Fetal Defect Marker Proficiency Test Mailout
January 2011

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from January, 2011, 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 261**	**MS 262**	**MS 263**	**MS 264**	**MS 265**
	Gestational Age (weeks)	18	15	17	16	20
Maternal Race	Ethnic Group	Black	Hispanic	White	Asian	White
Maternal Weight	Pounds (lbs)	150	150	145	140	135
Maternal Age	Years	28	25	22	27	20
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	38.99 ± 2.61	13.10 ± 0.90	32.56 ± 2.43	29.24 ± 1.77	57.82 ± 3.69
	MOM ± Std. Dev.	0.78 ± 0.08	0.45 ± 0.04	0.81 ± 0.08	0.83 ± 0.07	0.91 ± 0.08
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	1.16 ± 0.14	0.32 ± 0.05	0.93 ± 0.09	0.75 ± 0.12	1.48 ± 0.14
	MOM ± Std. Dev.	1.02 ± 0.28	0.58 ± 0.19	1.02 ± 0.29	1.04 ± 0.36	0.83 ± 0.21
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	18.23 ± 1.75	57.98 ± 6.93	7.49 ± 0.62	25.07 ± 2.72	16.91 ± 1.82
	MOM ± Std. Dev.	0.85 ± 0.11	1.57 ± 0.20	0.31 ± 0.03	0.83 ± 0.11	0.95 ± 0.10
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	137.33 ± 5.27	262.97 ± 12.70	131.10 ± 8.90	115.09 ± 12.15	229.79 ± 21.61
	MOM ± Std. Dev.	0.85 ± 0.07	1.38 ± 0.22	0.76 ± 0.13	0.65 ± 0.10	1.16 ± 0.18
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Further Action G,U,A	NFA	NFA	NFA	NFA	NFA
	NTD Risk 1 in	14,350	9,900	11,600	10,000	5,900
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(+) (86%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	G = 71% U = 57% A = 79%	NFA	NFA	NFA
	Risk Est. 1 in	3,400	101	10,000	4,300	3,250
2. Quad Test	Pos. (+) or Neg. (-)	(-) (100%)	(+) (93%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	G = 78% U = 63% A = 78%	NFA	NFA	NFA
	Risk Est. 1 in	5,000	70	20,000	8,800	4,150
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	18,800	5,000	3,880	18,600	19,000

*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 = 29 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 261 Wk 18.0	This specimen was obtained from a 28 year old black woman (Gravida = 2, Parity = 1) in her 18th week gestation with a body weight of 150 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 MS261 was not paired with an amniotic fluid sample.
MS 262 Wk 15.0	This specimen was obtained from a 25 year old Hispanic woman (Gravida = 2, Parity = 0) in her 15th week gestation with a body weight of 150 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 (93%) on the basis of low AFP and uE3, and elevated hCG and inhibin-A levels. Recommendations for further action from labs performing the T21 quad screen were: genetic counseling, 78%, ultrasound, 63% and amniocentesis, 78%; while the triple tests were: genetic counseling, 71%; ultrasound, 57% and amniocentesis, 79%. Specimen MS262 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 = 0.58).
MS 263 Wk 17.0	This specimen was obtained from a 22 year old white woman (Gravida = 3, parity = 2) in her 17th week gestation with a body weight of 145 lbs. She had a family (sibling) history of pregnancy complications. However, her sample screened negative for NTD. Her aneuploidy screens were also negative for both Trisomy-18 and Trisomy-21. Her MShCG sample was extremely low (see below for further discussion). This sample was not paired to an amniotic fluid specimen.
MS 264 Wk 16.0	This specimen was obtained from a 27 year old Asian woman (Gravida = 2, Parity = 1) in her 16th week gestation with a body weight of 140 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 265 Wk 20.0	This specimen was obtained from a 20 year old white woman (Gravida = 3, parity = 1) in her 20th week gestation with a body weight of 135 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. Although all MS-analyte MOMs were normal, her AFAFP MOM (AFAFP MOM = 3.01) was paired to an amniotic fluid specimen, which was elevated. Please see Critique below for further discussion of MS265 and AF265.

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=29; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 261 Wk 19.0	AFP = 7.06 ± 1.01 µg/ml MOM = 0.91 ± 0.09	The AF261 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF261 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 262 Wk 15.0	AFP = 9.98 ± 1.32 µg/ml MOM = 0.58 ± 0.06	The AF262 sample was targeted for a low level AFAFP value in the routine gestational age range. Most labs called AF262 a normal low MOM AFAFP specimen. This AFAFP sample was matched to maternal serum specimen MS262, which also showed low levels of AFP (MOM = 0.45).
AF 263 Wk 18.0	AFP = 8.80 ± 1.02 µg/ml MOM = 0.92 ± 0.10	The AF263 sample was targeted for a negative NTD screen for AFAFP in the routine 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 264 Wk 21.0	AFP = 6.20 ± 0.84 µg/ml MOM = 1.19 ± 0.15	The AF264 sample was targeted as an NTD negative screen in the upper gestational age screening range. All labs categorized AF264 as a negative NTD screen specimen. This specimen had no maternal serum counterpart.
AF 265 Wk 20.0	AFP = 19.05 ± 2.37 µg/ml MOM = 3.01 ± 0.38	The AF265 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 AF265 specimen was paired with maternal serum sample MS265, which was not elevated (MOM = 0.91). Please see Critique below for further discussion of MS265 and AF265.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 18	**Sample #**	**FT 261**	**FT 262**	**FT 263**	**FT 264**	**FT 265**
	Gestational Age (weeks)	11.9	11.2	12.4	13.0	11.4
Maternal Race	Ethnic Group	White	Hispanic	Asian	Black	White
Maternal Weight	Pounds (lbs)	150	140	140	160	125
Maternal Age	Years	25	28	28	29	21
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	53	45	60	67	47
	NT Thickness (mm)	2.90	1.10	1.40	1.05	1.09
	NT - MOM	2.22 ± 0.24	0.97 ± 0.09	0.96 ± 0.10	0.66 ± 0.06	0.92 ± 0.09
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	148.08 ± 27.69	87.00 ± 13.68	68.80 ± 9.17	64.62 ± 8.85	77.33 ± 10.85
	MOM ± Std. Dev.	2.01 ± 0.28	1.06 ± 0.12	0.96 ± 0.09	1.03 ± 0.13	0.91 ± 0.09
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	383.68 ± 102.34	661.97 ± 130.95	1502.66 ± 246.34	1146.64 ± 246.82	773.23 ± 153.03
	MOM ± Std. Dev.	0.42 ± 0.08	0.95 ± 0.18	1.55 ± 0.53	0.86 ± 0.20	0.91 ± 0.16
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (88%)	(-) (100%)	(-) (100%)
	Recommended Action NFA**	G = 94% U = 35% A = 65% C = 53%	NFA	NFA	NFA	NFA
	Risk Estimate	1 in 8	9,600	12,400	8,400	10,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (88%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	297	10,000	10,000	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.

***Results from methods that give IU/ml were converted to ng/ml as described in section D.1 below.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:
N = 18 all-lab Consensus Values.

Sample#	Summary Comments:
FT 261 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 FT261 risk estimate for Trisomy-21 was 1 in 8, while the Trisomy-18 risk was 1 in 297.
FT 262 Wk 11.2	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 11.2 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 FT262 risk estimate for Trisomy-21 was 1 in 9,600, while the Trisomy-18 risk was 1 in 10,000.
FT 263 Wk 12.4	This specimen was obtained from a 28 year old Asian woman of average body weight (140 lbs). Her gestational age at the time of screening was 12.4 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 FT263 risk estimate for Trisomy-21 was 1 in 12,400, while the Trisomy-18 risk was 1 in 10,000.
FT 264 Wk 13.0	This specimen was obtained from a 29 year old black woman of average body weight (160 lbs.). Her gestational age at the time of screening was 13.0 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 FT264 risk estimate for Trisomy-21 was 1 in 8,400, while the Trisomy-18 risk was 1 in 10,000.
FT 265 Wk 11.4	This specimen was procured from a 21 year old white woman with a body weight of 125 lbs. Her gestational age at the time of screening was 11.4 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 FT265 was 1 in 10,000, 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 were consistent with the targeted values for the NTD and the Trisomy Screens for risks, and outcomes. Maternal serum sample MS265 was targeted as a non-elevated specimen for NTD (Figs. 1 and 3), but was matched to an elevated AF265 sample (Fig. 2; see discussion below). All labs agreed that specimen MS265 was screen negative for NTD and both Trisomy screens, but that AF265 was elevated for AFP. The NTD recommended action for the mock specimen AF265 in real patient screening would have been genetic counseling, stage-II ultrasound, fetal hemoglobin, and acetylcholinesterase. Sample MS262 was obtained from a Hispanic woman with a prior family (sibling) history of pregnancy complications. The T21 MOM results for specimen MS262 (MSAFP-MOM = 0.45, MSuE3-MOM = 0.58, MShCG-MOM = 1.57, DIA-MOM = 1.38) were consistent with a T21 positive screen; thus, most labs (86% triple and 93% quad) classified this specimen as T21 screen positive and recommended further action. The MS262 sample produced a risk from the quad test of 1 in 70 and a triple test risk of 1 in 101, both of which were greater than expected from the maternal age alone (1 in 1000). The T21-related recommended action for MS262 triple screen was genetic counseling, 71%; ultrasound, 57%; and amniocentesis, 79%; while the quad test recommended action was genetic counseling, 78%; ultrasound, 63% and amniocentesis was 78%. The remaining samples, MS261, and MS263, and MS264 produced negative screens for NTD, T21, and T18; corrections for body weight were not indicated for these samples. The MS263 specimen, a special case of reduced levels of MShCG, will be further discussed below.

Although the MS265 sample was screen negative for NTD, T21, and T18, the amniotic fluid sample linked to this specimen was problematic. The AF265 sample was determined to have an elevated AFP value by all participating laboratories. This mock patient had been referred to a tertiary care medical center for an amniocentesis due to a family history of pregnancy complications and poor outcomes in several extended and close family members. The maternal serum sample was obtained prior to the amniocentesis, and following amniocentesis, the post-procedure AF specimen (untainted by color) together with the MS sample was then sent to a prenatal biomarker screening center. The AF265 (but not MS265) sample was determined to be screen positive for NTD. One possible cause of an unexplained elevated AFAFP is due to a fetal bleed from needle penetration during the invasive amniocentesis procedure. Less than 1% contamination of fetal blood into the amniotic fluid is sufficient to cause the AFAFP elevation reported by all participating laboratories. In a real-time situation, a fetal hemoglobin and acetylcholinesterase assays would be indicated. The final outcome in this mock patient showed that level-II diagnostic ultrasound showed no presence of a neural tube defect or any other anomaly and a diagnostic Ache band was lacking following gel electrophoresis. In retrospect, AF265 would be deemed a false positive amniotic fluid sample based on the later diagnostic results.

Though the mock normal specimen AF265 with elevated AFAFP would have been later shown to be a false-positive NTD amniotic fluid screen, this is not always the case in clinical practice. A large prognostic study of unexplained elevated amniotic fluid AFP was conducted in a medical center utilizing 5,743 pregnancy case histories (55). This large population study, derived from multiple pregnancy outcomes, found that unexplained elevated amniotic fluid AFP was actually predictive of preeclampsia and preterm delivery. Elevations of AFAFP in prior studies had established such elevations to be associated with multiple congenital disorders such as fetal open neural tube defects, abdominal wall defects, congenital nephrosis, intestinal atresias, and cystic hydromas (56). However, amniotic fluid AFP levels can also be elevated when no fetal anomaly is present and when a diagnostic AF acetylcholinesterase band is absent (57). Acetylcholinesterase, which is derived from neural tissue, is present in AF only in association with open neural tube defects or ventral body wall defects. If fetal blood enters the AF during amniocentesis, its presence can often be observed by discoloration of the fluid. However, this was not the case in the mock AF265 specimen presently discussed. Fetal blood contamination, although not visible, is suspected when an AF-acetylcholinesterase band is absent following gel electrophoresis (58). In the large case study discussed above, which utilized over 5,000 amniocenteses neither discolored fluid nor false-positive acetylcholinesterase levels were identified in many of the patients under study. In that study, differences between the groups were not explained by the number of needle sticks, presence of discolored fluids, rupture of membranes, bleeding after amniocentesis, infectious complications, or occurrence of spontaneous abortion within the first month after the procedure.

The MS263 specimen also presents an interesting case in that the MShCG levels were markedly reduced (MOM = 0.31), while the remaining second trimester biomarkers were slightly low or normal as follows: AFP MOM = 0.81; uE3 MOM = 1.02; and DIA MOM = 0.76. In cases of screen positive maternal serum samples for T18, MShCG, MSAFP, and uE3 levels are significantly reduced to 50% or less than those of non-affected cases. In contrast in MS263, only MShCG was markedly decreased. Physiological levels of hCG rise during the first trimester of pregnancy, fall precipitously by the start of the second trimester, and level out for the remaining of the second and third trimester with a slight upturn at term. The rise in early first trimester and peak of hCG at 9 weeks gestation, and the subsequent decrease to the 20^{th} week have been well-documented in the biomedical literature. Thus, low levels of MShCG during the first trimester of the pregnancy could be associated with genetic disorders, miscalculation of LMP pregnancy dates, possible miscarriage, blighted ovum, or an ectopic pregnancy. Discussion of some of these pregnancy complications together with adverse pregnancy outcomes will be addressed below in addition to hCG levels in non-pregnant and periconceptual situations.

The hCG molecule is a glycoprotein of 244 amino acids with a molecular mass of 36.7 KDa. The hCG glycoprotein represents a heterodimeric complex consisting of an alpha subunit (identical in LH, FSH, and TSH) consisting of 92 amino acids, and a beta subunit constituting 145 amino acids. Together, the two subunits create a small hydrophobic core pocket surrounded by a high surface area-to-volume ratio with the majority of surface amino acids being hydrophilic. The human trophic hormone binds to the hCG receptor resulting in signal transduction cascades that maintain the corpus luteum in order to prevent luteal deterioration during the beginning of pregnancy. This receptor-induced cascade causes the corpus luteum to secrete progesterone which enriches and thickens the uterine endometrium to receive the fertilized egg and sustain the growing embryo. It is the dimeric hCG and the free

beta subunit forms that are employed as biomarkers in the triple and quad screening platforms as outlined by Kaplan and Pesce (28). The hCG hormone is initially produced by the developing embryo cells following fertilization and later by the syncytiotrophoblast cells of the placenta. hCG has been suggested as a causative agent in the placental development of local maternal immunotolerance that allows the developing embryo to reside as an allograft in the body of the mother (28).

