal anaemia, exists. In fetuses prior to 24 weeks with NIHF, a chromosomal anomaly is a common association. TAM is a leukaemic condition, almost entirely limited to children with Down syndrome. The presentation of TAM prenatally is unusual but cases may present ultrasonographically with NIHF and associated fetal hepatosplenomegaly. We report a case presenting in this manner with NIHF detected at 29 weeks' gestation and discuss the subsequent diagnosis and management of in utero TAM.

(3) Title: A prenatally sonographically diagnosed conotruncal anomaly with mosaic type trisomy 21 and 22q11.2 microdeletion/DiGeorge syndrome.

Source: Genet Couns 2009, 20:373-377.

Authors: Balci S, Altugan FS, Alehan D, Aypar E, Baltaci V

Abstract: A prenatally sonographically diagnosed conotruncal anomaly with mosaic type trisomy 21 and 22q11.2 microdeletion/DiGeorge syndrome: We report a prenatally sonographically diagnosed conotruncal and urogenital anomaly. Postnatally, the patient presented with seizures, hypocalcemia, hypoparathyroidism and thymic aplasia and diagnosed as DiGeorge syndrome. Echocardiography showed malalignment VSD, supravalvular pulmonary stenosis and overriding aorta. Chromosome and FISH studies showed the association of mosaic type trisomy 21 and 22q11.2 microdeletion. The present patient is the second case of mosaic type of Down syndrome associated with 22q11.2 microdeletion. In addition the patient also had clinical and laboratory features of DiGeorge syndrome.

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

(1) Title: Combining biochemical and ultrasonographic markers in predicting preeclampsia: a systematic review

Source: Clin Chem 2009, 56:361-375

Authors: Giguere Y, Charland M, Bujold E, Bernard N, Grenier S, Rousseau F, Lafond J, Legare F, Forest JC

Abstract: BACKGROUND: Early identification of pregnant women at risk for preeclampsia is a priority to implement preventive measures. Some biochemical and ultrasonographic parameters have shown promising predictive performance, but so far there is no clinically validated screening procedure. CONTENT: Using a series of keywords, we reviewed electronic databases (Medline, Embase, all records to May 2009) reporting the performance of biological and ultrasonographic markers to predict preeclampsia, both single markers and combinations of markers. We analyzed the data according to gestational age and risk levels of the studied populations. We evaluated the methodological quality of included publications using QUADAS (quality assessment of diagnostic accuracy studies). We identified 37 relevant studies that assessed 71 different combinations of biochemical and ultrasonographic markers. Most studies were performed during the second trimester on small-scale high-risk populations with few cases of preeclampsia. Combinations of markers generally led to an increase in sensitivity and/or specificity compared with single markers. In low-risk populations, combinations including placental protein 13 (PP13), pregnancy-associated plasma protein A (PAPP-A), a disintegrin and metalloprotease-12 (ADAM12), activin A, or inhibin A measured in first or early second trimester and uterine artery Doppler in second trimester appear promising (sensitivity 60%-80%, specificity >80%). In high-risk populations, the combination of PP13 and pulsatility index in first trimester showed 90% sensitivity and 90% specificity in a single study limited to severe preeclampsia. SUMMARY: Combinations of biochemical and ultrasonographic markers improved the performance of early prediction of preeclampsia. From a perspective of integrative medicine, large population-based studies evaluating algorithms combining multiple markers are needed, if screening approaches are to be eventually implemented.

(2) Title: Distributions of current and new first-trimester Down syndrome screening markers in twin pregnancies

Source: Prenat Diagn 2010, 2010:18

Authors: Koster MP, Wortelboer EJ, Stoutenbeek P, Visser GH, Schielen PC

Abstract: OBJECTIVES: To study the distributions of pregnancy-associated plasma protein A (PAPP-A), the free beta subunit of human chorion gonadotrophin (fbeta-hCG), A Disintegrin and Metalloprotease 12 (ADAM12) and Placental Protein 13 (PP13) in first trimester twin pregnancies. METHODS: Serum marker concentrations were measured in monochorionic and dichorionic twin pregnancies and singleton controls to study differences in multiples of the gestation-specific normal medians (MoMs). RESULTS: Median PAPP-A and fbeta-hCG MoMs were 2.03 and 1.87 for monochorionic twins (n = 116) and 2.18 and 1.89 for dichorionic twins (n = 650). Furthermore, ADAM12 and PP13 MoMs were 1.66 and 1.56 for monochorionic twins (n = 51) and 1.64 and 1.53 for dichorionic twins (n = 249). No statistically significant differences between monochorionic and dichorionic twin pregnancies were found. Correlations between markers in these pregnancies did not differ from singletons. CONCLUSION: For first-trimester screening, different parameters for monochorionic and dichorionic twin pregnancies is not necessary. Furthermore, if ADAM12 and PP13 will be adopted as screening

markers, the presented median MoM values, standard deviations and correlation coefficients for twin pregnancies may contribute to a proper twin risk estimation. Copyright (c) 2010 John Wiley & Sons, Ltd.

(3) Title: [Noninvasive prenatal test in the first trimester of pregnancy (NT and estimation of beta-hCG and PAPP-A) in the diagnosis of fetal abnormalities in Polish population--comparison of the biochemistry own normal ranges and literature reported data]

Source: Ginekol Pol. 2009, 80:851-855

Authors: Mandryka-Stankewycz S, Perenc M, Dec G, Sieroszewski P

Abstract: THE AIM OF STUDY: Estimation of Polish population standards of the concentrations of pregnancy-associated plasma protein--A (PAPP-A) and free beta--human chorionic gonadotropin (beta-HCG) in the maternal blood between 10.0 and 13.6 week of pregnancy and comparison of the biochemistry own normal ranges and literature reported data. Estimation the sensitivity of the fetal nuchal translucency measurement, biochemical concentrations of PAPP-A and free beta-HCG in detection of the fetal chromosomal abnormalities. MATERIAL AND METHODS: 582 women in the age 14 to 46 years old with singleton pregnancies were included to the study The screening was performed between 10.0 and 13.6 week of gestation. The fetal nuchal translucency serum concentrations of PAPP-A and free beta-HCG were measured. The specific risk was calculated using the Fetal Medicine Foundation software (FTS) by accredited sonographers. RESULTS: Standards for serum concentrations of PAPP-A and free beta-HCG in normal pregnancies were determined. The measurement sensitivity of the fetal nuchal translucency in detection of the fetal chromosomal abnormalities was 80% and sensitivity of serum concentrations of PAPP-A and free beta-HCG was 40% and 80%. CONCLUSIONS: There is no significant differences between estimated biochemistry standards (PAPP-A and free beta-HCG) for Polish population and literature reported data. Observed differences in measurements of fetal NT, serum concentrations of PAPP-A and free beta-HCG in a control group and the group with the aneuploidies confirmed usefulness of these methods for the first trimester prenatal screening.

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

(1) Title: Multiplex tumor marker detection with new chemiluminescent immunoassay based on silica colloidal crystal beads

Source: Analyst. 2010, 135(1):177-181

Authors: Pei XP, Chen BA, Li L, Gao F, Jiang Z

Abstract: A new multiplex chemiluminescent immunoassay (CLIA) based on silica colloidal crystal beads (SCCBs) was developed for tumor marker detection. As the code is the characteristic reflection peak originating from the stop-band of colloid crystal, they avoid photobleaching, the potential interference of encoding fluorescence with analyte-detection fluorescence and chemical instability. Meanwhile our SCCBs suspension array improved the luminescence analysis efficiency by using chemiluminescent detection of enzyme labels. By forming a sandwich immunocomplex on SCCBs, the proposed suspension array was used for simultaneous multiplex detection of tumor markers in one test tube. The results showed that the linear range was 0.5-100ng ml(-1) and 1.0-120ng ml(-1) for carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) with a detection limit of 0.12ng ml(-1) and 0.16ng ml(-1) at 3 sigma. The proposed array showed the storage stability and the accuracy for sample detection were acceptable, and the results were in acceptable agreement with the reference electrochemiluminescence method. This technique provided an automated, simple, sensitive and low-cost approach for multianalyte immunoassay.

(2) Title: A sensitive electrochemical immunosensor for alpha-Fetoprotein detection with colloidal gold-based dentritical enzyme complex amplification

Source: Electroanalysis. 2010, 22(2):244-250

Authors: Liu XP, Wu HW, Zheng Y, Wu ZS, Jiang JH, Shen GL, Yu RQ

Abstract: A sensitive and specific electrochemical immunosensor was developed with alpha-fetoprotein (AFP) as the model analyte by using gold nanoparticle label for enzymatic catalytic amplification. A self-assembled monolayer membrane of mercaptopropionic acid (MPA) was firstly formed on the electrode surface through

gold-sulfur interaction. Monoclonal mouse anti-human AFP was covalently immobilized to serve as the Capture antibody. In the presence of the target human AFP. gold nanoparticles coated with polyclonal rabbit anti-human AFP were bound to the electrode via the formation of a sandwiched complex. With the introduction of goat anti-rabbit IgG conjugated with alkaline phosphatase, the dentritical enzyme complex was formed through selective interaction of the secondary antibodies with the colloidal gold-based primary antibody at the electrode. thus affording the possibility of signal amplification for AFP detection. Current response arising front the oxidation of enzymatic product was significantly amplified by the dentritical enzyme complex. The current signal vas proportional to the concentration of AFP from 1.0 ng mL(-1) to 500 ng mL(-1) with a detection limit of 0.8 ng mL(-1). This system could be extended to detect other target molecules with the corresponding antibody pairs.

(3) Title: Quantitative, label-free detection of five protein biomarkers using multiplexed arrays of silicon photonic microring resonators

Source: Anal 2010, 82:69-72

Authors: Washburn AL, Luchansky MS, Bowman AL, Bailey RC

Abstract: Because of the inherent complexity of biochemical pathways commonly altered in disease states, it has become accepted that multiplexed analyses can provide a more informative biomolecular understanding of disease onset and progression. Importantly, compared to conventional single-parameter assays, the detailed biomolecular insight gleaned from multiparameter measurements has the potential to greatly improve disease diagnostics, prognostics, and theragnostics. We have previously reported the utility of silicon photonic microring resonators for the sensitive quantitation of a single disease biomarker and herein demonstrate the first example of optical microcavity resonator arrays performing quantitative, label-free, multiplexed analyses of clinically relevant protein biomarkers. In this report, the concentrations of prostate specific antigen (PSA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), tumor necrosis factor-alpha (TNF-alpha), and interleukin-8 (IL-8) are simultaneously determined in three unknown protein cocktail solutions. This letter demonstrates that multiple immunoassays can be performed concurrently on a microresonator platform without any accompanying loss of sensitivity or measurement precision, and therefore, this report lays the groundwork for future applications involving multiplexed analysis of clinically relevant samples.

E). Special Abstract Selection:

(1) Title: Lack of Association between Unexplained Elevated Maternal Serum Alpha Fetoprotein and/or Human Chorionic Gonadotropin and the Occurrence of Placental Thrombotic Lesions..

Source: Placenta 2010, 2010:2

Authors: Salim R, Okopnik M, Garmi G, Nachum Z, Zafran N, Shalev E

Abstract: OBJECTIVE: To investigate the significance of unexplained elevated maternal serum alpha fetoprotein (MSAFP) and/or human chorionic gonadotropin (HCG) on the occurrence of placental thrombotic changes. STUDY DESIGN: Between January 2007 to April 2009, placentas of all women who delivered and had unexplained elevated MSAFP and/or HCG (above 2 MOM) were sent to histological examination. Women were divided into 2 groups. Group A included women who had uneventful pregnancies and delivered at term. Group B included women with antepartum complications attributed to thrombosis. Women in both groups (A and B) had elevated MSAFP and/or HCG. Group C was a frequency matched group of women who had normal MSAFP and HCG levels with uneventful pregnancies and delivered at term. MAIN OUTCOME MEASURE: Incidence of placental thrombotic lesions in each group. RESULTS: Of 9695 women who delivered during the study period there were 76 women with elevated MSAFP and or HCG, 48 in group A and 28 in Group B. Group C, included 30 women. The number of placentas in which any thrombotic lesion was identified was 22 (45.8%), 19 (67.9%) and 10 (33%) respectively. Changes differed significantly only between group B and C ($p = 0.03$). Although the rate of changes in group A was higher than in group C it did not reach statistical significance even when considering only women with two abnormal results (MSAFP and HCG) or when a cutoff of 2.5 MOM or more was set. CONCLUSION: Placental histopathological changes are associated with pregnancy complications and can only marginally be attributed to unexplained elevated MSAFP and/or HCG.