In weeks 11-13 of pregnancy hCG and other reproductive hormones have been studied in women with normal pregnancies, threatened abortion, and spontaneous abortions (29). Although there were no significant differences between MShCG levels in normal pregnancies versus threatened abortions, abnormally low MShCG have been reported in women with spontaneous abortions that were initially not detected. When MShCG levels were measured earlier at 6-8 weeks, it was determined that low or reduced MShCG levels served to predict a poor pregnancy outcome, even when associated with an embryo with a positive heartbeat as indicated by ultrasound (30). In another study during the 11 to 13 week period, measurements of low MS-free beta-hCG levels combined with increased nuchal translucency demonstrated an association of reduced free beta-hCG levels with adverse pregnancy outcomes and complications (31). This latter study was noteworthy in that 22,695 pregnancies were studied and the outcome results were linked to a Morbidity Database and a Birth Defects Registry. Following that report, another hospital-clinic-based study of 70 twin gestations was conducted at 11-13 weeks showing that low or reduced levels of free beta hCG correlated with very early preterm birth (<32 weeks gestation) (32). In this study, low free beta hCG was a strong predictor of very early preterm birth, but not classical preterm birth (>32 weeks gestation) in twin pregnancies. Lastly, a study of low free-beta hCG levels in the first trimester demonstrated that the beta subunit had no value in the prediction of pre-eclampsia later in pregnancy (33).

In second trimester screening studies, low MShCG, alone or in combination with one or more biomarkers was to be found a high risk factor for adverse outcomes of pregnancy (34). Combinations of low MShCG with either low MSAFP and/or MSuE3, were strong predictors of missed abortions and perinatal complications. In a further study, low MShCG was found to be significantly associated with small-for-gestational age infants but not birthweight in full-term infants (35). However, low MShCG alone (<0.4 MOM) had no influence on pregnancy outcomes regarding: 1) gestational age at delivery; 2) neonatal full-term birthweight; 3) premature membrane rupture; and 4) overall pregnancy loss (36, 37). Finally, it was recommended that low MShCG required a body weight correction factor for risk assessment in screening black women for Down syndrome and other trisomies (38).

In the course of prenatal screening, a low or reduced MShCG level has been found in association with pregnancies involving chromosome abnormalities and gene defects. In one study involving three clinical case reports of triploidy (69,XXX) detected following amniocentesis, it was found that both low MShCG and MSuE3 levels were usually present (39). The authors of this report suggested that placental insufficiency was a contributing cause of this disorder due to the histological observations that were found and that a known marker for placental insufficiency, MSuE3, was also very low. A subsequent study of fetal triploidy was undertaken in order to identify the parental origin of the extra haploid set of chromosomes and to correlate the clinical findings with biomarker levels (40). The authors of the latter study showed that the extra haploid sets of chromosomes were maternally-derived and that many triploid pregnancies exhibited very low levels of both MShCG and MSuE3, often with normal levels of AFP present. In another report, abnormally low MShCG levels were found associated with prenatal diagnosis of 5p deletion chromosome syndrome (41). A karyotype following amniocentesis revealed a terminal deletion in the short arm of one chromosome-5 as the root cause of this disorder. One of the most common occurrences of low MShCG levels in prenatal screening is associated with Trisomy-18, a condition in which all biomarkers in the triple screen are low or reduced, while in the quad test, only dimeric inhibin A is not affected. Trisomy-18, also known as Edward's syndrome, is a genetic disorder caused by a meiotic nondisjunction producing an extra chromosome 18 (42).

The most frequent occurrence of low or reduced hCG levels in periconceptual women is associated with ectopic pregnancies. This condition is a non-uterine-pregnancy in which the fertilized egg does not implant in the uterus but rather in another location, usually the upper portion of the Fallopian tubes (a tubal pregnancy). In rare instances, the egg may implant in the abdomen, ovary, or in the uterine cervix (44). Since a fertilized egg cannot survive outside the progesterone-supplemented uterus, an ectopic pregnancy cannot proceed normally and maternal tissues/structures are destroyed by proteolytic enzymes of the implantation event which can cause infertility and/or be life-threatening. Vaginal bleeding, amenorrhea, and abdominal pain are usually the signs of an ectopic pregnancy; thus, low MShCG is used as a diagnostic marker in this disorder (45, 46). In fact, low and declining

MShCG levels are used as a discriminating measure of tubal rupture and the progression status of ectopic pregnancies (44, 47). Declining levels of MShCG often correlate with trophoblastic tissue in regression because the egg is unable to properly implant and the trophoblastic tissue begins to undergo autophagy (48).

Other causes of low MShCG in pregnant and pre- and post-pregnant adult women can be related to conditions which include: 1) hydatidiform molar pregnancy (gestational trophoblastic disease); 2) in vitro fertilization and assisted reproduction procedures; and 3) autoantibodies to hCG that cause infertility. Gestational trophoblastic disease is viewed as any type of abnormal proliferation of trophoblast tissue at the onset and during pregnancy presenting as abnormal placental tissue due to a faulty fertilization (49). Following an assisted reproduction procedure such as embryo transfer, measurement of hCG levels are used to monitor fertilization success for two weeks following the surgical procedure and after low dose hCG administration (32, 50-52). Overall, aberrant serum levels of hCG have been associated with recurrent pregnancy loss, abortion, infertility, extended anovulatory periods, and in patients exhibiting anti-hCG autoantibodies in infertile women following an abortion (28, 53).

Clinically, it has been recommended that pregnant women with unexplained low hCG, uE3, or inhibin-A levels in the second trimester should receive normal antenatal care, as this pattern of analytes has not been associated with adverse perinatal outcomes (54). However, in the second trimester screening setting, low levels in 2 of 3 analytes in the triple test, and 3 or 4 analytes in the quad test are likely to trigger a screen risk result for chromosomal or genetic defects such as Trisomy-18, especially when MShCG and MSuE3 are involved. In comparison, using first trimester screening, an unexplained low PAPP-A (<0.4 MOM) and/or a low hCG (<0.5 MOM) has been associated with an increased frequency of adverse obstetrical outcomes; at present, however, no specific protocol for treatment is available (54).

B) <u>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 (Figs. 7-10) for each of the five MS samples. As shown in Figs. 7A and 8A, AFP and uE3 mass measurements in serum among the individual kits mostly agreed, although Siemens DPC Immulite was about 10% lower for AFP, and 10-20% higher for uE3. In contrast, when the kit specific uE3 MOMs were compared, DPC immulite 2000/2500 and New DPC 2000/2500 ranged from 20 to 40% higher than other kits (Fig. 8B). Regarding the hCG kits (Fig. 9), the two Beckman instruments (Access2 and UNICEL DXL) yielded similar mean hCG values, while the DPC Immulite/2000 results were 10-20% lower than those from the other assay platforms. Finally, the method comparison for Inhibin-A displayed in Fig. 10 shows that the results from the Beckman Access/2 or Unicel were similar and that the Diagnostic Systems Lab (DSL) assay platform was 25-30% lower.

Interestingly, when the AFP measurements in amniotic fluid were compared, the differences among the various methods seemed somewhat larger than in serum (Fig. 7B). In particular, results from the Beckman Unicel DXL instrument were about 30-40% lower than those from the Abbot AxSym, with the results from the other instruments somewhere in between. Since these specimens are derived from actual AF samples, these levels would be comparable to real patient testing**.**

C) <u>Second Trimester Screening Software Utilized:</u>

The alpha and Benetech software packages were each used by 58% of the labs; Robert Maciel (RMA) software was employed by 29%; and in-house and "other" softwares comprised 14%. Labs using programs classified as "other" are presumably proprietary software packages.

D) <u>First Trimester Screen:</u>

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 FT Table 2, Section II, the all lab measurement of the 11.9 week Caucasian FT261 specimen for total hCG resulted in a mass mean of 148.08 IU/ml ± 27.69, with an elevated MOM of 2.01± 0.28 (Table 2). Furthermore, the all-lab mass mean for PAPP-A was 383.68 ± 102.34 ng/ml with a MOM of 0.42 ± 0.08. This resulted in an all-lab T21 risk assessment of 1 in 8 for the FT261 specimen. Since analyte MOM values for the first trimester Down syndrome screen detection are associated with raised NT, low PAPP-A, and high hCG MOMs, the FT261 results (Fig. 13) were consistent with a T21 positive screen. Together with the low PAPP-A of 0.42 MOM, the elevated NT and hCG clearly produced a positive screen. Thus, the FT261 sample resulted in a 100% T21 positive screen assessment. Further actions by the labs included genetic counseling, 94%; ultrasound, 35%; and amniocentesis/CVS = 65/53%. Finally, the FT261 specimen screened negative for T18 (1 in 297) using a risk cutoff of 1 in 100.

The all lab measurement of the 11.2 week Hispanic FT262 specimen for total hCG resulted in a mass mean of 87.00 ± 13.68 IU/ml, with a MOM of 1.06 ± 0.12; the all-lab mass mean for PAPP-A was 661.97 ± 130.95 ng/ml with a MOM of 0.95 ± 0.18; and the all-lab T21 risk assessment was 1 in 9,600. The risk cut-off level for Hispanics ranges from 1 in 169 to 1 in 270 among the participating labs. Thus, the FT262 sample resulted in a 100% T21 negative screen assessment. No further action was indicated. Finally, the FT262 specimen also screened negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Figs. 13, 14).

In the FT263 Asian sample, the gestational age all-lab mean was reported as 12.4 weeks. Assay measurements for FT263 resulted in an all-lab total hCG mass measurement of 68.80 ± 9.17 IU/ml (MOM = 0.96 ± 0.09), while the all-lab PAPP-A mass assessment was 1502.66 ± 246.34 ng/ml (MOM = 1.55 ± 0.53). All labs agreed that the FT263 sample was screen negative for T21 with a risk assessment of 1 in 12,400 (Fig. 13). The all-lab T18 risk assessment for FT263 was 1 in 10,000; hence, the FT263 specimen resulted in a negative screen for T18 (Fig. 14).

As shown in Table 2 for the FT264 black 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 64.62 ± 8.85 IU/ml (MOM = 1.03 ± 0.13) and an all-lab PAPP-A mass measurement of 1146.64 ± 246.82 ng/ml (MOM = 0.86 ± 0.20). The all-lab T21 screen consensus for FT264 was negative with a risk assessment of 1 in 8,400. Similarly, the risk assessment for T18 was 1 in 10,000.

For the Caucasian FT265 specimen, the gestational age all-lab mean was reported as 11.4 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 77.33 ± 10.85 IU/ml (MOM = 0.91 ± 0.09) while the all-lab PAPP-A mass assessment was 773.23 ± 153.03 ng/ml (MOM = 0.91 ± 0.16). The all-lab FT T21 risk assessment was 1 in 10,000 and all labs agreed that the FT265 sample was screen negative for T21 (Fig. 13). The FT265 specimen also resulted in a negative screen for T18 with an all-lab risk assessment of 1 in 10,000.

D. 1.) <u>First Trimester Assay kit Performance:</u>

In order to compare the new Beckman 2/Unicel assays (65% users) for PAPP-A with those of the older DPC and DSL assay platforms, a conversion factor was calculated from participating labs from the last four PT mailouts. Using 20 data points, Beckman Access 2/Unicel (y-axis) data for PAPP-A were plotted (Fig. 15A) versus DPC Immunlite 2000 (x-axis) data, yielding a linear correlation with an R^2 value of 0.9586 and a slope of 0.232. In Fig. 15B, Beckmann Access2/Unicel PAPP-A values (y-axis) were plotted against DSL PAPP-A values (y-axis), yielding a second degree polynomial correlation with an R^2 value of 0.9959. Using the respective correlation equation allowed us to convert mIU/ml values into ng/ml and to directly compare Beckman 2 PAPP-A mass units of ng/ml to the mIU/mL mass units generated by DPC Immulite and DSL (Fig. 12A). However, for grading purposes, each lab's results were compared to their own peer group without conversion.

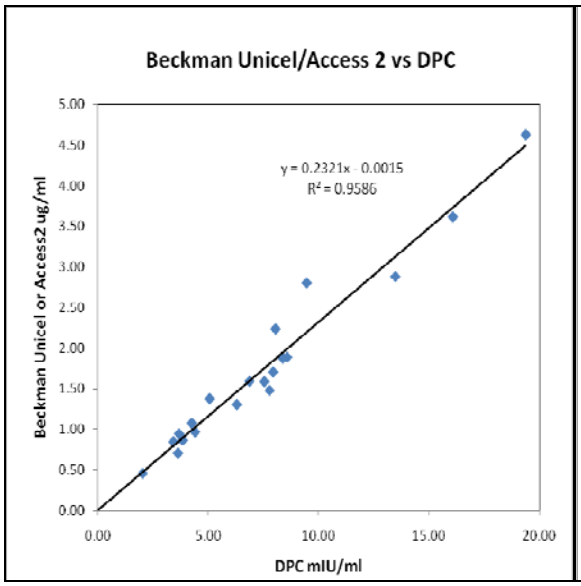

Fig. 15A

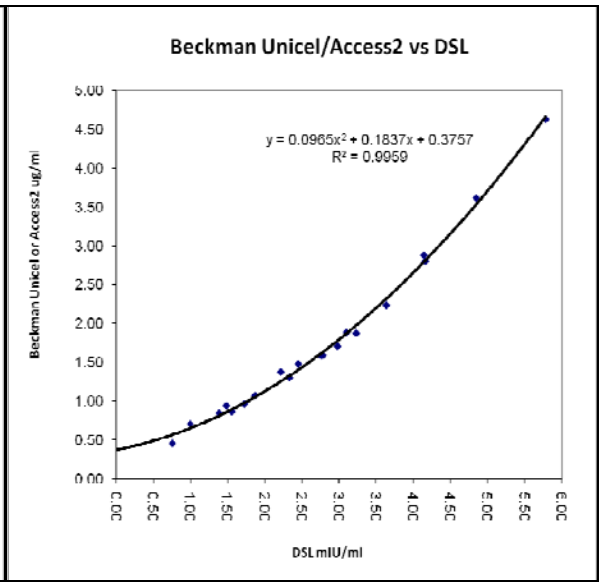

Fig. 15B

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11, 12 for each of the five FT samples. As shown in Fig. 11, hCG measurements between the two kits differed somewhat, with the Beckman Unicel/Access instruments measuring approximately 30% above the Siemens/Immulite instruments. Furthermore, the results from the three PAPP-A kits (see above) varied widely with the mean/all kit median values from Diagnostic Systems Lab (DSL) and Beckman Access/Unicel DXL being around 40% lower than 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 (Fig. 12B).

E) <u>First Trimester Screening Software Utilized:</u>

The alpha and Benetech software packages were each used by 29% and 24% of the labs, respectively; Robert Maciel (RMA) software was employed by 29%; and in-house software comprised 18%. 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 (this website no longer exists – This page cannot be found)

4) www.pregnancy-info.net/slpha_feto_protein.html (this website no longer exists – Error 404 Page not found)

5) www.healthopedia.com/alpha-fetoprotein

Teachings on Alpha-fetoprotein

Vol. 5, Part 1

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

The many physiologic roles of human alpha fetoprotein (HAFP) and its correlation with perinatal distress/pregnancy outcome are rarely addressed together in the biomedical literature, even though HAFP has long been used as a biomarker for fetal birth defects. Although the well being of the fetus can be monitored by the measurement of gestational age-dependent HAFP in biologic fluid levels (serum, amniotic fluid, urine, and vaginal fluids) throughout pregnancy, the majority of clinical reports reflect largely second trimester and (more recently) first trimester testing due to regulatory clinical restrictions. However, reports of third-trimester and pregnancy term measurement of HAFP levels performed in clinical research and/or investigational settings have gradually increased over the years and have expanded our base knowledge of AFP-associated pregnancy disorders during these stages. The different structural forms of HAFP (isoforms, epitopes, molecular variants, etc.) detected in the various biologic fluid compartments have been limited by antibody recognition of specific epitopic sites developed by the kit manufacturers based on antibody specificity, sensitivity, and precision. Concomitantly, the advances in elucidating the various biologic actions of AFP are opening new vistas towards understanding the physiologic roles of AFP during pregnancy. The present review surveys HAFP as a biomarker for fetal distress during the perinatal period in view of its structural and functional properties. An attempt is then made to relate the AFP fluid levels to adverse pregnancy complications and outcomes. Hence, the present review was divided into two major sections: (I) AFP structure and function considerations and (II) the relationship of AFP levels to the distressed fetus during the third trimester and at term.

INTRODUCTION

A. Human alpha-fetoprotein (HAFP) is a tumor associated fetal glycoprotein involved with both ontogenic and oncogenic growth (1, 2). The fetal protein is a 69-kDa single-polypeptide chain that contains 3%-5% carbohydrate and is produced in the yolk sac and fetal liver. It exhibits a triplicate domain structure configured by intramolecular loops dictated by disulfide bridging, resulting in a helical V- or U-shaped form observed in electron dot maps (3). Mammalian AFP has been classified as a member of a three-domain, cystein-rich translated protein of the albuminoid gene family that currently consists of four members: albumin (ABL), vitamin D-binding protein, AFP, and α-ALB (4, 5). In the clinical laboratory, HAFP has long been employed both as a postoperational tumor monitoring agent and as a gestational age-dependent fetal defect marker demonstrating utility in screening for neural tube defects and aneuploidies (Table 1; (6-8). However, the main biologic role of AFP during pregnancy remains controversial to this day.

Presently, the vast biomedical literature has amassed concerning the use of HAFP during pregnancy as a biomarker in human maternal serum (MS) and amniotic fluid. Such studies have addressed the measurement of serum levels of AFP outside the normal levels in the sera of pregnant women; such values are indicative of multiple congenital malformations of the embryo and fetus. The first developmental abnormalities to be associated with abnormal AFP levels

were neural tube defects and brain/spinal cord malformations (9, 10). Later, other types of birth defects were found to reflect discordant AFP levels, including chromosomal abnormalities (anaploidies) and various anatomic congenital disorders (11, 12). It was subsequently determined that assaying additional analytes together with HAFP increased the prenatal screening detection rates (Table 2). While MS-AFP levels associated with neural tube fetal defects are elevated, the chromosomal disorders demonstrate low serum AFP concentrations. Following the association of increased AFP levels with neural tube defects, additional structural anomalies have been classified within the AFP-elevated level category (6, 13). AFP serum levelsduring pregnancy also have been used as an ancillary aid in the diagnosis of pregnancy-related hematologic disorders (anemias), placental abnormalities, fetal death, growth restriction/retardation, and preterm labor (14). However, there exists a paucity of reports that have attempted to correlate such pregnancy anomalies with the multiple biologic activities attributed to AFP in the last decade.

B. The focus of the present report has addressed the increasing number of reports associated with abnormal AFP serum and amniotic fluid levels concomitant with perinatal complications and adverse pregnancy outcomes. This review has attempted to link the many functional roles of AFP with physicochemical stress/shock conditions of the fetus during the perinatal period of pregnancy. Moreover, the various physiological roles attributed to AFP in the regulation of growth and differentiation during fetal/neonatal development has not kept pace with the increase of clinical research reports. Thus, the objectives of the present review are twofold. First, the biological activities of AFP during late pregnancy will be addressed, since many reviews have focused solely on AFP as a second-trimester fetal defect biomarker. Second, the various properties of AFP in light of its investigational utility as a clinical biomarker during perinatal development will be surveyed. Thus, the multitude of fetal malformations, congenital anomalies, and genetic diseases with adverse pregnancy outcomes associated with AFP in late pregnancy will be addressed. For more intense accounts of the physicochemistry and genetics of AFP, the reader is directed to earlier reviews on AFP (15-19). Recently, a report on the functional mapping of the various domains and motifs of the HAFP molecule has been put forward (20). In view of the above discussion, the present review has been divided into two major sections. These will include the following: (I) structural and functional aspects of AFP and (II) the relevance of AFP fluid levels as a biomarker in the perinatal period to adverse pregnancy outcome.

Table 1. Prenatal Screening Timeline for AFP Employed as a Biomarker Alone or in Combination with Other Analytes[a]

Year	Observation/Event	Reference
1972	Elevated AFP in amniotic fluid for neural tube defects. Indication: Potential biomarker.	Brock, DJH *et al* Lancet Vol. 2: 191-194, 1972
1973	Elevated AFP in MS for neural tube defects. Indication: Potential biomarker.	Leek, AE *et al* Lancet Vol. 2: 385-36, 1973
1980	Early antenatal diagnosis of ventral wall defect using AFP.	Wald, NJ *et al* Lancet 1: 368, 1980
1981	Maternal weight influence on MS-AFP level in prenatal screening.	Haddow, JE *et al*, Clin. Chem. Vol. 27: 133, 1981.
1981	Amniotic fluid acetylcholinesterase diagnoses for neural tube defects with elevated AFP levels.	Collaboration Study, Lancet, Vol. 2: 321-323, 1981.
1984	Low maternal serum AFP levels discovered in prenatal Down syndrome pregnancy samples.	Merkatz IR, *et al* Amer. J. Obstet. Gynecol. 148: 886-894, 1984.
1987	Combination of maternal age and AFP levels useful in Down syndrome pregnancies.	Cuckle, HS *et al* Brit. J. Obstet. Gynecol. 94: 387, 1987.
1989	Screening for Down syndrome using AFP, μE3, and hCG (triple biomarker test).	Wald, NJ Amer. J. Human Genetics, Vol. 44:586, 1989.
1990	Triple marker screen used to detect Trisomy-18 chromosomal disorders.	Canick, JA, *et al*. Prenat. Diag. 10:546, 1990.
1991	Low MS-AFP in congenital diaphragmatic defects.	Resta, RG. Amer. J. Med. Genet. 40:129, 1991.
1992	Prenatal screen in MS using multiple markers for fetal distress.	Haddow *et al*, New Eng. J. Med. Vol. 327: 588, 1992.
1994	Four-marker serum screening for Down syndrome (quad test) using AFP, estriol, hCG, Inhibin-A.	Wald, NJ. Prenat. Diagn. 14: 707-716, 1994.
2003	Comparison of triple vs. quad test for Down syndrome using AFP, estriol, hCG, and Inhibin-A.	Wald, NJ *et al*. Lancet 361: 835-836, 2003.
2003	First- and second-trimester screening for Down syndrome, integrated testing (SURUSS trials).	Wald, NJ *et al*. Health technology assessment. Vol. 7 (11):1-30, 2003.
2004	Combined (sequential) first- and second-trimester screening for Down syndrome using PAPP-A, B-hCG, followed by the AFP triple test.	Platt, LD *et al*. Vol. 104: 661-666, 2004.
2004	Frist-trimester PAPP-A and B-hCG, NT comparison levels for Down syndrome (FASTER trials).	Dugoff, L. *et al*. Amer. J. Obstet. Gynecol. 2004.
2005	First and second combined screen for Down syndrome (FASTER trial follow-up).	Malone, FD *et al* New Eng. J. Med. 353:2001, 2005.

[a]MS, maternal serum; hCG, human chorionic gonadotropin; Estriol, unconjugated estriol; SURRUSS, prenatal screening clinical trials held in United Kingdom; PAPP-A, pregnancy-associated plasma protein-A; B-hCG, beta-hCG; NT, nuchal translucency; FASTER, prenatal screening clinical trials held in the United States.

Table 2. Detection Rate Percent (%) Produced by Increasing the Number of Analyte Screening Components in Prenatal Assays with the False-Positive Rate Constant at 5%[a]

Screening components (agents)[b]	% Detection rate[b]
1. Maternal age alone	35-40
2. Maternal age plus MS-AFP in second trimester	40-45
3. MS-AFP plus MS hCG in second trimester	50-55
4. MS-AFP plus MS hCG plus MS E3 in second trimester (triple test)	65-70
5. MS-AFP, MS hCG, MS E3, and Inhibin-A in second trimester (Quad test)	70-80
6. Combined test: first-trimester maternal age, NT, PAPP-A, hCG	80-85
7. Serum integrated test: PAPP-A plus Quad	85-90
8. Integrated: first trimester (NT, PAPP-A, hCG) plus second trimester Quad	90-95

[a]Serum integrated test indicates maternal serum PAPP-A in first trimester followed by Quad test in second trimester. Integrated indicates maternal serum PAPP-A and hCG in first trimester followed by Quad test in the second trimester. MS E3, maternal serum unconjugated estriol.

[b]Range of three to four studies each. See Table 1 for reference citations.

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New York State Fetal Defect Markers Proficiency Test,
January 2011
Summary of First Trimester Results

	FT261	FT262	FT263	FT264	FT265
FT Gestational Age All Lab Mean:					
Mean	11.9	11.2	12.4	13.0	11.4
SD	0.09	0.13	0.09	0.07	0.09
%CV	0.8%	1.2%	0.7%	0.5%	0.8%
X+3*SD	12.2	11.6	12.7	13.2	11.7
X-3*SD	11.6	10.8	12.1	12.8	11.2
N	18	18	18	18	18

	FT261	FT262	FT263	FT264	FT265
FT NT MoMs All Lab Mean:					
Mean	2.22	0.97	0.96	0.66	0.92
SD	0.24	0.09	0.10	0.06	0.09
%CV	11.0%	9.7%	10.0%	9.7%	10.3%
X+3SD	2.95	1.25	1.25	0.85	1.21
X- 3SD	1.49	0.69	0.67	0.47	0.64
N	17	17	17	17	17
All Median	2.13	0.97	0.93	0.65	0.91

New York State Fetal Defect Markers Proficiency Test,
January 2011
Summary of First Trimester Results

	FT261	FT262	FT263	FT264	FT265
FT hCG All Lab Mean:					
mean	148.08	87.00	68.80	64.62	77.33
SD	27.69	13.68	9.17	8.85	10.85
%CV	18.7%	15.7%	13.3%	13.7%	14.0%
X+3SD	231.2	128.0	96.3	91.2	109.9
X-3SD	65.0	46.0	41.3	38.1	44.8
N	17	17	17	16	17
mean/All kit median	1.07	1.05	1.03	1.04	1.05
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	115.2	73.2	61.4	55.9	65.8
SD	18.5	10.5	7.1	4.3	6.4
%CV	16.1%	14.3%	11.6%	7.7%	9.7%
X+3SD	170.8	104.5	82.8	68.8	84.9
X-3SD	59.6	41.8	40.1	43.0	46.7
N	5	5	5	5	5
median	103.0	69.0	59.0	56.9	62.6
mean/All kit median	0.83	0.88	0.92	0.90	0.89
FT hCG kit average:					
mean	138.5	83.0	66.6	62.2	74.0
SD	33.0	13.9	7.4	8.9	11.5
all kit median	138.5	83.0	66.6	62.2	74.0

	FT261	FT262	FT263	FT264	FT265
FT hCG Beckman Unicel or Access (BCU or BCX/BC1) mean:					
mean	161.8	92.8	71.9	68.6	82.1
SD	17.1	10.4	8.3	7.4	8.4
%CV	10.6%	11.3%	11.5%	10.8%	10.3%
X+3SD	213.2	124.1	96.7	90.8	107.4
X-3SD	110.4	61.4	47.0	46.3	56.9
N	12	12	12	11	12
median	163.7	91.0	69.7	68.3	82.8
mean/All kit median	1.17	1.12	1.08	1.10	1.11

	FT261	FT262	FT263	FT264	FT265
FT hCG MoMs All Lab Mean:					
Mean	2.01	1.06	0.96	1.03	0.91
SD	0.28	0.12	0.09	0.13	0.09
%CV	13.7%	11.5%	9.3%	12.8%	9.8%
mean+3*SD	2.83	1.42	1.23	1.43	1.18
mean- 3*SD	1.18	0.69	0.69	0.63	0.64
N	16	16	16	15	16
All Median	2.04	1.08	0.99	1.04	0.94

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	FT261	FT262	FT263	FT264	FT265
FT PAPP-A All Lab Mean:					
Mean	383.68	661.97	1502.66	1146.64	773.23
SD	102.34	130.95	246.34	246.82	153.03
%CV	26.7%	19.8%	16.4%	21.5%	19.8%
mean + 3SD	690.70	1054.81	2241.68	1887.09	1232.33
mean- 3SD	76.66	269.12	763.64	406.18	314.12
N	17	17	17	16	17
All Lab Median	343.00	603.12	1410.55	1075.85	725.92
mean/All kit median	0.78	0.93	1.06	1.09	0.98

	FT261	FT262	FT263	FT264	FT265
***FT PAPP-A DPC Immullite or 2000 or 2500 (DPB or D or F/DP5) Mean:**					
Mean	489.73	903.64	1974.40	1618.51	1067.66
SD	51.33	41.54	77.34	45.38	70.25
%CV	0.10	0.05	0.04	0.03	0.07
X + 3SD	643.71	1028.27	2206.42	1754.66	1278.40
X - 3SD	335.76	779.02	1742.38	1482.36	856.92
N	3	3	3	3	3
Kit Median	471.16	893.59	1986.78	1622.38	1090.87
mean/All kit median	1.00	1.27	1.39	1.54	1.35

FT PAPP-A Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	313.71	582.51	1397.10	1034.38	688.70
SD	23.79	36.48	81.09	81.30	44.63
%CV	7.6%	6.3%	5.8%	7.9%	6.5%
X + 3SD	385.06	691.95	1640.37	1278.27	822.59
X - 3SD	242.35	473.07	1153.84	790.49	554.81
N	11	11	11	10	11
Kit Median	310.0	585.0	1403.4	1039.1	681.0
mean/All kit median	0.64	0.82	0.99	0.99	0.87

***FT PAPP-A Diagnostic Systems Lab (DS1) Mean:**					
Mean	534.18	711.64	1417.94	1048.96	788.72
SD	23.42	52.58	201.88	117.36	52.64
%CV	4.4%	7.4%	14.2%	11.2%	6.7%
X + 3SD	0.87	1.53	3.40	2.51	1.68
X - 3SD	0.42	0.75	1.52	1.19	0.96
N	3	3	3	3	3
Kit Median	520.66	718.64	1505.66	1089.69	790.72
mean/All kit median	1.09	1.00	1.00	1.00	1.00

FT PAPP-A kit average:					
mean	445.87	732.60	1596.48	1233.95	848.36
SD	116.60	161.59	327.45	333.12	196.39
all kit median	489.73	711.64	1417.94	1048.96	788.72

***Note: The above 2 tables contain converted values (mIU/ml->ng/ml) from equations obtained based on in house correlation data.**
(see critique)

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FT PAPP-A MoM All Lab Mean:

Mean	0.42	0.95	1.55	0.86	0.91
SD	0.08	0.18	0.53	0.20	0.16
%CV	18.4%	19.1%	34.2%	23.8%	17.2%
mean + 3SD	0.65	1.50	3.15	1.47	1.38
mean- 3SD	0.19	0.41	-0.04	0.25	0.44
N	14	14	16	13	14
All Lab Median	0.42	0.96	1.46	0.88	0.89
mean/ All kit median	0.96	0.96	1.05	0.94	0.97

FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:

Mean	1.18	2.48	3.47	2.20	2.57
SD	0.38	0.57	1.23	0.60	0.88
%CV	32.7%	22.9%	35.5%	27.1%	34.2%
X + 3SD	2.33	4.18	7.18	3.98	5.21
X - 3SD	0.02	0.78	-0.23	0.41	-0.07
N	3	3	3	3	3
mean/All kit median	2.70	2.51	2.35	2.40	2.73

FT PAPP-A MoM Beckman Unicel or Access (BCU or BCX/BC1) Mean:

Mean	0.44	0.99	1.48	0.92	0.94
SD	0.05	0.14	0.17	0.14	0.13
%CV	12.4%	14.4%	11.6%	14.9%	13.9%
X + 3SD	0.60	1.42	1.99	1.32	1.33
X - 3SD	0.27	0.56	0.96	0.51	0.55
N	10	10	10	9	10
Kit Median	0.43	0.96	1.50	0.96	0.89
mean/All kit median	1.00	1.00	1.00	1.00	1.00

FT PAPP-A MoM Diagnostic System Labs (DS1) Mean:

Mean	0.42	0.96	1.27	0.86	0.92
SD	0.11	0.19	0.16	0.18	0.16
%CV	25.3%	19.5%	12.7%	21.0%	17.7%
X + 3SD	0.74	1.52	1.76	1.40	1.40
X - 3SD	0.10	0.40	0.79	0.32	0.43
N	3	3	3	3	3
Kit Median	0.40	0.99	1.19	0.84	0.95
mean/ All kit median	0.97	0.97	0.86	0.94	0.97

FT PAPP-A MoM kit average:

mean	0.68	1.48	2.07	1.32	1.48
SD	0.43	0.87	1.22	0.76	0.95
all kit median	0.44	0.99	1.48	0.92	0.94

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	MS 261	MS 262	MS 263	MS 264	MS 265
Gestational Age All Lab Mean:					
Mean	18.0	15.0	17.0	16.0	20.0
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	16.0	20.0
X-3*SD	18.0	15.0	17.0	16.0	20.0
N	28	28	28	28	28

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP All Lab Mean:					
mean	38.99	13.10	32.56	29.24	57.82
SD	2.61	0.90	2.43	1.77	3.69
%CV	6.7%	6.9%	7.5%	6.1%	6.4%
mean+3SD	46.8	15.8	39.9	34.6	68.9
mean-3SD	31.2	10.4	25.3	23.9	46.8
N	28	28	28	28	28
median	38.9	13.2	33.4	29.1	58.5
mean/all kit median	0.99	1.00	0.99	0.98	0.97

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	39.7	13.0	32.4	29.8	59.3
SD	1.9	0.5	2.7	1.3	3.6
%CV	4.7%	3.8%	8.4%	4.3%	6.1%
mean + 3SD	45.4	14.5	40.6	33.6	70.2
mean - 3SD	34.0	11.5	24.2	25.9	48.4
N	9	9	9	9	9
Median	39.7	13.0	33.7	29.2	59.2
mean/All kit median	1.01	0.99	0.98	1.00	0.99

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP MoMs All Lab Mean:					
mean	0.78	0.45	0.81	0.83	0.91
SD	0.08	0.04	0.08	0.07	0.08
%CV	10.2%	9.9%	9.9%	8.8%	8.4%
mean+3SD	1.02	0.58	1.05	1.05	1.14
mean-3SD	0.54	0.32	0.57	0.61	0.68
N	28	28	28	28	28

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP DPC Immulite or 2000 (DPB or DPD/DP5) mean:					
mean	36.3	12.2	30.6	27.2	53.7
SD	2.0	0.9	2.2	1.3	2.2
%CV	5.5%	7.6%	7.1%	4.7%	4.1%
mean+3SD	42.3	15.0	37.1	31.0	60.4
mean-3SD	30.3	9.4	24.0	23.4	47.0
N	8	8	8	8	8
median	36.4	12.3	30.6	27.3	54.2
mean/all kit median	0.92	0.93	0.93	0.91	0.90

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP Beckman Access (BCX/BC1) mean:					
mean	40.9	13.8	34.2	29.9	60.1
SD	2.1	0.4	1.0	1.1	1.7
%CV	5.2%	3.3%	2.8%	3.8%	2.8%
mean+3SD	47.4	15.1	37.0	33.3	65.0
mean-3SD	34.5	12.4	31.3	26.5	55.1
N	8	8	8	8	8
median	41.6	13.8	34.5	30.0	60.0
mean/all kit median	1.04	1.05	1.04	1.00	1.01

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP kit average:					
mean	39.0	13.0	32.4	29.0	57.7
SD	2.4	0.8	1.8	1.5	3.5
all kit median	39.7	13.0	32.4	29.8	59.3

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 All Lab Mean:					
mean	1.16	0.32	0.93	0.75	1.48
SD	0.14	0.05	0.09	0.12	0.14
%CV	11.7%	14.6%	10.1%	16.3%	9.8%
mean+3SD	1.57	0.47	1.21	1.12	1.91
mean-3SD	0.76	0.18	0.65	0.38	1.04
N	27	27	27	27	27
mean/all kit median	1.06	1.06	1.05	1.09	1.06

MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	1.09	0.31	0.88	0.67	1.40
SD	0.11	0.05	0.08	0.07	0.09
%CV	10.1%	14.9%	9.7%	10.3%	6.3%
mean+3SD	1.42	0.44	1.13	0.88	1.66
mean-3SD	0.76	0.17	0.62	0.47	1.13
N	9	9	9	9	9
Median	1.08	0.29	0.87	0.64	1.39
mean/all kit median	0.94	0.94	0.94	0.91	0.94

MS uE3 BeckmanAccess (BCX/BC1) mean:					
mean	1.10	0.31	0.88	0.69	1.40
SD	0.08	0.04	0.07	0.07	0.12
%CV	6.9%	11.4%	8.2%	9.7%	8.9%
mean+3SD	1.33	0.41	1.10	0.89	1.77
mean-3SD	0.87	0.20	0.66	0.49	1.03
N	8	8	8	8	8
median	1.10	0.31	0.88	0.69	1.40
mean/all kit median	0.95	0.94	0.94	0.92	0.94

MS uE3 DPC Immulite 2000 or 2500(DPD or F/DP5) mean:					
Mean	1.34	0.36	0.98	0.94	1.57
SD	0.07	0.06	0.05	0.07	0.04
%CV	5.5%	16.6%	4.9%	7.6%	2.2%
mean+3SD	1.56	0.54	1.13	1.15	1.68
mean-3SD	1.12	0.18	0.84	0.72	1.47
N	5	5	5	5	5
Median	1.32	0.38	1.00	0.98	1.57
mean/all kit median	1.15	1.12	1.06	1.26	1.06

MS uE3 New generation DPC Immulite 2000 or 2500(DPD or F/DP6) mean:					
Mean	1.23	0.34	1.03	0.80	1.66
SD	0.13	0.03	0.06	0.08	0.11
%CV	10.5%	7.9%	5.6%	9.8%	6.9%
mean+3SD	1.56	0.54	1.13	1.15	1.68
mean-3SD	1.12	0.18	0.84	0.72	1.47
N	5	5	5	5	5
Median	1.25	0.36	1.02	0.77	1.71
mean/All Kit Median	1.05	1.06	1.10	1.08	1.11

MS UE3 kit average:					
mean	1.19	0.33	0.94	0.78	1.51
SD	0.12	0.03	0.08	0.12	0.13
all kit median	1.16	0.33	0.93	0.74	1.49

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs All Lab Mean:					
Mean	1.02	0.58	1.02	1.04	0.83
SD	0.28	0.19	0.29	0.36	0.21
%CV	27.1%	32.0%	28.8%	34.8%	25.0%
X+3SD	1.86	1.14	1.90	2.13	1.45
X-3SD	0.19	0.02	0.14	-0.05	0.21
N	27	27	27	27	27
mean/All Kit Median	1.00	0.95	0.94	0.98	0.95

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoM (DPD or F/DP5) Mean:					
Mean	1.48	0.83	1.38	1.61	1.09
SD	0.04	0.12	0.11	0.11	0.11
%CV	2.8%	14.8%	7.6%	6.9%	10.5%
X+3SD	1.60	1.19	1.69	1.94	1.44
X-3SD	1.35	0.46	1.06	1.28	0.75
N	5	5	5	5	5
mean/All Kit Median	1.45	1.36	1.27	1.51	1.26

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs (BCU/BC1) Mean:					
Mean	0.84	0.47	0.82	0.79	0.68
SD	0.10	0.07	0.09	0.08	0.06
%CV	11.4%	15.1%	11.4%	9.9%	9.4%
X+3SD	1.12	0.68	1.10	1.02	0.88
X-3SD	0.55	0.25	0.54	0.56	0.49
N	9	9	9	9	9
mean/All Kit Median	0.82	0.76	0.76	0.74	0.79

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoM (DPD or F/DP6) Mean:					
Mean	1.20	0.75	1.34	1.33	1.05
SD	0.18	0.13	0.28	0.24	0.12
%CV	15.3%	17.5%	21.2%	18.1%	11.3%
X+3SD	1.74	1.15	2.18	2.05	1.40
X-3SD	0.65	0.36	0.49	0.61	0.69
N	5	5	5	5	5
mean/All Kit Median	1.17	1.24	1.23	1.25	1.21

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs (BCX/BC1) Mean:					
Mean	0.84	0.45	0.83	0.80	0.69
SD	0.08	0.05	0.08	0.08	0.07
%CV	9.1%	11.3%	9.4%	9.6%	9.8%
X+3SD	1.07	0.60	1.07	1.03	0.89
X-3SD	0.61	0.30	0.60	0.57	0.49
N	8	8	8	8	8
mean/All Kit Median	0.83	0.74	0.77	0.75	0.79

	MS 261	MS 262	MS 263	MS 264	MS 265
MS UE3 MoM kit average:					
mean	1.09	0.62	1.09	1.13	0.88
SD	0.31	0.19	0.31	0.41	0.22
all kit median	1.02	0.61	1.08	1.06	0.87

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG All Lab Mean:					
mean	18.23	57.98	7.49	25.07	16.91
SD	1.75	6.93	0.62	2.72	1.82
%CV	9.6%	12.0%	8.3%	10.8%	10.8%
mean+3SD	23.5	78.8	9.4	33.2	22.4
mean-3SD	13.0	37.2	5.6	16.9	11.4
N	28	28	28	28	28
mean/all kit median	0.98	0.94	0.98	0.97	0.96

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	18.69	60.36	7.59	25.52	17.48
SD	1.89	4.34	0.52	2.86	1.68
%CV	10.1%	7.2%	6.8%	11.2%	9.6%
mean+3SD	24.58	79.13	9.15	33.13	21.75
mean-3SD	12.79	46.97	6.30	19.09	13.75
N	9	9	9	9	9
median	18.50	59.90	7.70	25.80	17.60
mean/All kit median	1.00	0.98	0.99	0.99	0.99

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG Beckman Access (BCX/BC1) mean:					
mean	18.7	63.1	7.7	26.1	17.8
SD	2.0	5.4	0.5	2.3	1.3
%CV	10.5%	8.5%	6.1%	9.0%	7.5%
mean+3SD	24.6	79.1	9.1	33.1	21.7
mean-3SD	12.8	47.0	6.3	19.1	13.8
N	8	8	8	8	8
median	19.3	64.7	7.8	25.7	17.9
mean/all kit median	1.00	1.02	1.01	1.01	1.01

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	17.1	49.8	7.1	22.8	15.3
SD	1.2	2.8	0.6	1.7	1.2
%CV	6.8%	5.5%	9.1%	7.3%	7.9%
mean+3SD	20.6	58.0	9.0	27.7	18.9
mean-3SD	13.6	41.5	5.2	17.8	11.6
N	8	8	8	8	8
median	17.0	48.9	7.0	22.6	15.7
mean/all kit median	0.91	0.81	0.92	0.88	0.87

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG kit average:					
mean	18.2	57.7	7.5	24.8	16.8
SD	0.9	7.0	0.3	1.8	1.4
all kit median	18.7	60.4	7.6	25.5	17.5

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG MoMs All Lab Mean:					
mean	0.85	1.57	0.31	0.83	0.95
SD	0.11	0.20	0.03	0.11	0.10
%CV	13.1%	13.0%	9.5%	12.7%	11.0%
mean+3SD	1.19	2.18	0.40	1.14	1.27
mean-3SD	0.52	0.96	0.22	0.51	0.64
N	27	27	27	27	27

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A all lab mean:					
Mean	137.33	262.97	131.10	115.09	229.79
SD	5.27	12.70	8.90	12.15	21.61
%CV	3.8%	4.8%	6.8%	10.6%	9.4%
mean + 3SD	153.1	301.1	157.8	151.5	294.6
mean- 3SD	121.5	224.9	104.4	78.6	165.0
N	24	24	25	27	27
All Lab Median	137.5	264.7	133.0	119.2	234.0
mean/all kit median	1.00	1.01	1.01	0.98	0.98

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Beckman Access (BCX/BC1) mean:					
Mean	138.6	266.8	135.7	121.4	240.6
SD	4.1	9.3	4.3	5.1	9.7
%CV	3.0%	3.5%	3.2%	4.2%	4.0%
mean + 3SD	151.0	294.6	148.6	136.6	269.7
mean- 3SD	126.2	238.9	122.7	106.2	211.4
N	12	12	12	12	12
median	139.9	265.2	134.8	122.8	239.9
mean/All kit median	1.01	1.02	1.04	1.03	1.03

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	137.1	261.6	130.4	117.5	233.7
SD	5.3	12.9	5.9	5.4	11.6
%CV	3.8%	4.9%	4.5%	4.6%	5.0%
mean + 3SD	152.9	300.2	148.0	133.8	268.6
mean- 3SD	121.3	223.0	112.7	101.2	198.8
N	11	11	11	11	11
median	136.6	262.6	131.5	119.0	231.2
mean/all kit median	1.00	1.00	1.00	1.00	1.00

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Diagnostic System Labs (DS1) Mean:					
Mean	94.5	192.0	93.2	89.6	186.7
SD	26.3	33.3	19.2	6.4	16.0
%CV	27.8%	17.3%	20.6%	7.2%	8.6%
mean + 3SD	173.3	291.9	150.8	108.8	234.7
mean- 3SD	15.7	92.1	35.6	70.4	138.7
N	4	4	4	4	4
median	96.0	191.2	97.2	89.8	187.6
mean/all kit median	0.69	0.73	0.71	0.76	0.80

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A kit average:					
mean	123.4	240.1	119.7	109.5	220.3
SD	25.0	41.7	23.1	17.3	29.3
all kit median	137.1	261.6	130.4	117.5	233.7