(2) Title: Estimating the effect of gestational age on test performance of combined first-trimester screening for Down syndrome: a preliminary study

Source: J Perinat Med 2010, 2010:2

Authors: van Heesch PN, Struijk PC, Laudy JA, Steegers EA, Wildschut HI

Abstract: Abstract Objective: To establish how different methods of estimating gestational age (GA) affect reliability of first-trimester screening for Down syndrome. Methods: Retrospective single-center study of 100 women with a viable singleton pregnancy, who had first-trimester screening. We calculated multiples of the median (MoM) for maternal-serum free beta human chorionic gonadotropin (free beta-hCG) and pregnancy associated plasma protein-A (PAPP-A), derived from either last menstrual period (LMP) or ultrasound-dating scans. Results: In women with a regular cycle, LMP-derived estimates of GA were two days longer (range -11 to 18), than crown-rump length (CRL)-derived estimates of GA whereas this discrepancy was more pronounced in women who reported to have an irregular cycle, i.e., six days (range -7 to 32). Except for PAPP-A in the regular-cycle group, all differences were significant. Consequently, risk estimates are affected by the mode of estimating GA. In fact, LMP-based estimates revealed ten "screen-positive" cases compared to five "screen-positive" cases where GA was derived from dating-scans. Conclusion: Provided fixed values for nuchal translucency are applied, dating-scans reduce the number of screen-positive findings on the basis of biochemical screening. We recommend implementation of guidelines for Down syndrome screening based on CRL-dependent rather than LMP-dependent parameters of GA.

(3) Title: Reference centile chart for fetal nuchal translucency, maternal serum PAPP-A and free beta hCG..

Source: J Med Assoc Thai 2010, 93:154-160

Authors: Chawanpaiboon S, Cheunwattana P

Abstract: OBJECTIVE: To create reference centile chart of fetal nuchal translucency maternal serum pregnancy associated plasma protein-A (PAPP-A) and maternal serum free beta human chorionic gonadotropin (beta-hCG) in order to predict preliminarily Down syndrome in Thai fetuses during 10-14 weeks of gestation. MATERIAL AND METHOD: This was a prospective, descriptive cohort study. From 1 January 2004 to 31 December 2006, a total of 1,000 pregnant women during 10-14 weeks of gestation were participated in the present study. Pregnancy outcomes were reviewed from the records. The excluded cases were chromosomal and major structural abnormalities, twin pregnancy and cases resulting in miscarriage or intrauterine death. All women had a scan for nuchal translucency (NT) and had blood taken for measurement of maternal serum PAPP-A and free beta-hCG level. RESULTS: The mean NT was 1.6 +/- 0.8 mm (range 0.3-14 mm). The 5th, 50th and 95th centile of PAPP-A and free beta-hCG during 11-14 weeks of gestation were 1.54-69, 14-28, 51-57 and 24.8-17, 78-47, 181.6-126.5 mIU/mL, respectively. The distribution and the 5% and 95%, lower and upper limits of NT, PAPP-A and free beta-hCG was presented. CONCLUSION: The present study shows that NT measurements increase with increasing gestational age. The mean serum PAPP-A rises and the mean serum-free beta hCG decreases from 10 to 14 weeks of gestation in normal Thai fetuses. These results can be used for reference value to predict fetal Down Syndrome.

(4) Title: Occult inflammation and/or ischemia may be responsible for the false positivity of biochemical Down syndrome screening test

Source: J Perinat Med 2010, 2010:18

Authors: Guven S, Karahan SC, Kandemir O, Ucar U, Cora AO, Bozkaya H

Abstract: Abstract Objective: To determine the possible underlying cause of a false-positive first or second trimester biochemical Down syndrome screening test result by means of second trimester amniotic fluid cytokine level analysis. Methods: A total of 74 consecutive patients undergoing amniocentesis for karyotype analysis at 16-20 weeks' gestation were included in this prospective age-matched case-control study. The study group (n=38) had abnormal first or second trimester screening test results and normal karyotype results, while controls (n=36) included those admitted for genetic amniocentesis for other reasons who had normal first or second trimester screening test and normal karyotype results. Four markers [interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-alpha, and ischemia-modified albumin (IMA)] were studied in amniotic fluid. Results: The mean age of the women in the study and control groups was 34.0+/-5.6 and 33.6+/-7.2 years, respectively. The women in the study and control groups had similar clinical and laboratory characteristics. The mean amniotic fluid IL-6 (414.84+/-83.96 vs. 343.02+/-110.59, p=0.002) and IL-8 (377.61+/-243.31 vs. 261.90+/-201.29, p=0.029), TNF-alpha (24.91+/-5.78 vs. 21.60+/-5.55, p=0.014), and IMA (1.19+/- 0.10 vs. 1.05+/-0.12, p<0.001) values were significantly increased in the study group when compared to controls. Conclusion: The higher amniotic

fluid cytokine and ischemia-modified albumin levels in patients with false-positive first or second trimester biochemical Down syndrome screening test may result from subclinical fetal membrane inflammation and/or ischemia.

(5) Title: Using population-based data to predict the impact of introducing noninvasive prenatal diagnosis for Down syndrome

Source: Genet Med 2010, 2010:5

Authors: Susman MR, Amor DJ, Muggli E, Jaques AM, Halliday J

Abstract: PURPOSE:: To compare the number and types of chromosome abnormalities prenatally diagnosed and the number of invasive procedures between current prenatal testing pathways and a pathway where noninvasive prenatal diagnosis for Down syndrome replaces Down syndrome screening tests. METHODS:: Numbers and types of chromosome abnormalities for each referral category were extracted from prenatal diagnostic testing reports routinely collected in Victoria, Australia, in 2006 and 2007. These data were then applied to the proposed implementation strategy. RESULTS:: If noninvasive prenatal diagnosis for Down syndrome had replaced Down syndrome screening tests in 2006 and 2007, in Victoria, there would have been 25 (7%) additional Down syndrome diagnosed, 6896 (84%) fewer invasive procedures, and 231 (56%) non-Down syndrome chromosome abnormalities no longer detected. These include trisomy 13, trisomy 18, sex chromosome abnormalities, balanced and unbalanced rearrangements, polyploidy, and mosaic results. CONCLUSIONS:: The potential loss of information about chromosome abnormalities other than Down syndrome with noninvasive prenatal diagnosis compared with full karyotyping with traditional prenatal diagnosis should be considered when planning for the implementation of new technologies.

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

June 2010

Teachings on Alpha-fetoprotein

Vol. 4, Part 9

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

Acquired Immunodeficiency Disorders (AIDS):

Although there may be many explanations for the lack of fetal infection in HIV-positive mothers in the first and second trimesters (200-206), a possible link to HAFP can be examined. In fact, a recent study has documented that the relative absence of HIV-I fetal transmission during pregnancy is associated with elevated MSAFP levels (205). The pioneering studies of Uriel and his associates in 1987-1989 demonstrated specific uptake of HAFP by receptors on human T-lymphocyte blast cells during antigen-induced transformation and in malignant lymphoid cells (207, 208). In the course of these detailed studies, Uriel's group reported an impairment in the ability of AIDs (HIV) patient's peripheral blood mononuclear cells to internalize AFP (209, 210). Their AFP endocytosis assay clearly revealed a defective uptake of AFP in AIDS, and in lymphoadenoapathy syndrome, and in mitogen-responsive T-cells of asymtomatic patients. They predicted that the reduced capacity to bind and internalize AFP in early-stage symptomatic free HIV/AIDS patients may have potential for a prognostic test. These investigators further reported good concordance of the defective AFP uptake with an impaired expression of IL-2 receptors on the lymphoid cells (211). Finally, these investigators noted that the conversion of HIV-negative to HIV-positive patients displayed a progressive deterioration in their AFP uptake capability.

Uriel's group then attempted to determine whether the AFP-uptake impairment was due to an expression of inhibited AFP receptor or to a target signal transduction defect. They studied AFP endocytosis in peripheral mononuclear cells (PMC) from disease-free HIV-positive patients

as well as HIV-transfected PMCs (*in vitro*) from healthy donors in phytohemaglutinin stimulated and non-stimulated instances. Their results showed that defective AFP endocytosis was a consequence of an abnormal mitogenic response of PMCs associated with the presence of HIV virus. Also, a lowered level of IL-2 receptor was found in this study (210). Thus, their results reflected both the status of T-cell activation associated with HIV infection and the PMCs' responsiveness to mitogenic stimulation. The study by Uriel and coworkers in 1994 demonstrated a considerable loss of membrane fluidity of the PMCs, as evidenced by elevated values of the cholesterol/phospholipid (CH/PL) ratio in cell membranes from AIDS patients (211). Relative to normal cells, the expression of AFP and IL-2 receptors also appeared considerably reduced in AIDS-related complex disorders and in AIDS patients. Thus, the HIV infection disrupted the fluidity of the cell membrane and altered the normal sequelae of lymphocyte antigen-activation and blast cell transformation.

Later, in 1997, another group of French investigators reported that AFP interacted with the HIV type 1 gp 120/160 viral coat proteins (212); thus, AFP inhibited the infection of primary monocyte-derived macrophages by certain HIV-1 viral strains. Serving as an inhibitor, AFP acted at the cell-surface CD4-independent stages of virus binding to the macrophages. AFP was found to inhibit the binding of HIV specifically at the V3 loop clade consensus peptide, and it interfered with viral post-binding events during HIV-1 infection of primary macrophages. Furthermore, a carbohydrate chain-related inhibition of HIV infection was found, which depended on the cell type (macrophage) and differences in the glycan structure of the specific cofactor receptors involved in HIV entry into cells (213). Subsequently, it was shown that HAFP specifically interacts at the primary macrophage cell surface and competes with the gp 120 V3C binding of HIV-1 to these cells (214). Antibodies to the CCR-5 chemokine receptor

inhibited AFP binding to these macrophages. Native AFP (not heat-denatured) specifically interacted with electroblotted V3C-bound ligand, the CCR5 cell surface receptor. Thus, these authors' data indicated that the AFP inhibitory effect during HIV infection was related to an AFP-virus interaction concurrent with HIV infection that was related to AFP binding to the CCR5 family of macrophage chemokine receptors. This observation may provide one possible explanation for the lack of vertical transmission of HIV-1 infection observed in the first and second trimesters of pregnancy (205).

Further data from the same French group revealed that HAFP was found to bind to CCR5 receptors at both high and low-affinity binding sites (5.15 and 100 nM KA, respectively), localized on monocyte-derived macrophages (215). The CCR5 chemokine receptor is known to cluster with the CD4 receptor and serve as a co-receptor for HIV intake and transfection (216-218). Both protein-to-protein interaction and lectin carbohydrate involvement were established as parts of the binding process; these experiments utilized treatments such as heat denaturation and neuraminidase exposure of AFP. As discussed above, HAFP was found to displace binding of the clade-B HIV-1 gp 120 VC3 loop to the CCR5 receptor on the macrophage; conversely, CCR5 ligands were also able to displace AFP from in its binding to the macrophage cell surface (219). Finally, it was shown that HAFP could bind to the CCR5 receptor expressed on HeLa cells, although not on HeLa cells lacking the CCR5 receptor. These data presented strong evidence that AFP binds directly to the CCR5 chemokine co-receptor associated with CD4 receptors expressed on primary macrophages (monocyte-derived) and on transfected CCR5 HeLa cells.

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Maternal serum α-fetoprotein and human chorionic gonadotropin levels in women with human immunodeficiency virus

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To the Editors: We have read with great interest the article by Gross et al[1] on maternal serum alpha-fetoprotein (MSAFP) and human chorionic gonadotropin (hCG) levels in pregnant women with human immunodeficiency virus (HIV). In their discussion, the authors proposed 2 hypotheses to explain their findings of elevated MSAFP with increased viral load and decreased CD4 counts with hCG. Having discounted chronic placental damage as the first possible cause of elevated MSAFP levels, the authors favored maternal AFP immunoregulatory activity during the course of the HIV disease. During the last 20 years, the immunoregulatory role of human AFP, in contrast to that of rodent AFP, has remained highly controversial and largely unconfirmed, with results difficult to duplicate among laboratories. Indeed, the references cited to support their contention of an AFP immunoregulatory role employed largely rodent cells, which were used as in vitro systems in studies performed during the 1970s. However, more recent research has addressed the physiologic and biochemical aspects of AFP interaction with monocyte/macrophage membrane receptor complexes. Studies by Uriel et al[2] have shown that monocytes/macrophages from patients with acquired immunodeficiency syndrome (AIDS)-related disorders display defective AFP-cell surface signaling pathways, loss of membrane fluidity, altered endocytotic trafficking, and a reduction in cell-surface cytokine receptors. More recently, human (H) AFP was found to bind to CCR5 receptors at both high- and low-affinity binding sites localized on human monocyte-derived macrophages in vitro.[3] The CCR5 chemokine receptor (M-trophic) serves as a coreceptor to the CD4 major receptor for HIV intake and transfection. In this latter report, HAFP was reported to displace the dade-B HIV gp 120-VC3 loop from its binding to the CCR5 receptor on the macrophage cell surface; conversely, CCR5 ligands were found to displace HAFP from its binding to the macrophage cell surface receptor. Therefore, we propose that HAFP binding to the macrophage/monocyte at the placental interface might enhance the fetal-to-maternal gradient of AFP placental transport, thus increasing

AFP levels in the maternal circulation. It is tempting to speculate that the positive correlation observed between increasing viral load and elevation of MSAFP is related to AFP sterochemical interference with viral fusion to the CD4+ cell surface receptor and its coreceptors, thus impairing viral transmission and down-regulating the CD4 counts. In this regard, it should be noted that all HIV-infected mothers in the study had normal pregnancy outcomes in that none of the screened infants, followed-up for 1 year, developed HIV. Therefore, AFP intervention may provide at least 1 explanation for the lower incidence of HIV vertical transmission from mother to fetus in the second trimester.[4]

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Maternal serum α-fetoprotein and human chorionic gonadotropin levels in women with human immunodeficiency virus

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Abstract

Objective: The purpose of this study was to establish whether there is a correlation between maternal serum genetic screen analyte results in pregnant women with human immunodeficiency virus and corresponding human immunodeficiency virus index values.

Study Design: Medical records of all pregnant women with human immunodeficiency virus who were delivered at Bronx Lebanon Hospital Center from January 2000 through December 2001 were reviewed for maternal serum screen results, viral load, CD4 counts and percent, antiretroviral therapy, opportunistic infections, substance abuse, and other demographic data. Statistical analysis was accomplished with the χ^2 test, Mann-Whitney *U* test, and Spearman rank correlation test, with a probability value of <.05 considered significant.

Results: Of the 98 women with human immunodeficiency virus who were delivered, 49 women (50%) had a maternal serum genetic screen available. Screened and unscreened women had similar severity of human immunodeficiency virus disease, CD4 count and percentage, and viral loads. Serum screen results showed elevations in maternal serum human chorionic gonadotropin (1.43 ± 1.04 multiples of the median [MoM]; range, 0.2-5.2 MoM) and maternal serum α-fetoprotein (1.29 ± 0.9 MoM; range, 0.5-3.3 MoM) compared with expected values in the general obstetric population. Maternal serum human chorionic gonadotropin was correlated inversely with CD4 count (*P*= .002) and CD4 percent (*P*< .0001). Maternal serum α-fetoprotein varied directly with viral load (*P*< .0001).

Conclusion: Increasing maternal serum human chorionic gonadotropin and maternal serum α-fetoprotein levels in patients with human immunodeficiency virus are correlated with increasing viral load and decreasing CD4 counts. (Am J Obstet Gynecol 2003;188:1052-6.)

Key words: Maternal serum α-fetoprotein, maternal serum human chorionic gonadotropin, maternal serum screening, human immunodeficiency virus, acquired immunodeficiency syndrome