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A MoM All Lab Mean:					
mean	0.85	1.38	0.76	0.65	1.16
SD	0.07	0.22	0.13	0.10	0.18
%CV	8.1%	16.2%	17.1%	14.9%	15.5%
mean+3SD	1.06	2.05	1.14	0.94	1.69
mean-3SD	0.65	0.71	0.37	0.36	0.62
N	24	27	27	27	27

New York State Fetal Defect Markers Proficiency Test,
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	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP All Lab Mean :					
mean	7.06	9.98	8.80	6.20	19.05
SD	1.01	1.32	1.02	0.84	2.37
%CV	14.3%	13.2%	11.6%	13.6%	12.4%
mean+3SD	10.1	13.9	11.9	8.7	26.2
mean-3SD	4.0	6.0	5.7	3.7	11.9
N	22	22	22	21	22
mean/all kit median	1.01	0.97	0.98	0.95	0.98

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	6.7	10.7	9.2	6.9	16.5
SD	0.6	0.7	0.6	0.6	0.9
%CV	8.7%	6.4%	6.4%	8.3%	5.3%
mean+3SD	8.4	12.7	10.9	8.6	19.1
mean-3SD	4.9	8.6	7.4	5.2	13.9
N	5	5	5	5	5
median	6.5	10.6	9.1	6.7	16.3
mean/all kit median	0.95	1.03	1.02	1.05	0.85

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP MoMs All Lab Mean:					
mean	0.91	0.58	0.92	1.19	3.01
SD	0.09	0.06	0.10	0.15	0.38
%CV	10.1%	9.6%	10.4%	12.7%	12.7%
mean+3SD	1.18	0.75	1.21	1.65	4.17
mean-3SD	0.63	0.42	0.63	0.74	1.86
N	22	22	22	21	22

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	6.2	8.7	8.0	5.2	18.5
SD	0.7	0.8	1.3	0.3	1.8
%CV	10.5%	9.4%	15.8%	6.4%	9.9%
X+3SD	8.7	11.9	10.0	7.1	24.8
X-3SD	6.0	8.2	7.7	5.4	16.4
N	7	7	7	6	7
median	6.0	8.7	7.6	5.1	18.0
mean/All kit median	0.89	0.84	0.88	0.79	0.95

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Beckman Access (BCX/BC1) mean:					
mean	7.4	10.0	8.8	6.2	20.6
SD	0.4	0.6	0.4	0.3	1.4
%CV	6.0%	6.1%	4.3%	4.7%	6.8%
mean+3SD	8.7	11.9	10.0	7.1	24.8
mean-3SD	6.0	8.2	7.7	5.4	16.4
N	6	6	6	6	6
median	7.2	9.95	8.85	6.3	20.5
mean/all kit median	1.05	0.97	0.98	0.95	1.05

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Abbott Axsym (ABB/AB2) mear:					
mean	8.7	12.2	10.1	7.3	21.7
N	2	2	2	2	2
mean/all kit median	1.24	1.18	1.12	1.11	1.11

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP kit average:					
mean	7.2	10.4	9.0	6.4	19.3
SD	1.1	1.4	0.9	0.9	2.3
all kit median	7.0	10.3	9.0	6.6	19.5