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Figure 1

Maternal Sera AFP MoM

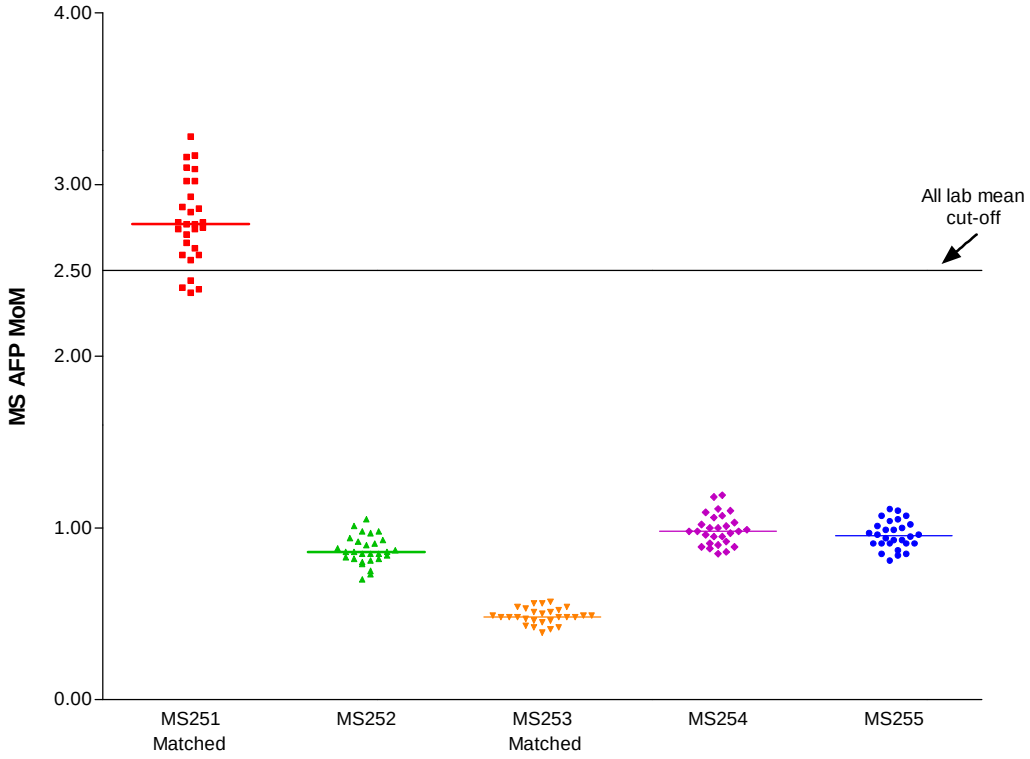


Figure 2

Amniotic Fluid AFP MoM

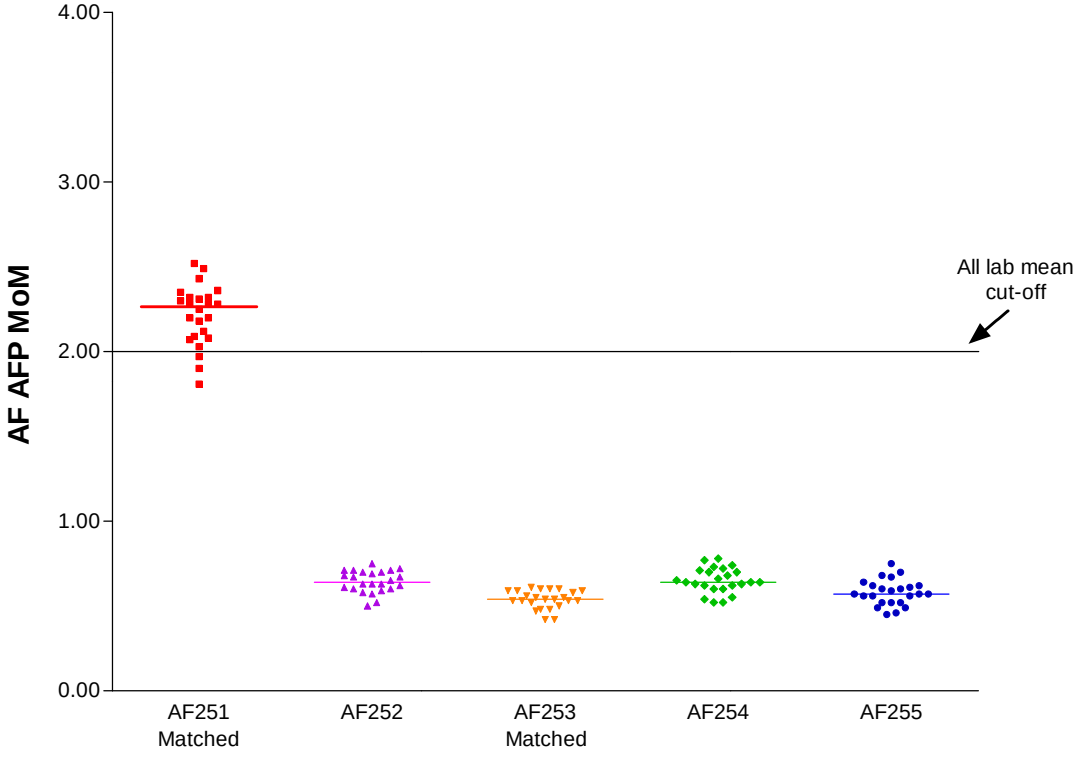


Figure 3

Graphic Distribution of Second Trimester
Neural Tube Defect Risk Estimates

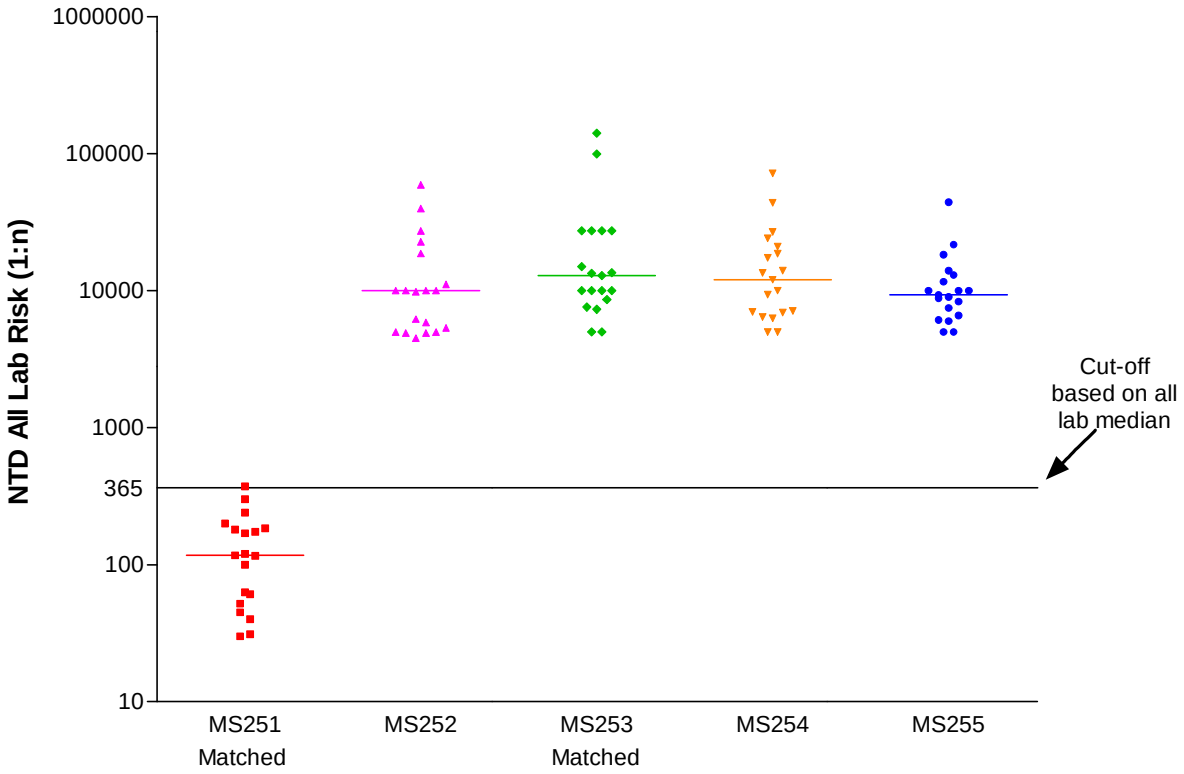


Figure 4

Graphic Distribution of Second Trimester
Trisomy 18 Risk Estimates

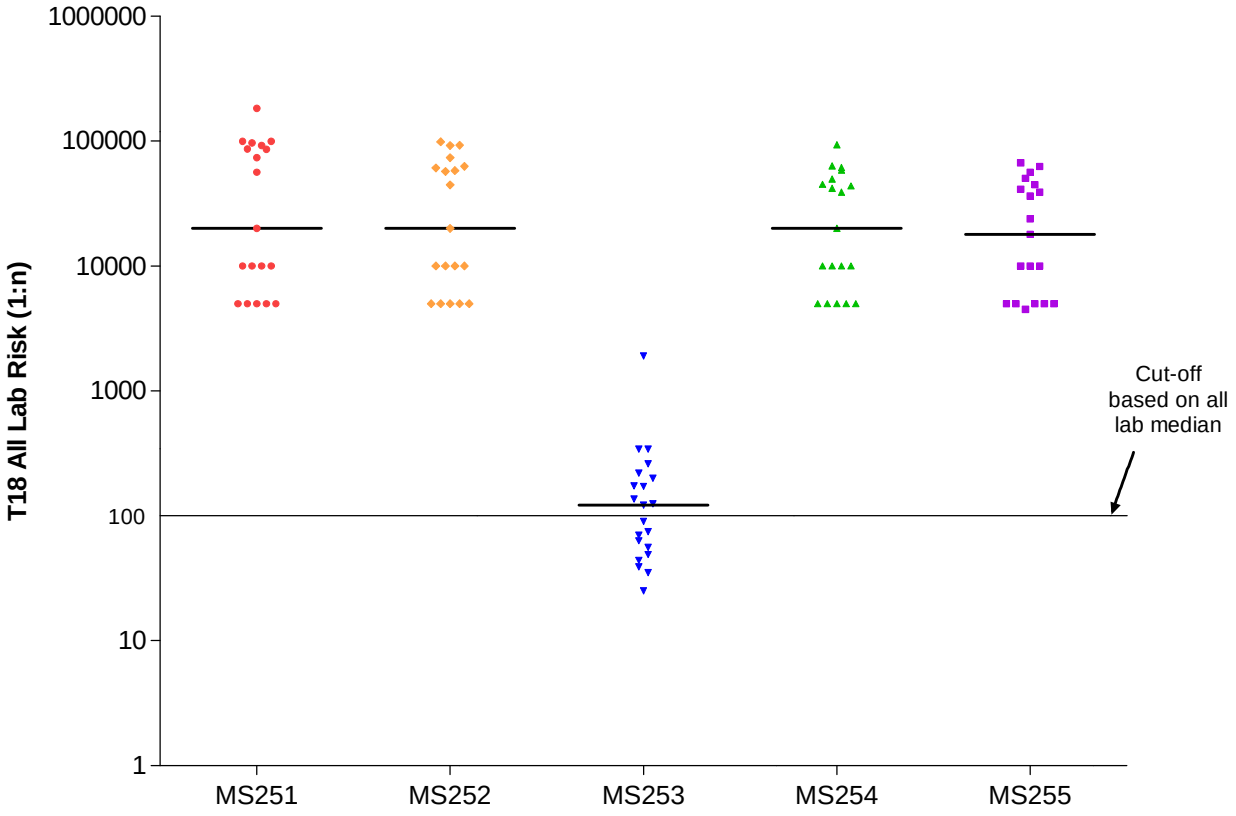


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

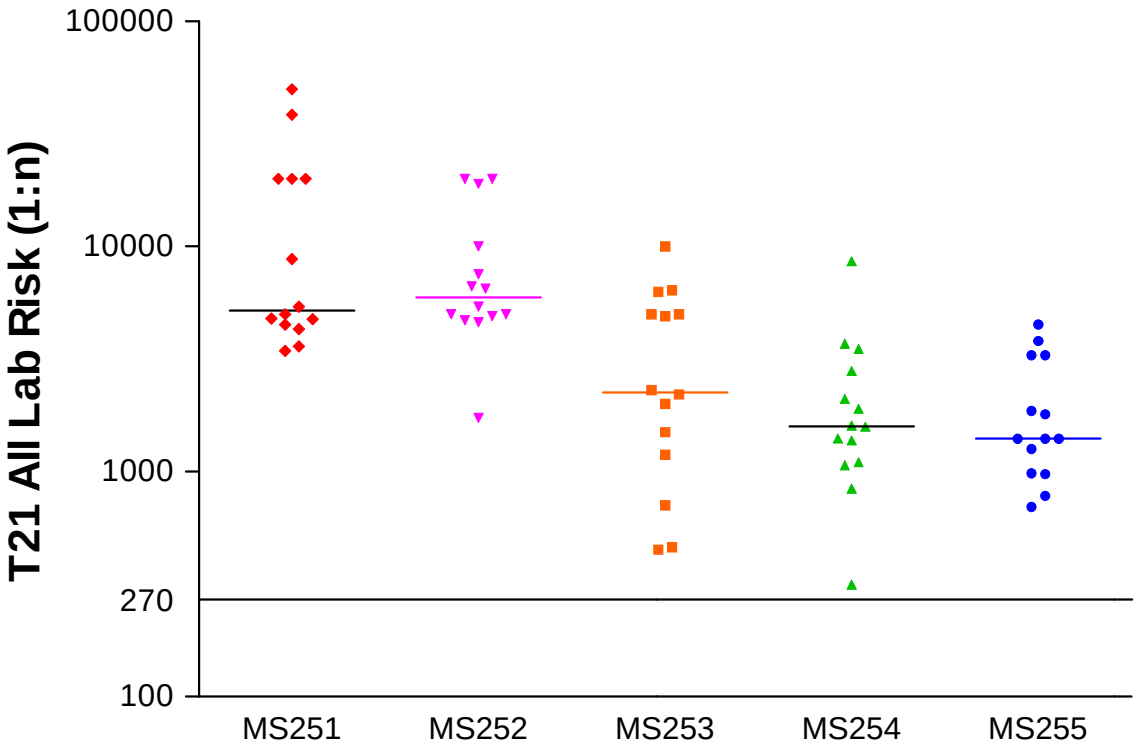


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

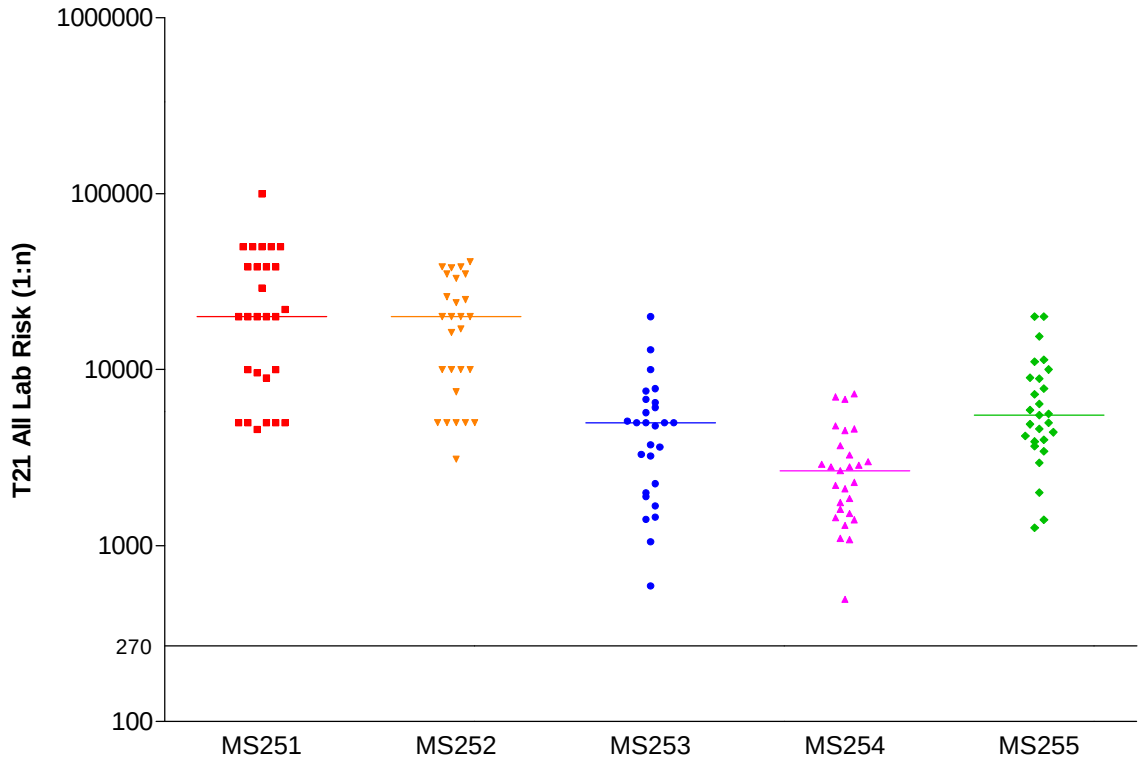


Figure 7