Figure 1

Maternal Sera AFP MoM

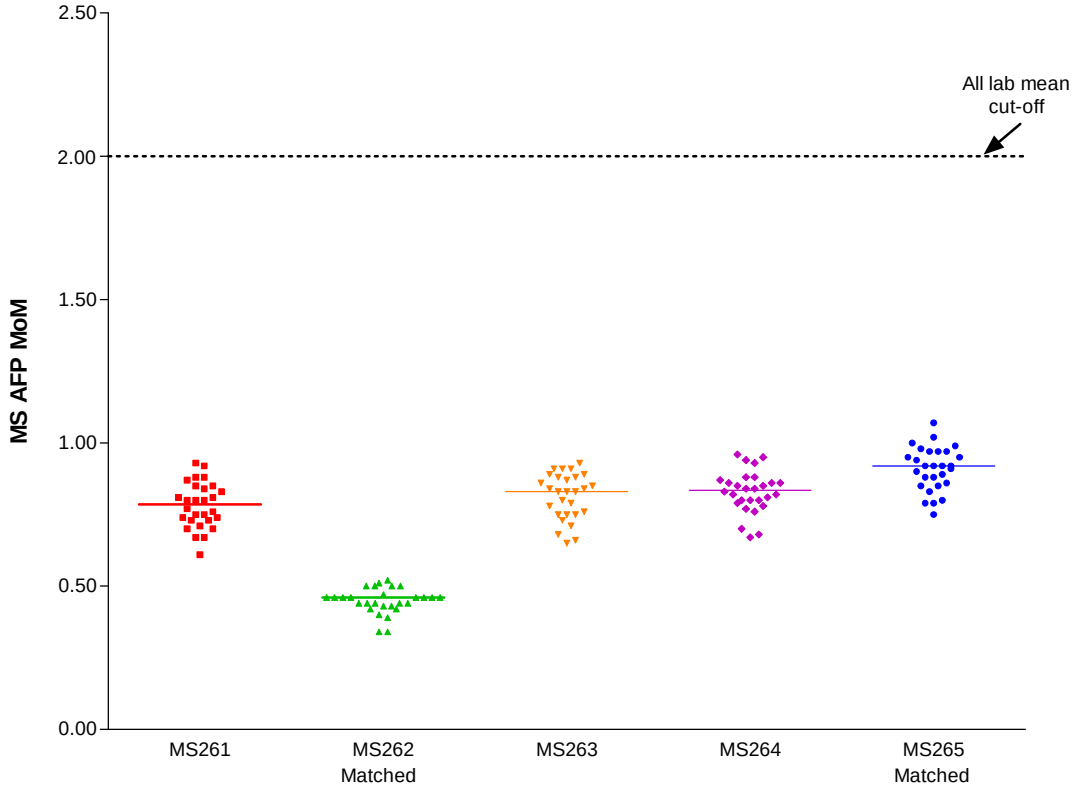


Figure 2

Amniotic Fluid AFP MoM

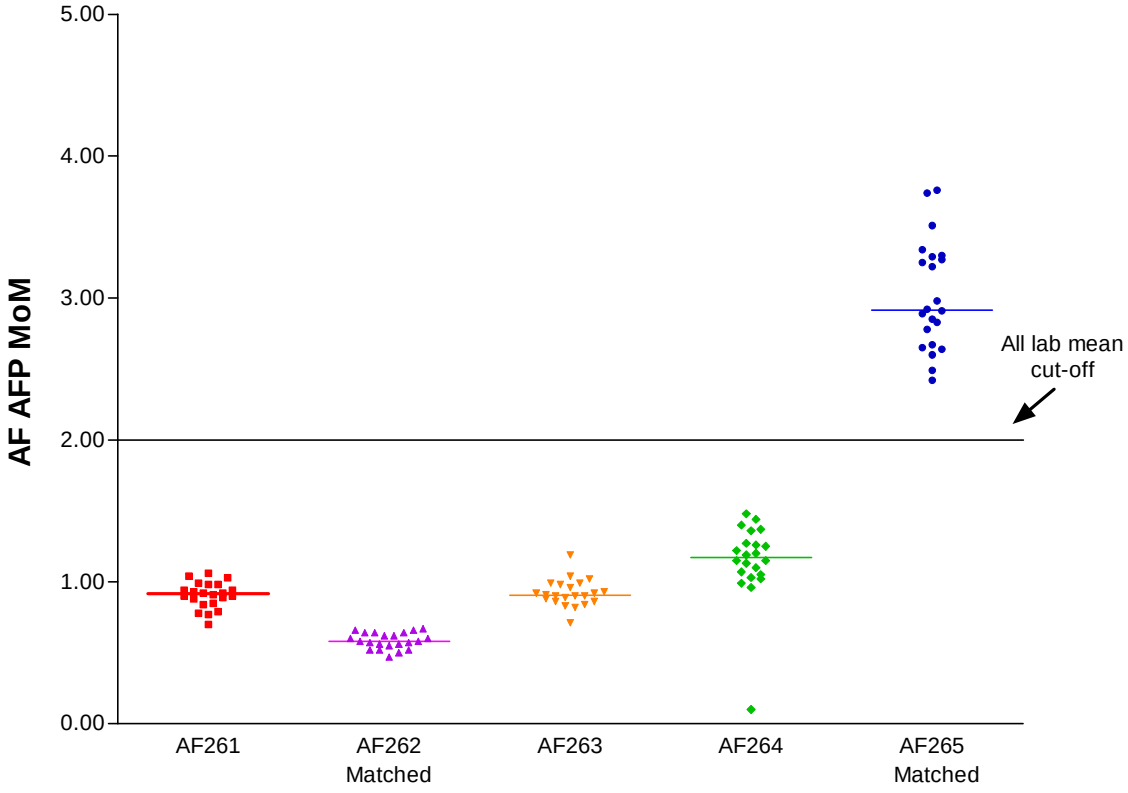


Figure 3

Graphic Distribution of Second Trimester Neural Tube Defect Risk Estimates

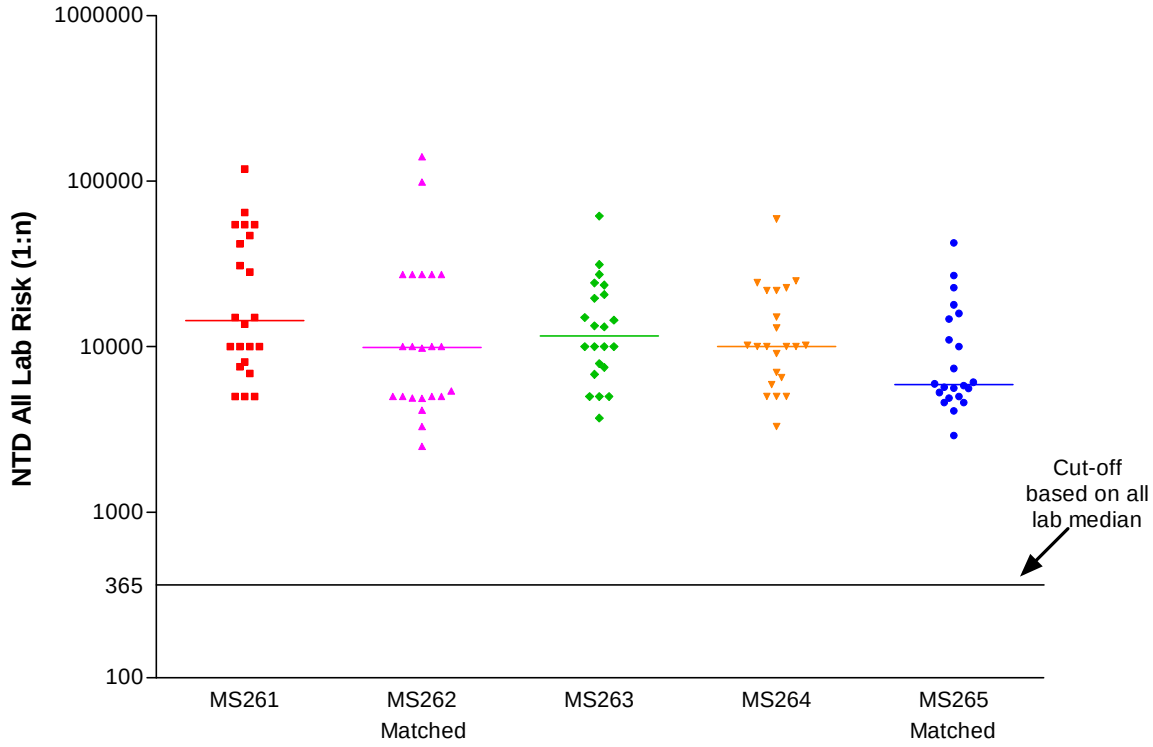


Figure 4

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates

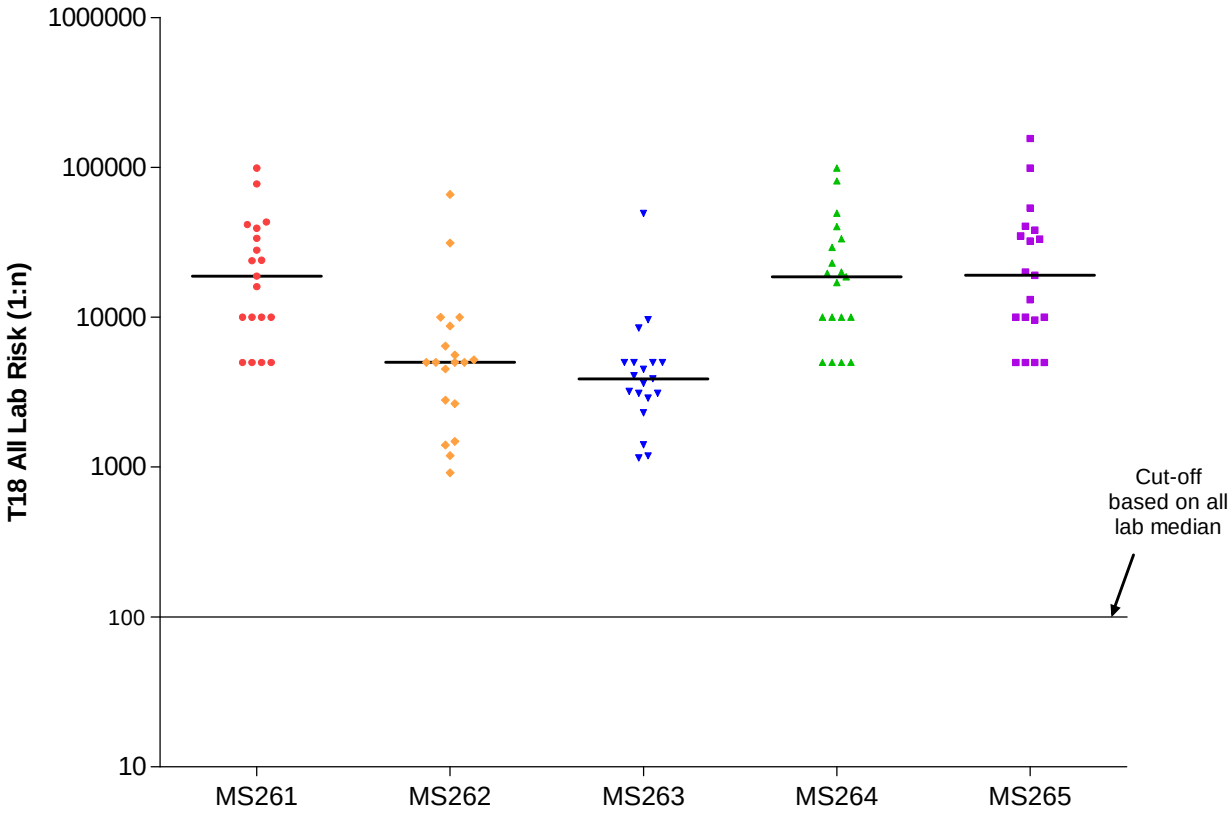


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

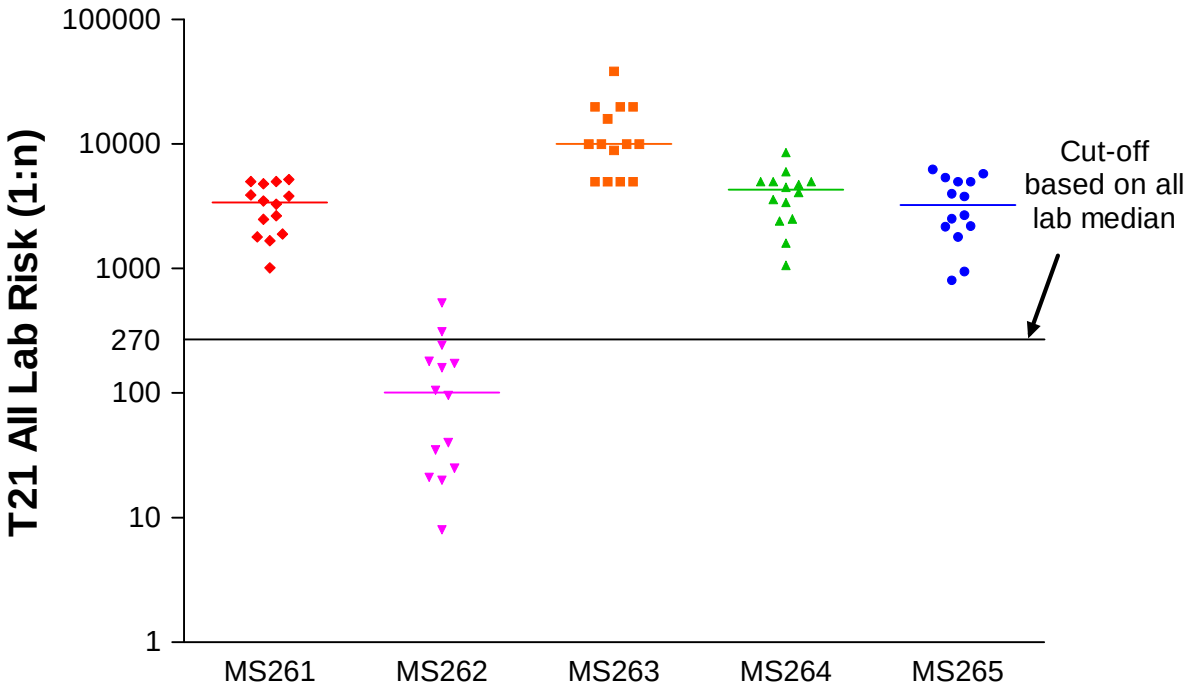


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

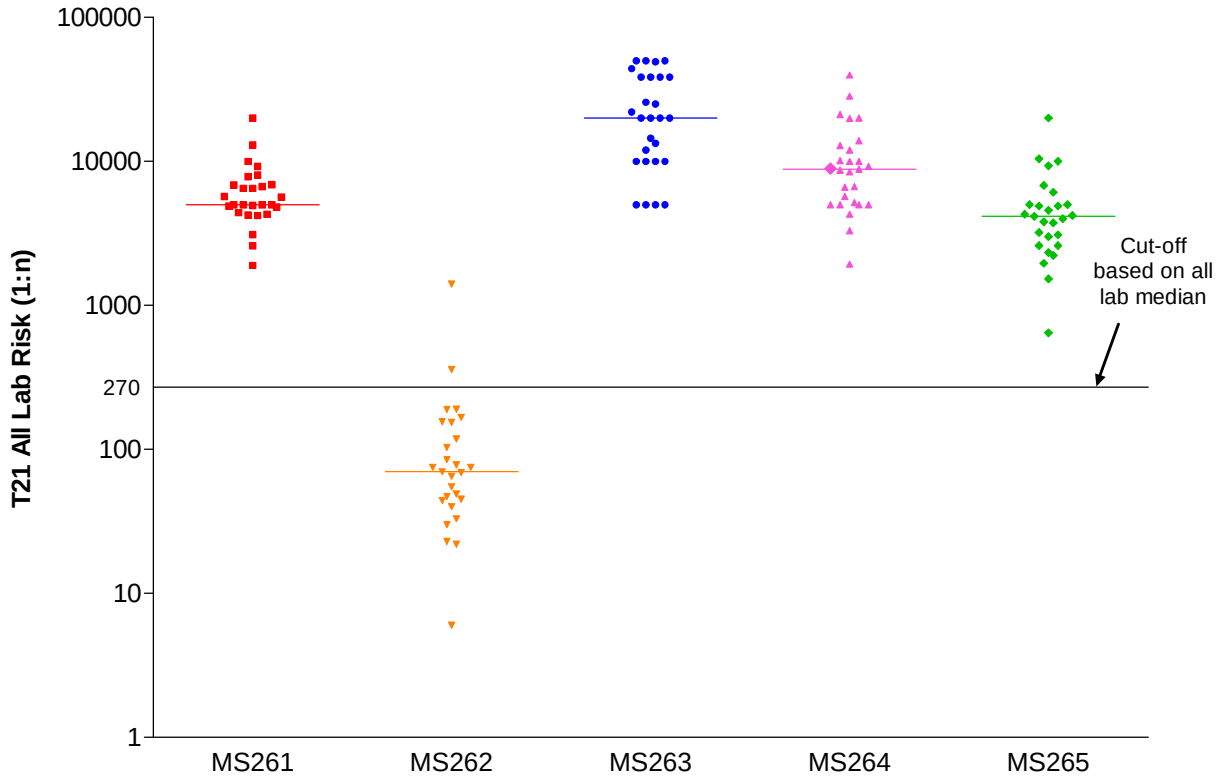


Figure 7A

MS AFP FEDM PT 1/11 Method Comparison

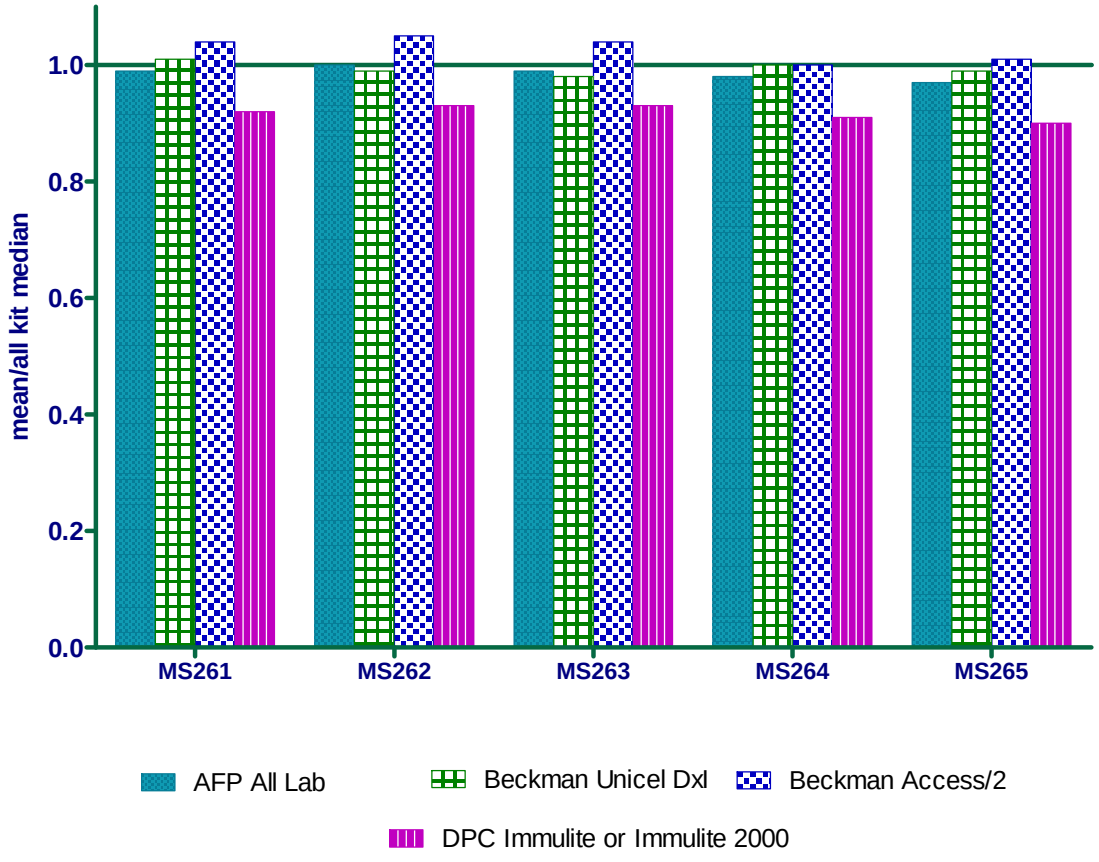


Figure 7B

AF AFP FEDM PT 1/11 Method Comparison