MS AFP FEDM PT 5/10 Method Comparison

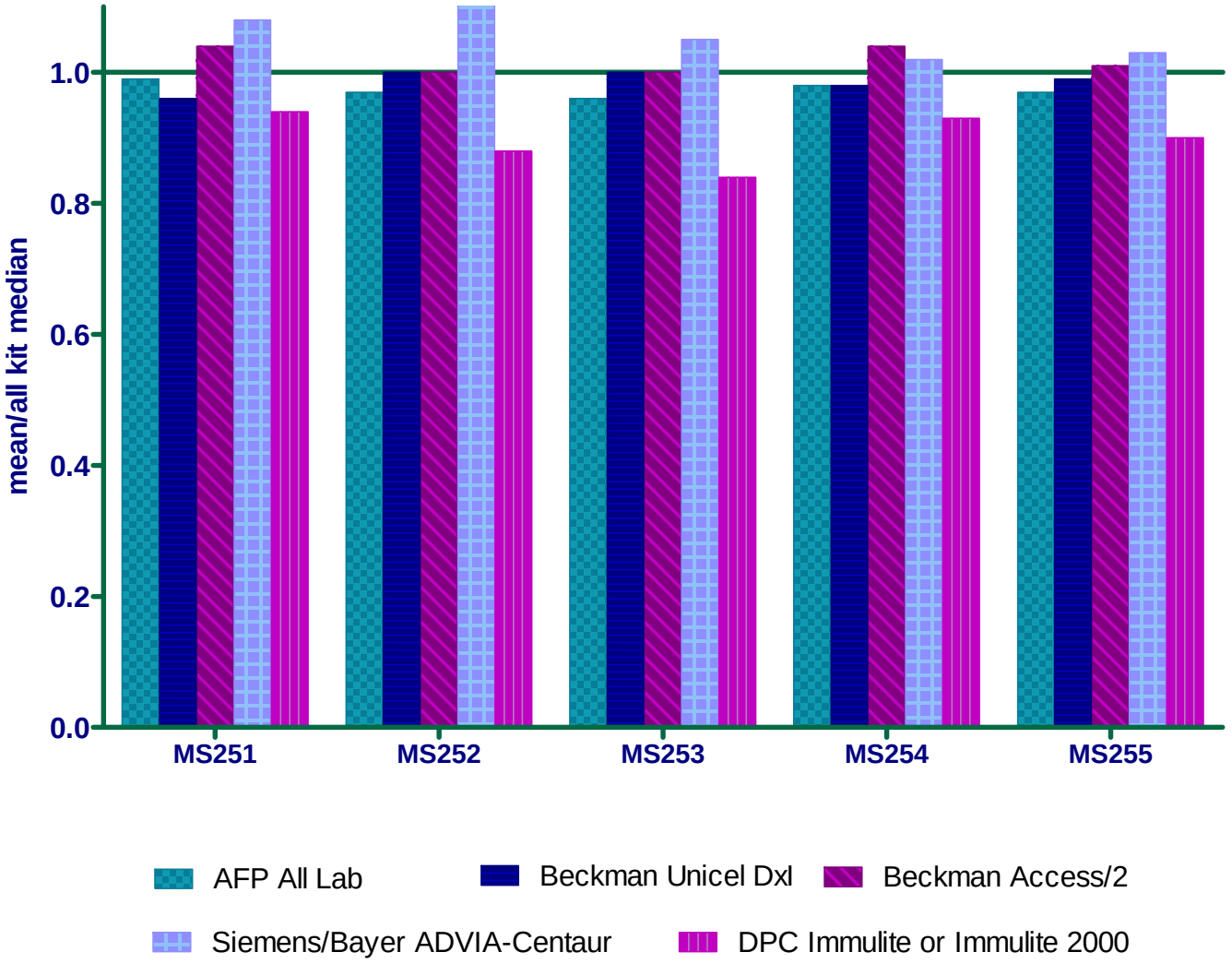


Figure 8A

MS uE3 FEDM PT 5/10 Method Comparison

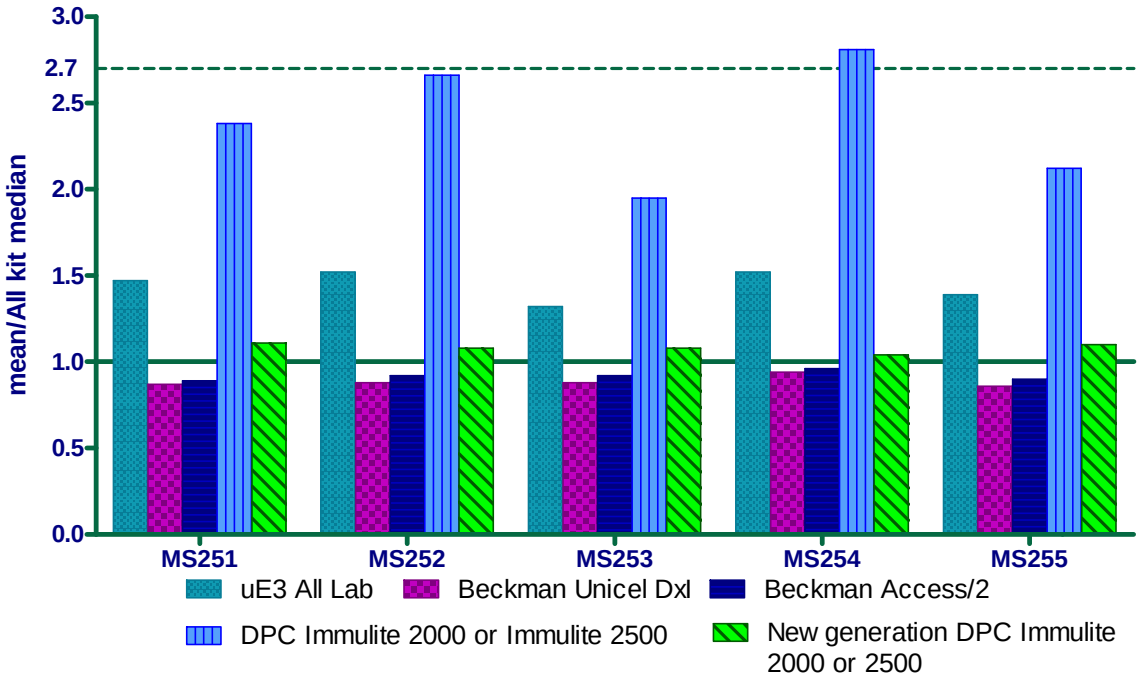


Figure 8 B

MS uE3 MOM FEDM PT 5/10 Method Comparison

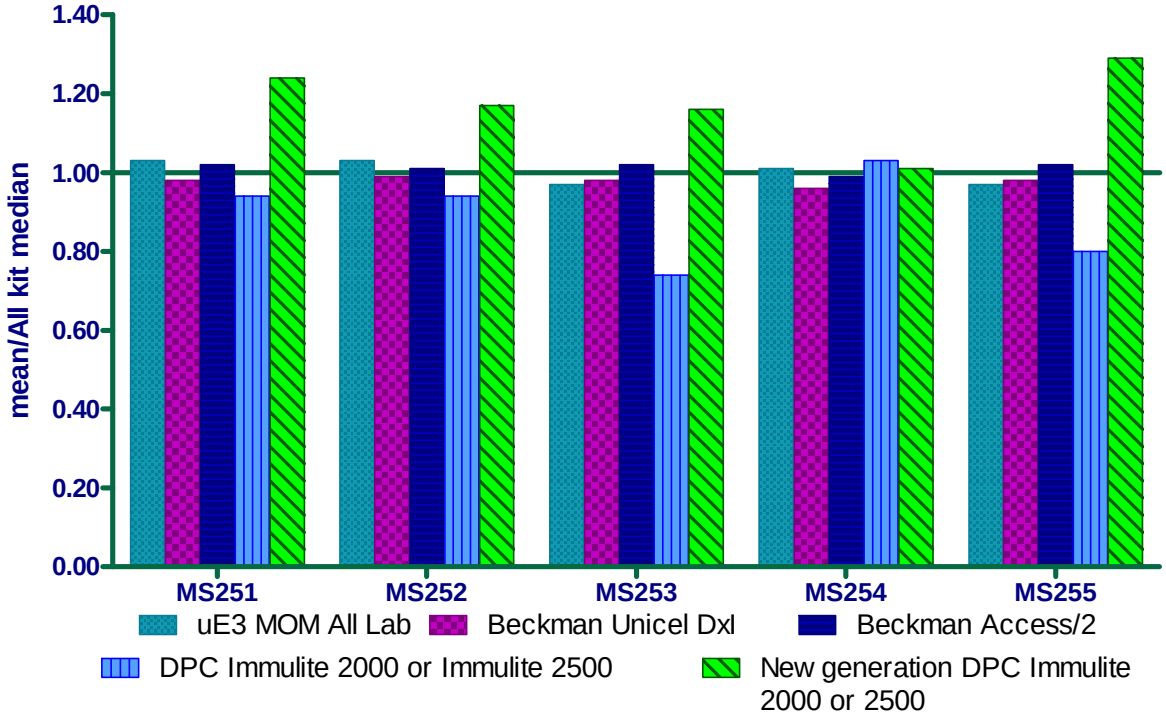


Figure 9

MS hCG FEDM PT 5/10 Method Comparison

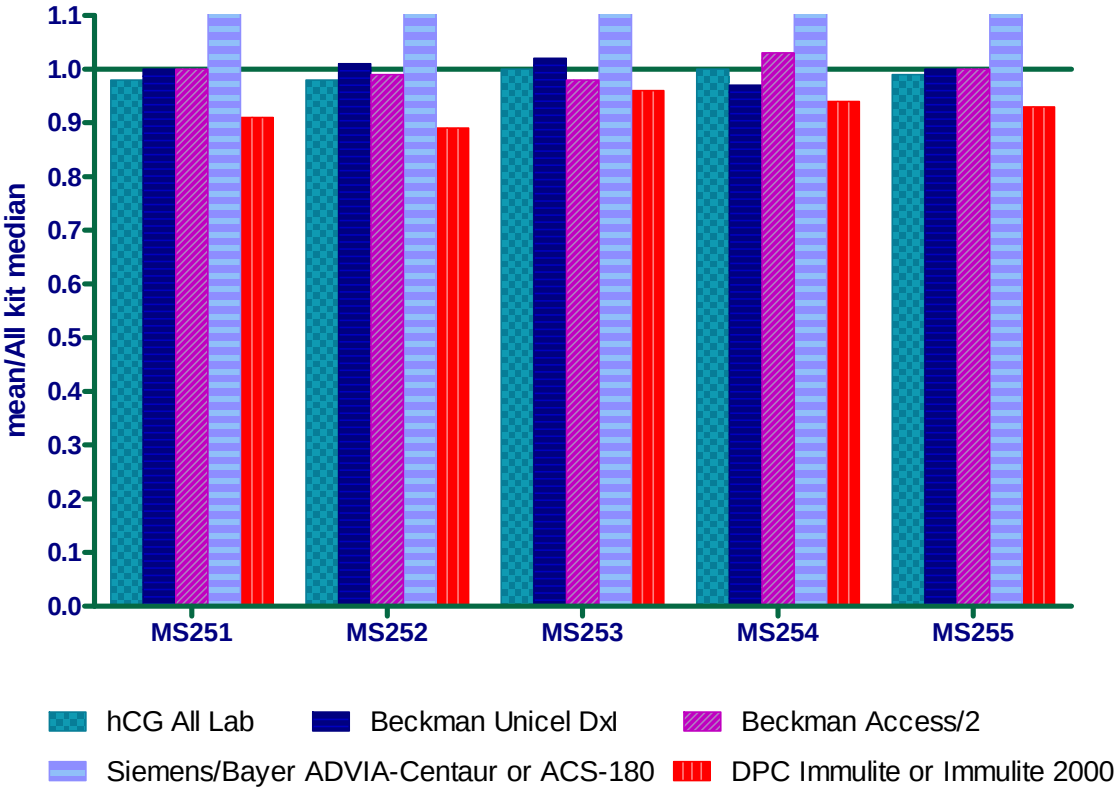


Figure 10

MS Inhibin A FEDM PT 5/10 Method Comparison

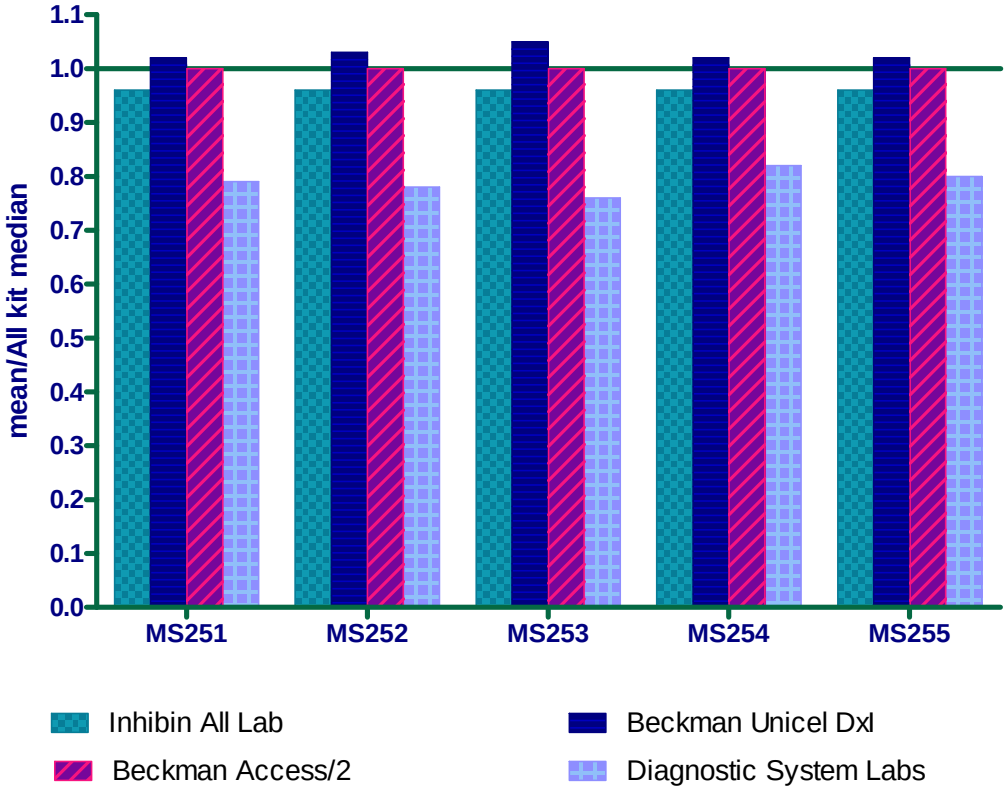


Figure 11

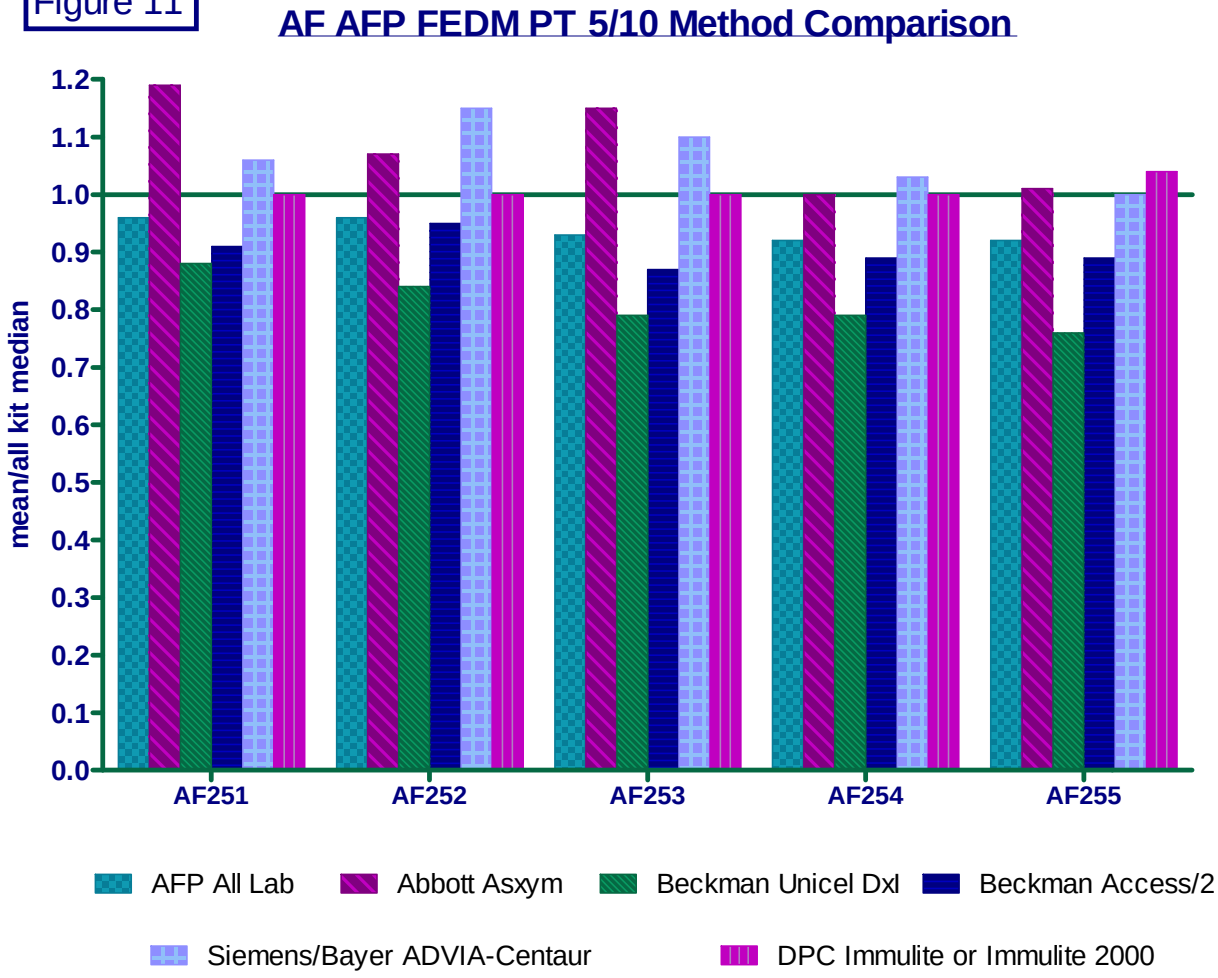

AF AFP FEDM PT 5/10 Method Comparison
mean/all kit median
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
AF251
AF252
AF253
AF254
AF255
AFP All Lab
Abbott Asxym
Beckman Unicel Dxl
Beckman Access/2
Siemens/Bayer ADVIA-Centaur
DPC Immulite or Immulite 2000

Figure 12

FT hCG FEDM PT 5/10 Method Comparison

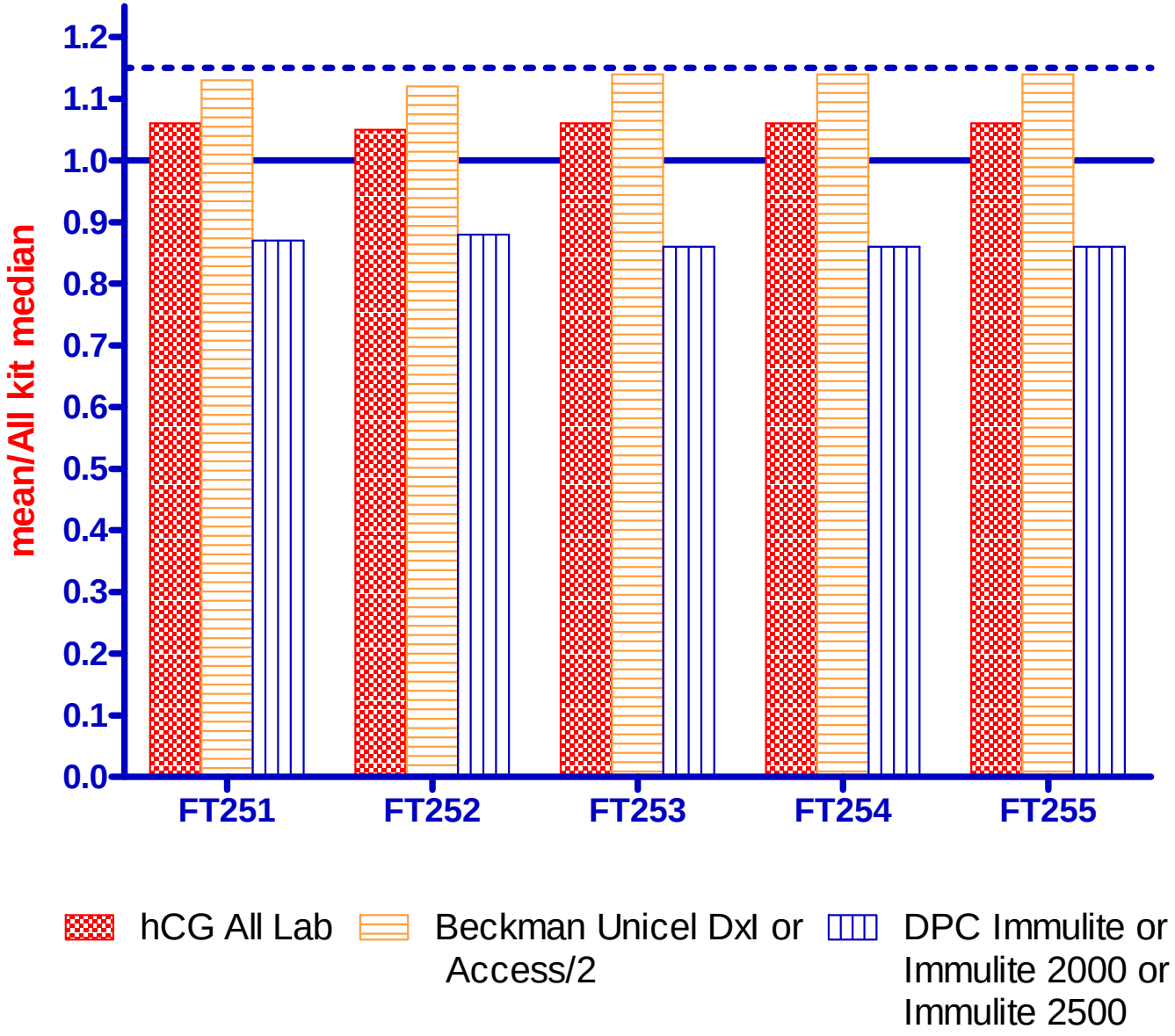
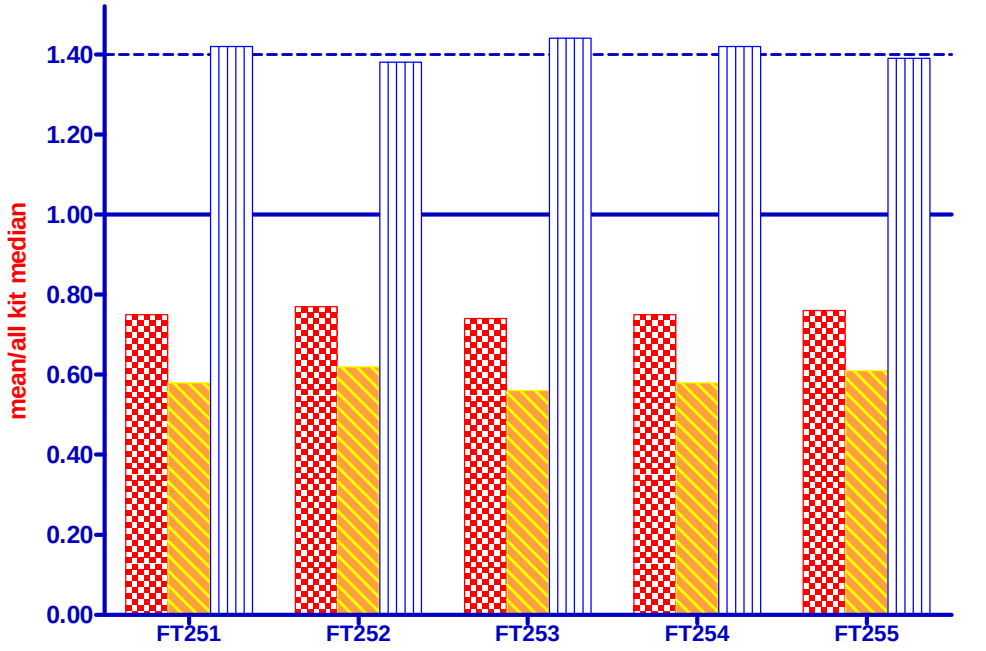


Figure 13A

FT_PAPP- A FEDM PT 5/10 Method Comparison

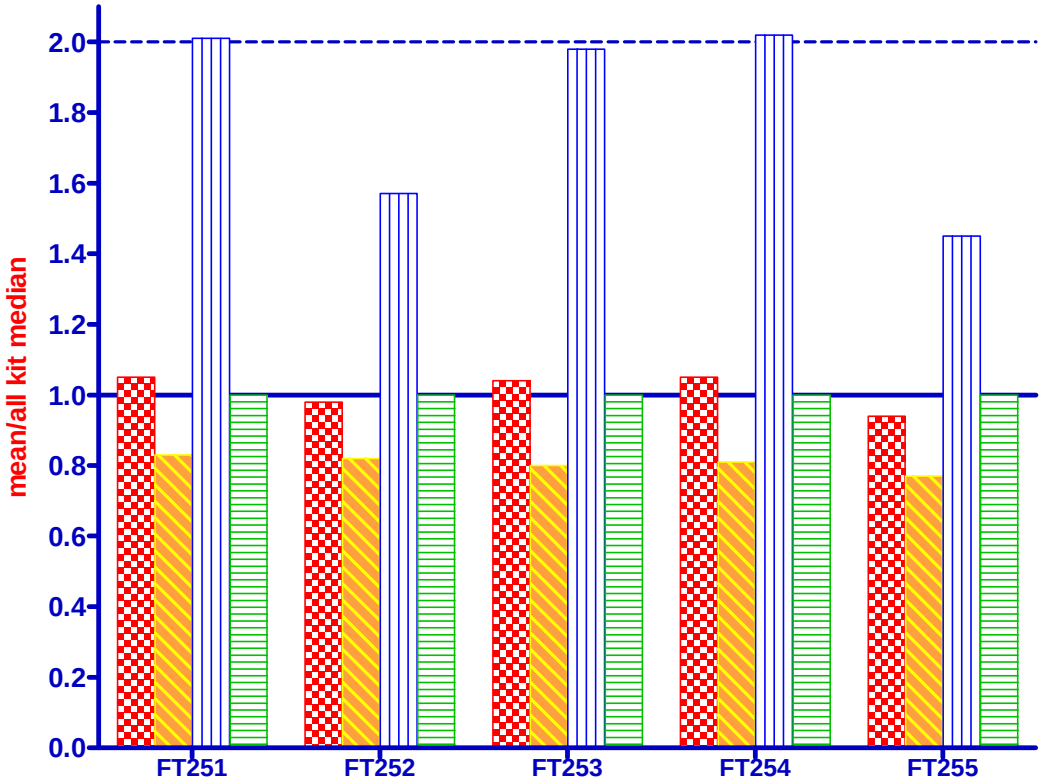


PAPPA All Lab Diagnostic Systems Lab DPC Immulite or Immulite 2000 or Immulite 2500

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

Figure 13B

FT_PAPP- A MOM FEDM PT 5/10 Method Comparison



PAPP-A MOM All Lab Diagnostic Systems Lab DPC Immulite or Immulite 2000 Beckman Unicel Dxl or Access/2