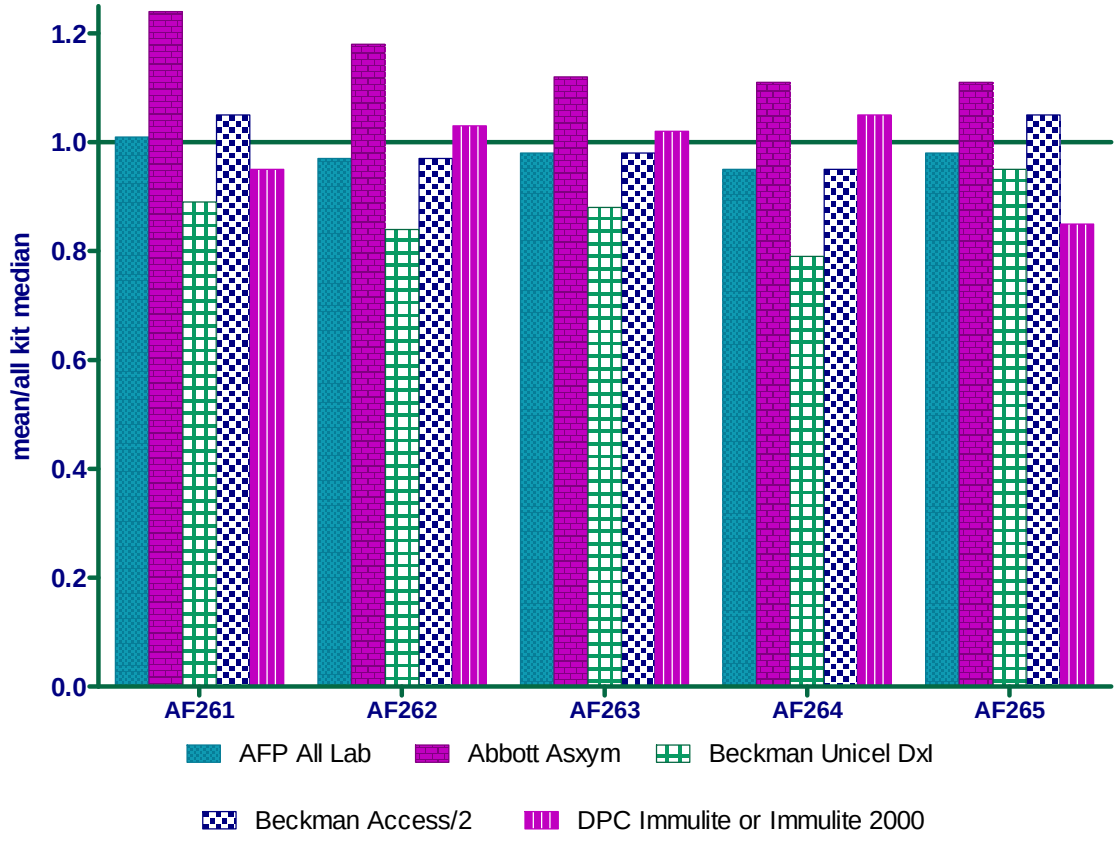


Figure 8A

MS uE3 FEDM PT 1/11 Method Comparison

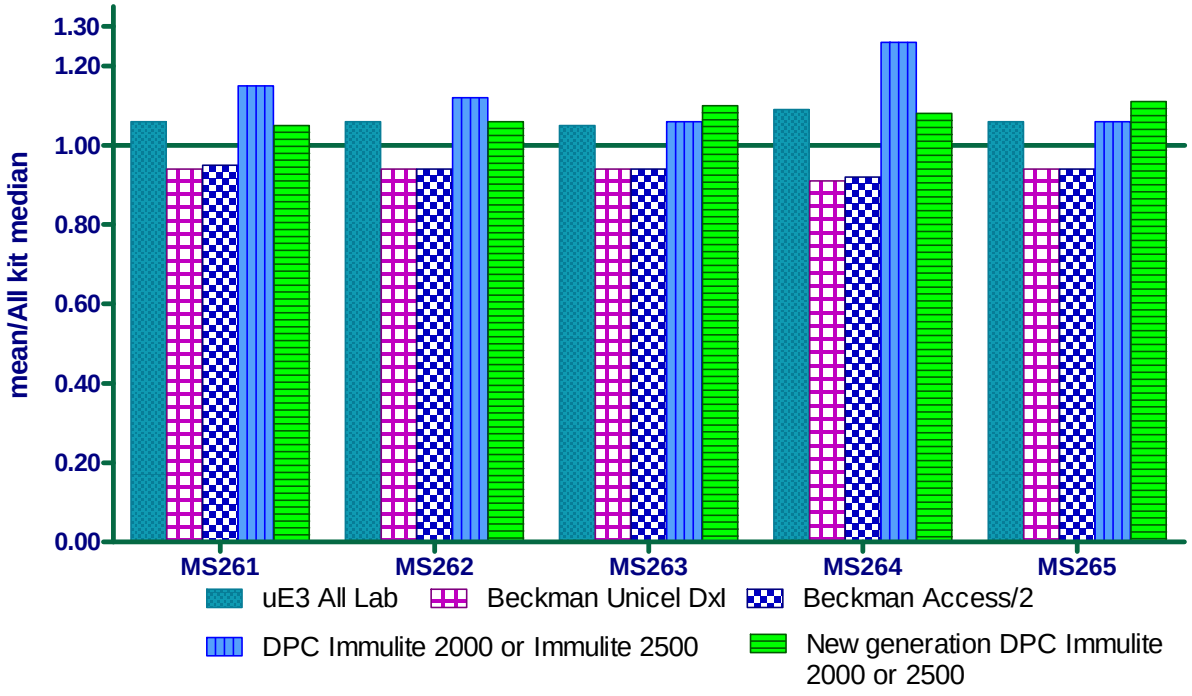


Figure 8 B

MS uE3 MOM FEDM PT 1/11 Method Comparison

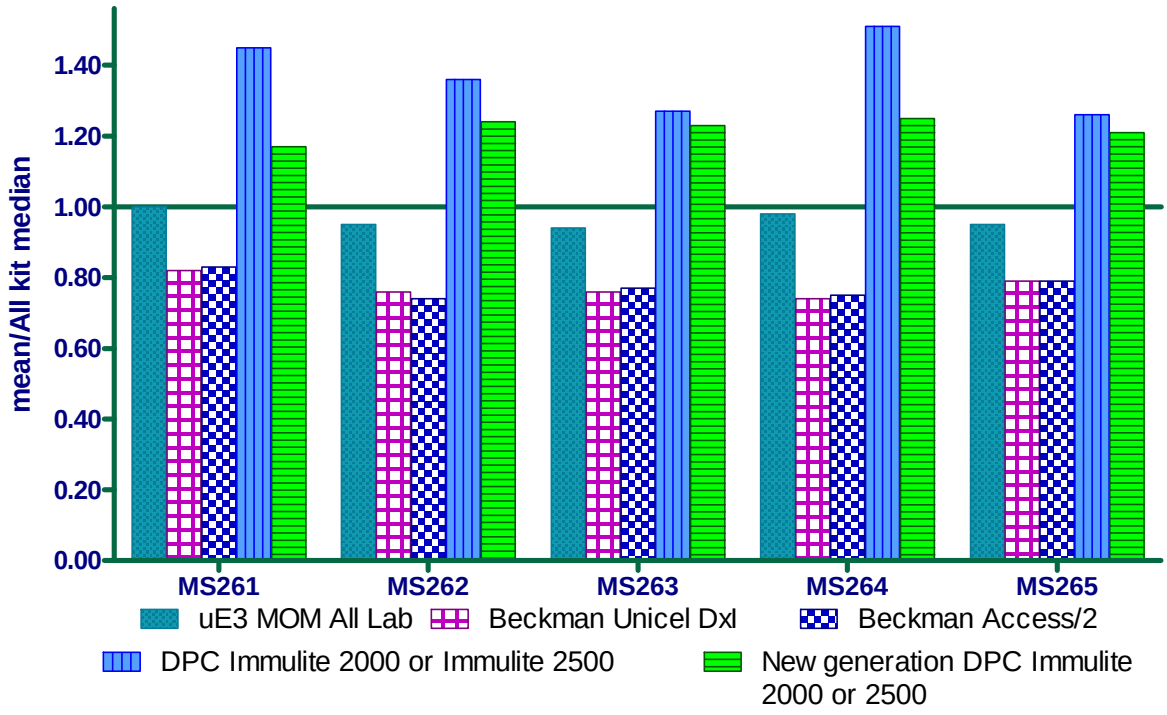


Figure 9

MS hCG FEDM PT 1/11 Method Comparison

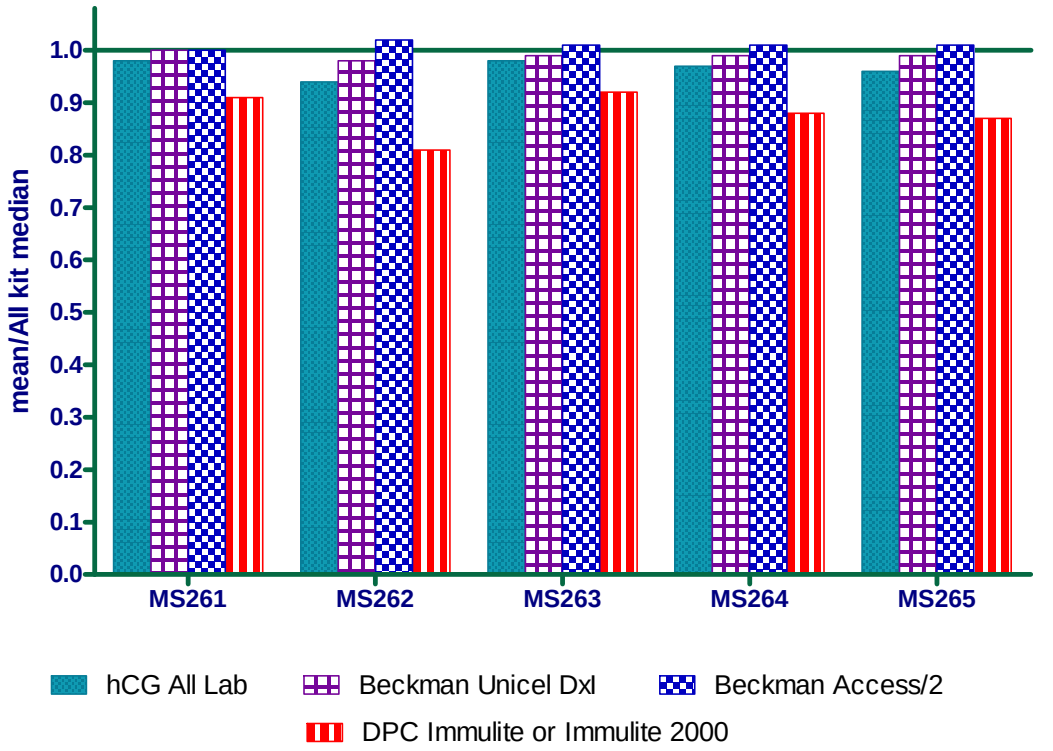


Figure 10

MS Inhibin A FEDM PT 1/11 Method Comparison

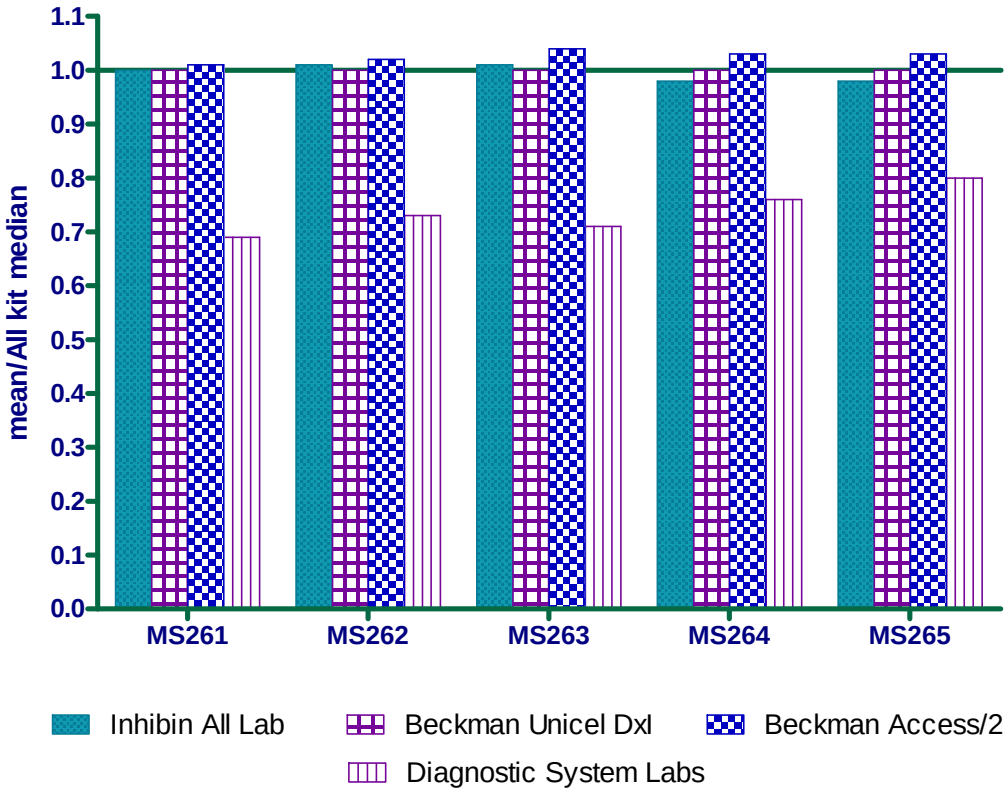


Figure 11

FT hCG FEDM PT 1/11 Method Comparison

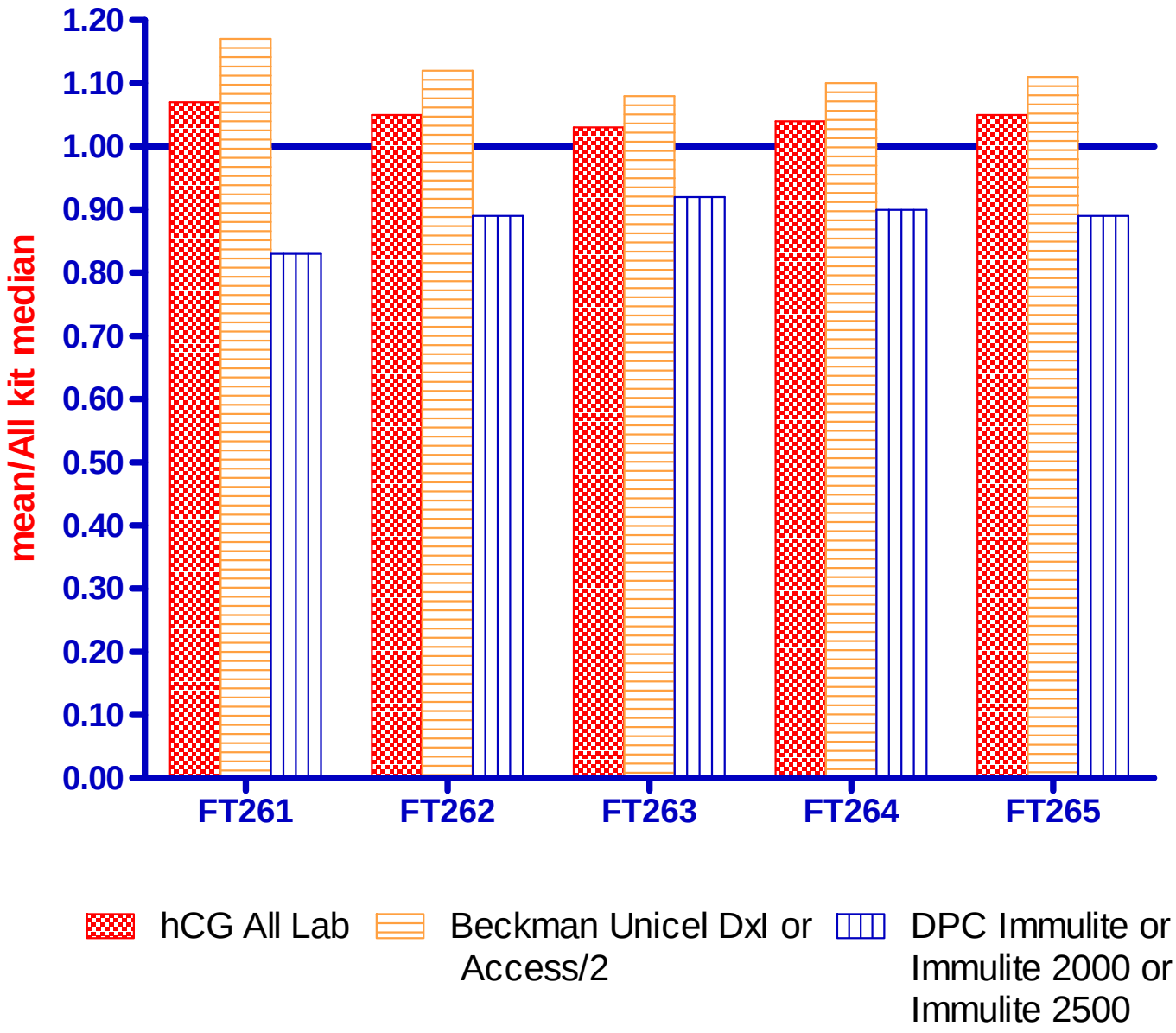
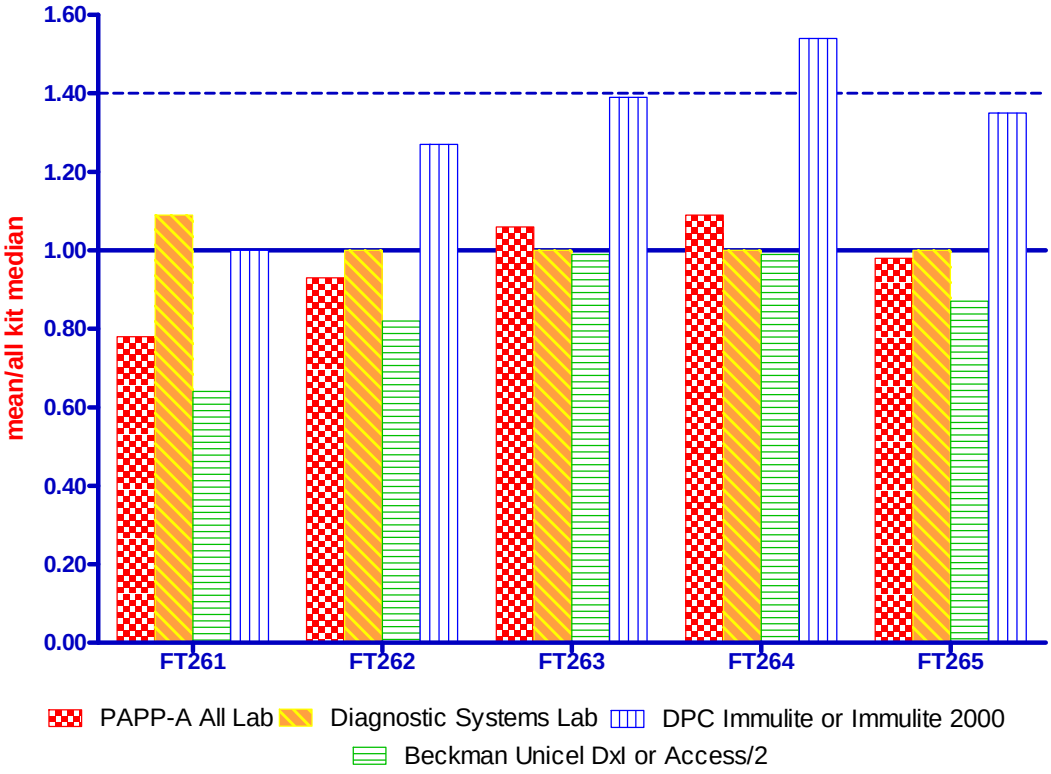


Figure 12A

FT PAPP- A FEDM PT 1/11 Method Comparison



*Please note: this graph is derived from converted values due to the difference in mass units used (mIU/ml -> ng/ml)