Figure 14

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

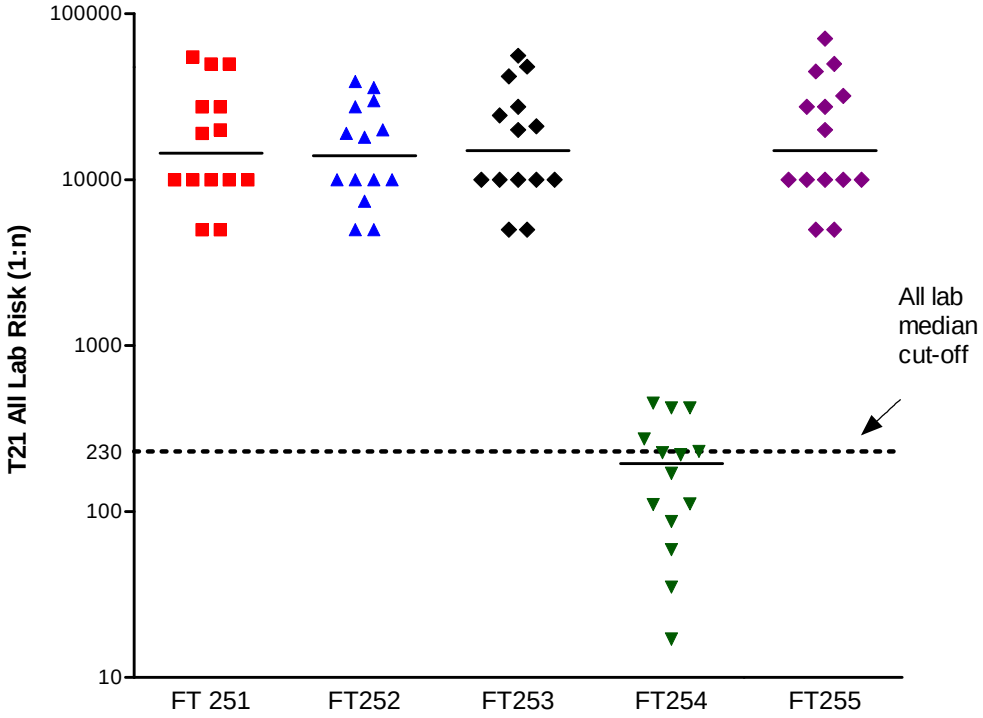
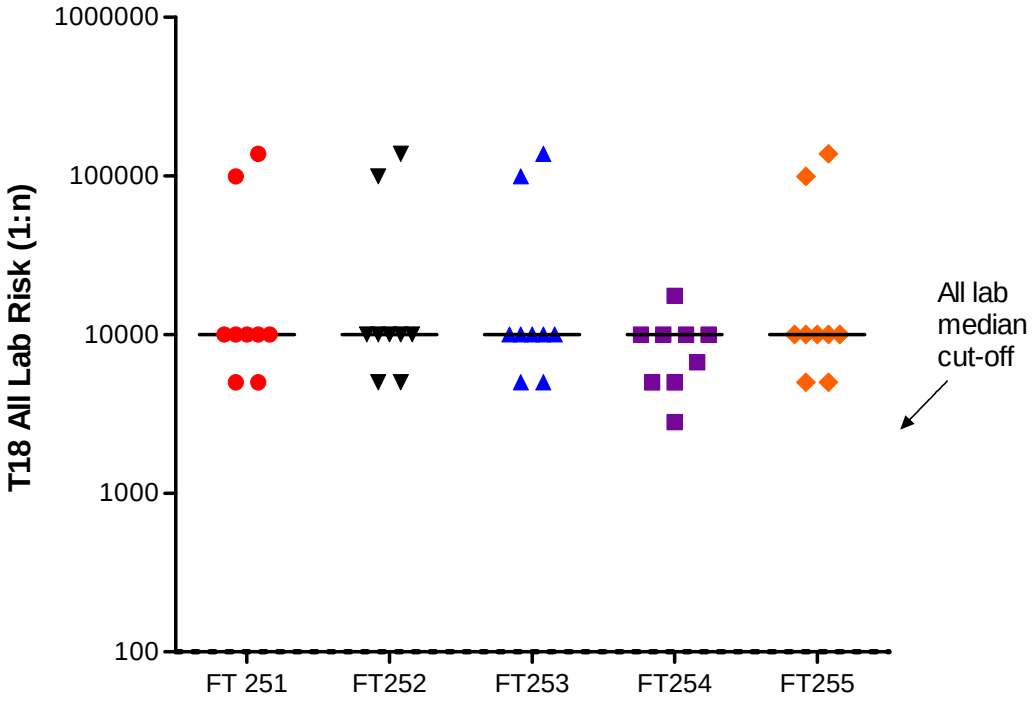


Figure 15

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates



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

	MS 251	MS 252	MS 253	MS 254	MS 255
Gestational Age All Lab Mean:					
Mean	18.0	15.0	17.0	19.0	16.0
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	18.0	15.0	17.0	19.0	16.0
X-3*SD	18.0	15.0	17.0	19.0	16.0
N	28	28	28	28	28

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP All Lab Mean:					
mean	123.3	26.0	18.3	49.3	30.6
SD	10.1	2.3	1.7	4.1	2.3
%CV	8.2%	8.8%	9.3%	8.4%	7.4%
mean+3SD	153.8	32.9	23.4	61.7	37.3
mean-3SD	92.9	19.1	13.2	36.9	23.8
N	28	28	28	28	28
median	124.8	25.9	18.8	49.6	30.8
mean/all kit median	0.99	0.97	0.96	0.98	0.97

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	120.3	26.8	19.2	49.0	31.3
SD	10.0	1.4	0.9	4.6	2.4
%CV	8.3%	5.2%	4.5%	9.4%	7.6%
mean + 3SD	150.3	31.0	21.7	62.8	38.4
mean - 3SD	90.3	22.7	16.6	35.2	24.1
N	7	7	7	7	7
Median	123.7	26.7	19.2	49.5	31.7
mean/All kit median	0.96	1.00	1.00	0.98	0.99

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP Beckman Access (BCX/BC1) mean:					
mean	129.4	26.8	19.0	52.0	31.7
SD	5.9	1.4	1.2	3.0	1.5
%CV	4.6%	5.3%	6.1%	5.8%	4.8%
mean+3SD	147.1	31.1	22.5	61.0	36.2
mean-3SD	111.7	22.6	15.6	42.9	27.1
N	9	9	9	9	9
median	128.1	27.3	19.5	52.4	32.1
mean/all kit median	1.04	1.00	1.00	1.04	1.01

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP Siemens/Bayer ADVIA-Centaur(COB/BA1) mean:					
mean	134.5	30.3	20.1	51.1	32.3
N	2	2	2	2	2
mean/all kit median	1.08	1.13	1.05	1.02	1.03

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP DPC Immulite or 2000 (DPB or DPD/DP5) mean:					
mean	116.8	23.6	16.1	46.6	28.3
SD	11.3	1.1	1.1	3.9	1.8
%CV	9.7%	4.9%	6.7%	8.4%	6.3%
mean+3SD	150.6	27.0	19.4	58.3	33.7
mean-3SD	82.9	20.1	12.9	34.9	22.9
N	8	8	8	8	8
median	115.5	23.4	15.8	44.6	28.1
mean/all kit median	0.94	0.88	0.84	0.93	0.90

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP kit average:					
mean	125.2	26.9	18.6	49.7	30.9
SD	8.1	2.7	1.7	2.4	1.8
all kit median	124.8	26.8	19.1	50.1	31.5

	MS 251	MS 252	MS 253	MS 254	MS 255
MS AFP MoMs All Lab Mean:					
mean	2.79	0.87	0.49	0.99	0.96
SD	0.25	0.08	0.05	0.09	0.08
%CV	8.9%	9.6%	9.6%	9.1%	8.4%
mean+3SD	3.53	1.12	0.63	1.26	1.20
mean-3SD	2.05	0.62	0.35	0.72	0.72
N	28	28	28	28	28

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	MS 251	MS 252	MS 253	MS 254	MS 255
MS uE3 All Lab Mean:					
mean	1.62	2.46	0.60	1.88	0.99
SD	0.84	1.42	0.25	1.11	0.47
%CV	51.5%	57.9%	41.2%	58.9%	47.5%
mean+3SD	4.13	6.73	1.33	5.21	2.40
mean-3SD	-0.88	-1.81	-0.14	-1.45	-0.42
N	27	27	27	27	27
mean/all kit median	1.47	1.52	1.32	1.52	1.39

MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	1.07	1.55	0.44	1.21	0.68
SD	0.07	0.07	0.03	0.07	0.05
%CV	6.7%	4.4%	6.6%	5.8%	7.9%
mean+3SD	1.29	1.75	0.52	1.42	0.84
mean-3SD	0.86	1.35	0.35	1.00	0.52
N	7	7	7	7	7
Median	1.05	1.58	0.43	1.22	0.64
mean/all kit median	0.87	0.88	0.88	0.94	0.86

MS uE3 BeckmanAccess (BCX/BC1) mean:					
mean	1.10	1.62	0.45	1.24	0.71
SD	0.04	0.05	0.03	0.06	0.03
%CV	3.8%	3.1%	6.1%	4.6%	4.1%
mean+3SD	1.23	1.77	0.54	1.41	0.80
mean-3SD	0.98	1.47	0.37	1.07	0.62
N	9	9	9	9	9
median	1.11	1.64	0.46	1.24	0.70
mean/all kit median	0.89	0.92	0.92	0.96	0.90

MS UE3 kit average:					
mean	1.62	2.43	0.59	1.86	0.99
SD	0.89	1.50	0.25	1.19	0.47
all kit median	1.24	1.76	0.49	1.30	0.79

MS uE3 DPC Immulite 2000 or 2500(DPD or F/DP5) mean:					
Mean	2.94	4.67	0.96	3.64	1.68
SD	0.23	0.54	0.17	0.46	0.33
%CV	7.8%	11.5%	17.5%	12.6%	19.5%
mean+3SD	3.63	6.29	1.46	5.02	2.67
mean-3SD	2.26	3.05	0.45	2.27	0.70
N	6	6	6	6	6
Median	2.97	4.78	0.93	3.49	1.59
mean/all kit median	2.38	2.66	1.95	2.81	2.12

MS uE3 New generation DPC Immulite 2000 or 2500(DPD or F/DP6) mean:					
Mean	1.37	1.89	0.53	1.35	0.88
SD	0.03	0.12	0.04	0.10	0.07
%CV	2.2%	6.4%	7.1%	7.5%	7.6%
mean+3SD	3.63	6.29	1.46	5.02	2.67
mean-3SD	2.26	3.05	0.45	2.27	0.70
N	4	4	4	4	4
Median	1.37	1.86	0.53	1.35	0.87
mean/All Kit Median	1.11	1.08	1.08	1.04	1.10

	MS 251	MS 252	MS 253	MS 254	MS 255
MS uE3 MoMs All Lab Mean:					
Mean	0.86	2.44	0.41	0.84	0.84
SD	0.20	0.56	0.10	0.08	0.19
%CV	22.9%	22.7%	25.0%	9.9%	22.9%
X+3SD	1.45	4.11	0.72	1.09	1.41
X-3SD	0.27	0.78	0.10	0.59	0.26
N	27	27	27	25	26

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	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG All Lab Mean:					
mean	22.87	32.28	13.51	21.43	31.66
SD	1.91	3.17	0.98	1.89	3.01
%CV	8.4%	9.8%	7.3%	8.8%	9.5%
mean+3SD	28.6	41.8	16.5	27.1	40.7
mean-3SD	17.1	22.8	10.6	15.8	22.6
N	27	27	27	27	27
mean/all kit median	0.98	0.98	1.00	1.00	0.99

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG Siemens/Bayer ADVIA-Centaur (COB/BA1) mean:					
mean	26.2	37.7	15.2	24.8	38.0
N	2	2	2	2	2
mean/all kit median	1.13	1.15	1.12	1.16	1.19

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	23.29	33.07	13.81	20.90	31.69
SD	1.75	2.19	0.64	1.14	2.48
%CV	7.5%	6.6%	4.6%	5.5%	7.8%
mean+3SD	28.15	41.06	16.40	25.64	37.91
mean-3SD	18.27	24.41	10.25	18.34	25.98
N	7	7	7	7	7
median	23.00	32.00	13.50	20.50	32.80
mean/All kit median	1.00	1.01	1.02	0.97	1.00

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	21.2	29.4	13.0	20.1	29.5
SD	1.0	2.0	0.9	1.8	2.5
%CV	4.8%	6.8%	6.6%	8.7%	8.5%
mean+3SD	24.2	35.3	15.6	25.3	36.9
mean-3SD	18.2	23.4	10.4	14.8	22.0
N	8	8	8	8	8
median	21.0	28.9	13.1	19.8	29.5
mean/all kit median	0.91	0.89	0.96	0.94	0.93

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG Beckman Access (BCX/BC1) mean:					
mean	23.2	32.7	13.3	22.0	31.9
SD	1.6	2.8	1.0	1.2	2.0
%CV	7.1%	8.5%	7.7%	5.5%	6.2%
mean+3SD	28.1	41.1	16.4	25.6	37.9
mean-3SD	18.3	24.4	10.2	18.3	26.0
N	9	9	9	9	9
median	22.5	33.3	13.2	21.4	31.8
mean/all kit median	1.00	0.99	0.98	1.03	1.00

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG kit average:					
mean	23.5	33.2	13.8	21.9	32.8
SD	2.1	3.4	1.0	2.1	3.6
all kit median	23.2	32.9	13.6	21.4	31.8

	MS 251	MS 252	MS 253	MS 254	MS 255
MS hCG MoMs All Lab Mean:					
mean	1.10	0.81	0.57	1.21	1.11
SD	0.12	0.10	0.07	0.18	0.13
%CV	11.3%	11.9%	12.9%	14.7%	11.8%
mean+3SD	1.47	1.10	0.79	1.75	1.51
mean-3SD	0.72	0.52	0.35	0.68	0.72
N	27	27	27	27	27

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	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A all lab/DSL mean:					
Mean	130.63	125.93	105.79	184.40	121.85
SD	17.23	18.22	16.06	23.62	16.46
%CV	13.2%	14.5%	15.2%	12.8%	13.5%
mean + 3SD	182.3	180.6	154.0	255.3	171.2
mean- 3SD	78.9	71.3	57.6	113.5	72.5
N	27	27	27	27	27
All Lab Median	136.0	130.8	109.0	188.5	128.0
mean/all kit median	0.96	0.96	0.96	0.96	0.96

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A Beckman Access (BCX/BC1) mean:					
Mean	136.2	131.2	110.0	191.1	126.7
SD	14.1	14.9	11.9	21.7	15.1
%CV	10.3%	11.4%	10.8%	11.4%	12.0%
mean + 3SD	178.5	176.1	145.5	256.3	172.1
mean- 3SD	94.0	86.4	74.4	126.0	81.2
N	13	13	13	13	13
median	139.2	132.5	110.8	197.1	128.4
mean/All kit median	1.00	1.00	1.00	1.00	1.00

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	139.3	134.9	115.7	194.0	129.5
SD	7.9	9.0	9.4	19.4	8.0
%CV	5.7%	6.7%	8.2%	10.0%	6.2%
mean + 3SD	163.1	161.8	144.0	252.3	153.6
mean- 3SD	115.5	107.9	87.3	135.8	105.4
N	8	8	8	8	8
median	139.5	133.5	112.6	195.3	130.2
mean/all kit median	1.02	1.03	1.05	1.02	1.02

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A Diagnostic System Labs (DS1) Mean:					
Mean	107.0	102.6	83.6	157.0	101.2
SD	10.3	14.9	9.2	8.5	10.1
%CV	9.6%	14.5%	11.1%	5.4%	9.9%
mean + 3SD	137.8	147.3	111.3	182.5	131.3
mean- 3SD	76.1	57.8	55.8	131.4	71.0
N	6	6	6	6	6
median	109.1	103.4	84.0	158.2	100.6
mean/all kit median	0.79	0.78	0.76	0.82	0.80

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A kit average:					
mean	127.5	122.9	103.1	180.7	119.1
SD	17.9	17.7	17.1	20.6	15.6
all kit median	136.2	131.2	110.0	191.1	126.7

	MS 251	MS 252	MS 253	MS 254	MS 255
MS Inhibin A MoM All Lab Mean:					
mean	0.78	0.66	0.65	1.16	0.72
SD	0.13	0.11	0.11	0.18	0.12
%CV	16.8%	16.8%	17.6%	15.4%	16.3%
mean+3SD	1.18	0.99	0.99	1.70	1.07
mean-3SD	0.39	0.33	0.30	0.63	0.37
N	27	27	27	27	27

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	AF 251	AF 252	AF 253	AF 254	AF 255
AF AFP All Lab Mean :					
mean	21.3	9.2	6.3	4.2	4.6
SD	2.6	1.1	0.9	0.5	0.6
%CV	12.3%	11.5%	14.2%	11.8%	13.5%
mean+3SD	29.2	12.3	9.0	5.7	6.4
mean-3SD	13.4	6.0	3.6	2.7	2.7
N	24	24	24	24	24
mean/all kit median	0.96	0.96	0.93	0.92	0.92
AF AFP Abbott Axsym (ABB/AB2) mear:					
mean	26.5	10.2	7.9	4.6	5.1
N	2	2	2	2	2
mean/all kit median	1.19	1.07	1.15	1.00	1.01
AF AFP DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	22.2	9.5	6.8	4.6	5.2
SD	1.3	0.4	0.4	0.3	0.2
%CV	5.9%	3.8%	5.7%	7.2%	4.4%
mean+3SD	26.1	10.6	8.0	5.5	5.9
mean-3SD	18.3	8.4	5.6	3.6	4.5
N	6	6	6	6	6
median	22.35	9.5	6.85	4.45	5.2
mean/all kit median	1.00	1.00	1.00	1.00	1.04

	AF 251	AF 252	AF 253	AF 254	AF 255
AF AFP MoMs All Lab Mean:					
mean	2.21	0.64	0.54	0.65	0.58
SD	0.18	0.06	0.05	0.07	0.08
%CV	8.0%	10.0%	10.2%	11.3%	13.0%
mean+3SD	2.75	0.84	0.70	0.87	0.81
mean-3SD	1.68	0.45	0.37	0.43	0.35
N	24	24	24	24	24

	AF 251	AF 252	AF 253	AF 254	AF 255
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	19.6	8.0	5.4	3.6	3.8
SD	2.3	0.8	0.5	0.3	0.4
%CV	11.8%	10.0%	9.3%	9.2%	10.1%
X+3SD	25.4	11.3	7.3	5.0	5.4
X-3SD	14.9	6.7	4.6	3.1	3.5
N	6	6	6	6	6
median	19.6	7.9	5.3	3.7	3.8
mean/All kit median	0.88	0.84	0.79	0.79	0.76
AF AFP Beckman Access (BCX/BC1) mean:					
mean	20.2	9.0	5.9	4.1	4.5
SD	1.8	0.8	0.4	0.3	0.3
%CV	8.7%	8.4%	7.5%	7.6%	7.4%
mean+3SD	25.4	11.3	7.3	5.0	5.4
mean-3SD	14.9	6.7	4.6	3.1	3.5
N	7	7	7	7	7
median	20.1	9.1	5.9	4.1	4.5
mean/all kit median	0.91	0.95	0.87	0.89	0.89
AF AFP Siemens/Bayer ADVIA-Centaur(COB/BA1)mean:					
mean	23.7	11.0	7.5	4.7	5.0
N	2	2	2	2	2
mean/all kit median	1.06	1.15	1.10	1.03	1.00
AF AFP kit average:					
mean	22.4	9.5	6.7	4.3	4.7
SD	2.8	1.1	1.0	0.5	0.6
all kit median	22.2	9.5	6.8	4.6	5.0

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	FT251	FT252	FT253	FT254	FT255
FT Gestational Age All Lab Mean:					
Mean	11.2	12.4	11.9	11.4	13.0
SD	0.14	0.09	0.10	0.11	0.06
%CV	1.2%	0.7%	0.8%	0.9%	0.5%
X+3*SD	11.6	12.7	12.2	11.8	13.2
X-3*SD	10.8	12.1	11.6	11.1	12.8
N	15	15	15	15	15

	FT251	FT252	FT253	FT254	FT255
FT NT MoMs All Lab Mean:					
Mean	0.98	0.97	0.93	2.11	0.99
SD	0.09	0.10	0.10	0.22	0.09
%CV	9.6%	10.2%	10.8%	10.3%	9.3%
X+3SD	1.26	1.26	1.24	2.76	1.27
X- 3SD	0.70	0.67	0.63	1.45	0.72
N	14	14	14	14	14
All Median	0.99	0.94	0.92	2.07	0.98

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	FT251	FT252	FT253	FT254	FT255
FT hCG All Lab Mean:					
mean	84.03	65.42	74.27	172.25	49.01
SD	13.10	8.49	12.19	33.37	6.99
%CV	15.6%	13.0%	16.4%	19.4%	14.3%
X+3SD	123.3	90.9	110.8	272.4	70.0
X-3SD	44.7	40.0	37.7	72.1	28.0
N	14	14	14	14	14
mean/All kit median	1.06	1.05	1.06	1.06	1.06
FT hCG kit average:					
mean	79.6	62.3	70.0	162.4	46.3
SD	14.8	10.2	14.1	32.5	9.1
all kit median	79.6	62.3	70.0	162.4	46.3

	FT251	FT252	FT253	FT254	FT255
FT hCG MoMs All Lab Mean:					
Mean	1.10	0.96	1.06	2.25	0.80
SD	0.14	0.10	0.15	0.42	0.10
%CV	13.2%	10.6%	14.3%	18.8%	12.6%
X+3SD	1.53	1.26	1.52	3.52	1.10
X- 3SD	0.66	0.65	0.61	0.98	0.50
N	13	13	13	13	13
All Median	1.07	0.95	1.05	2.22	0.81

	FT251	FT252	FT253	FT254	FT255
FT hCG Beckman Unicel or Access (BCU or BCX/BC1) mean:					
mean	90.0	69.5	80.0	185.4	52.7
SD	10.3	6.1	9.0	21.0	4.2
%CV	11.5%	8.8%	11.3%	11.3%	8.0%
X+3SD	121.0	87.9	107.1	248.3	65.3
X-3SD	59.0	51.2	52.8	122.4	40.1
N	10	10	10	10	10
median	89.7	67.3	78.9	178.6	52.7
mean/All kit median	1.13	1.12	1.14	1.14	1.14
FT hCG DPC Immulite or 2000 or 2500(DPB or D or F/DP5) mean:					
mean	69.1	55.2	60.1	139.5	39.8
SD	2.7	1.7	4.6	38.7	1.1
%CV	3.9%	3.1%	7.6%	27.7%	2.9%
X+3SD	77.2	60.4	73.8	255.5	43.2
X-3SD	61.0	49.9	46.3	23.4	36.4
N	4	4	4	4	4
median	68.9	55.8	60.1	130.4	39.8
mean/All kit median	0.87	0.88	0.86	0.86	0.86

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	FT251	FT252	FT253	FT254	FT255
FT PAPP-A All Lab Mean: (does not include Beckman)					
Mean	3.63	4.49	4.30	2.03	5.19
SD	2.09	2.33	2.62	1.16	2.78
%CV	57.5%	52.0%	61.0%	57.3%	53.6%
mean + 3SD	9.88	11.49	12.17	5.52	13.53
mean- 3SD	-2.63	-2.51	-3.57	-1.46	-3.15
N	8	8	8	8	8
All Lab Median	2.68	3.48	3.20	1.54	4.11
mean/All kit median	0.75	0.77	0.74	0.75	0.76

	FT251	FT252	FT253	FT254	FT255
FT PAPP-A DPC Immullite or 2000 or 2500 (DPB or D or F/DP5) Mean:					
Mean	6.87	8.05	8.38	3.85	9.46
N	2	2	2	2	2
Kit Median	6.87	8.05	8.38	3.85	9.46
mean/All kit median	1.42	1.38	1.44	1.42	1.39

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

FT PAPP-A Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	1590.57	2235.73	1874.99	863.93	2801.14
SD	112.95	189.63	170.48	71.85	199.66
%CV	7.1%	8.5%	9.1%	8.3%	7.1%
X + 3SD	1929.43	2804.61	2386.44	1079.47	3400.11
X - 3SD	1251.71	1666.84	1363.53	648.38	2202.18
N	7	7	7	7	7
Kit Median	1570.00	2219.00	1890.00	887.00	2810.00

FT PAPP-A kit average (does not include Beckman):					
mean	4.83	5.84	5.80	2.70	6.81
SD	2.89	3.12	3.64	1.62	3.74
all kit median	4.83	5.84	5.80	2.70	6.81

FT PAPP-A Diagnostic Systems Lab (DS1) Mean:					
Mean	2.79	3.64	3.23	1.56	4.16
SD	0.38	0.51	0.39	0.18	0.45
%CV	13.8%	13.9%	12.2%	11.3%	10.7%
X + 3SD	3.94	5.15	4.41	2.08	5.50
X - 3SD	1.63	2.12	2.05	1.03	2.83
N	5	5	5	5	5
Kit Median	2.66	3.45	3.10	1.50	4.07
mean/All kit median	0.58	0.62	0.56	0.58	0.61

FT PAPP-A MoM All Lab Mean:					
Mean	3.22	2.44	3.08	1.58	2.61
SD	1.34	0.75	1.32	0.68	0.81
%CV	41.7%	30.7%	42.7%	42.9%	31.0%
mean + 3SD	7.25	4.69	7.03	3.61	5.03
mean- 3SD	-0.81	0.20	-0.86	-0.45	0.18
N	14	14	14	14	14
All Lab Median	2.84	2.28	2.70	1.39	2.67