Figure 12B

FT PAPP- A MOM FEDM PT 1/11 Method Comparison

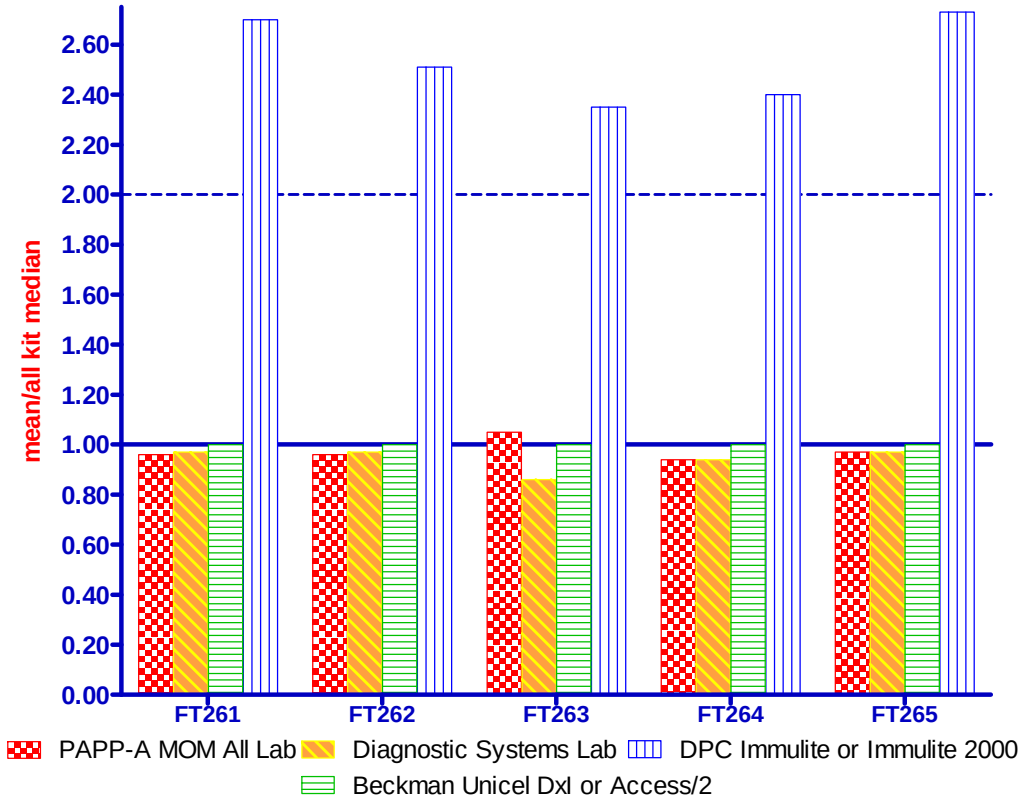


Figure 13

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

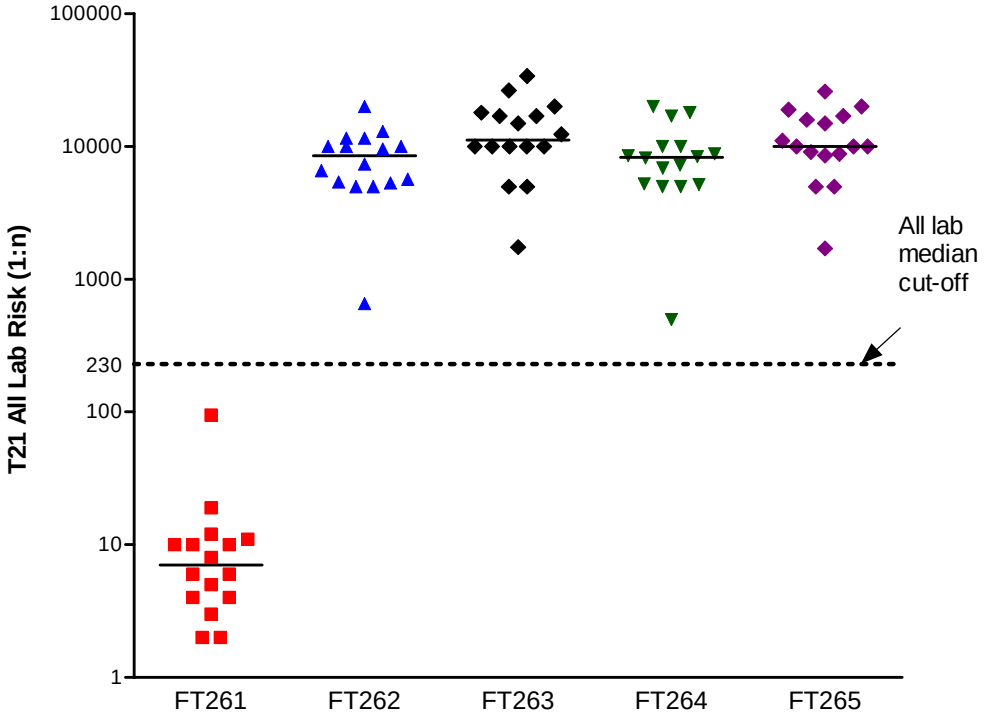


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates

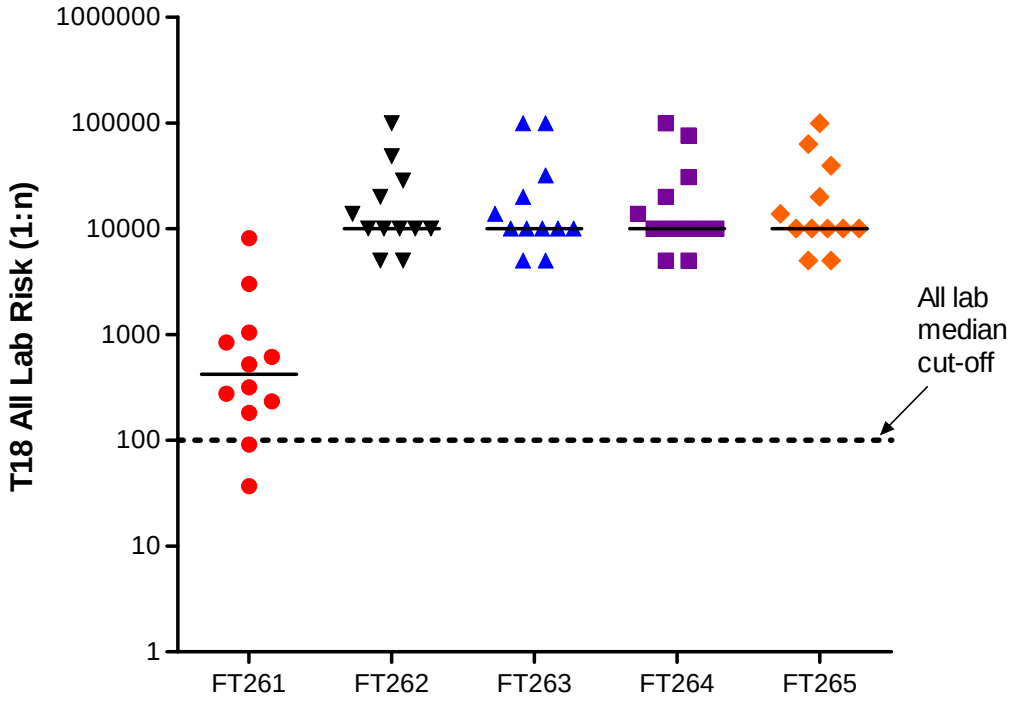


Figure 1

Maternal Sera AFP MoM

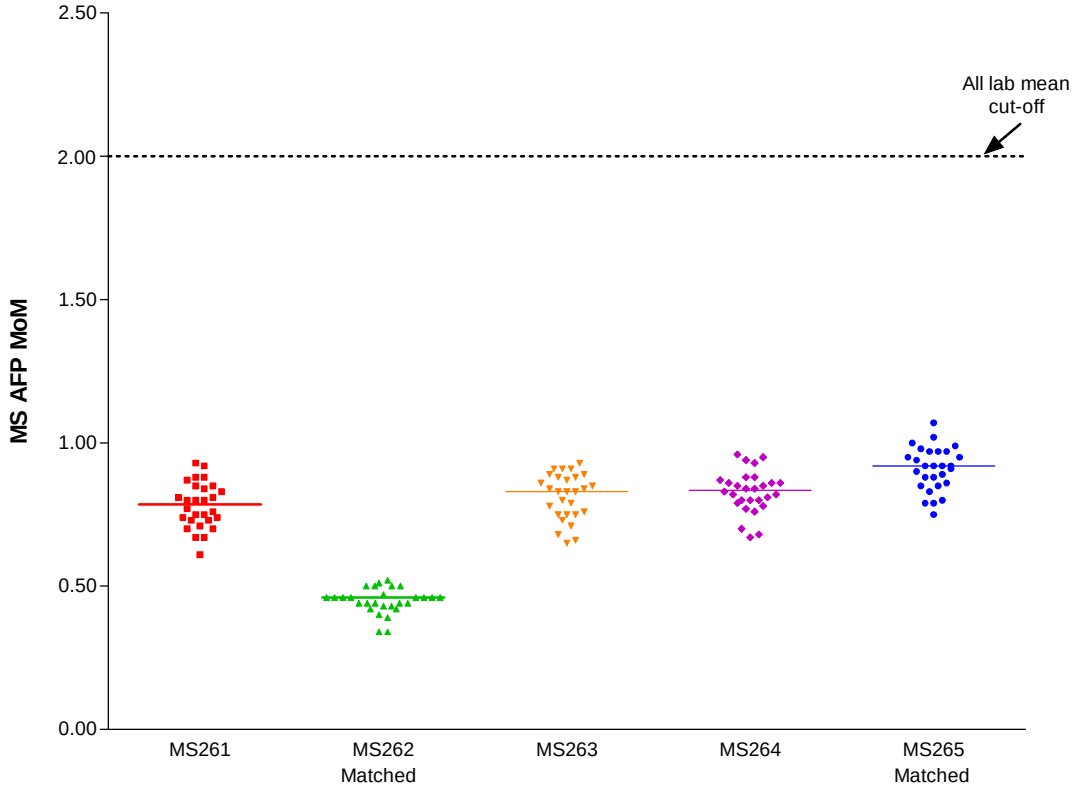


Figure 2

Amniotic Fluid AFP MoM

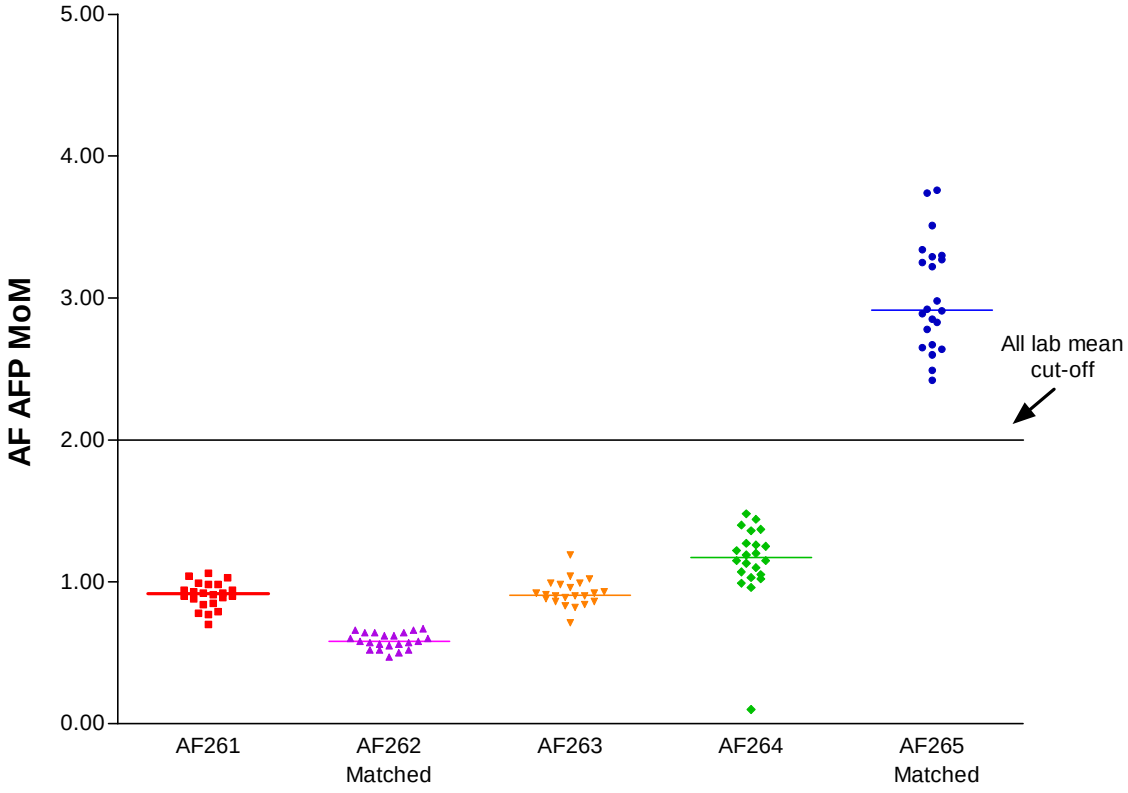


Figure 3

Graphic Distribution of Second Trimester Neural Tube Defect Risk Estimates

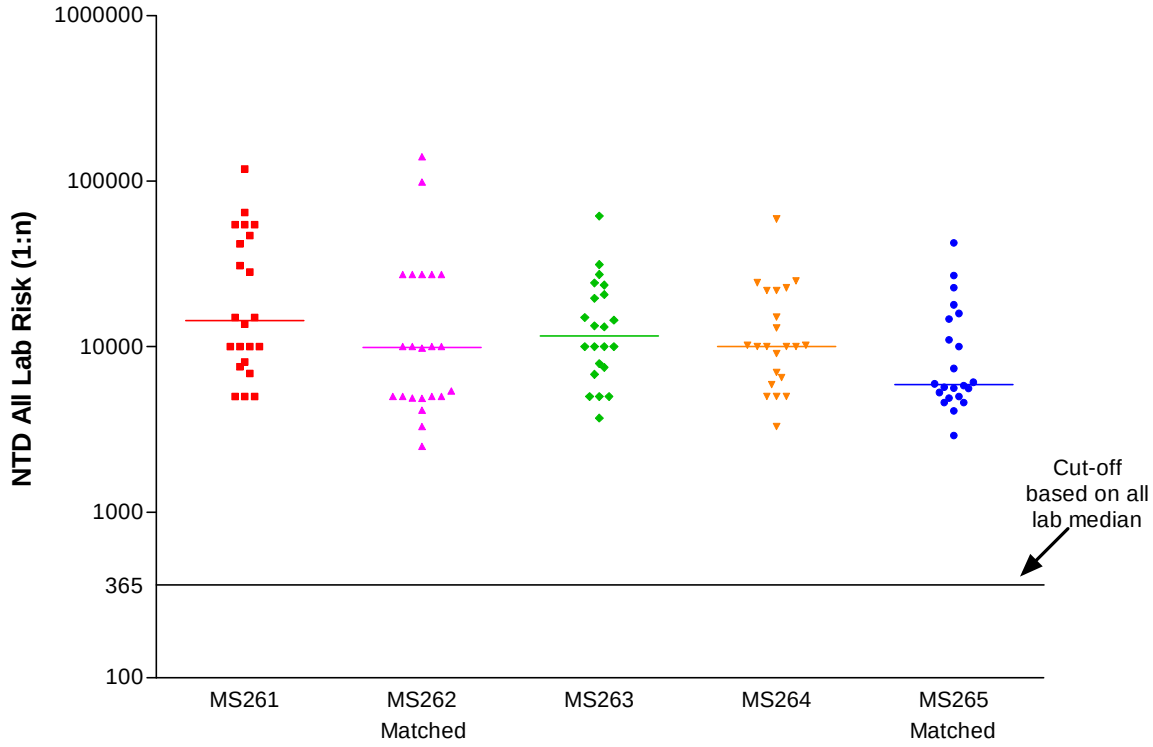


Figure 4

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates

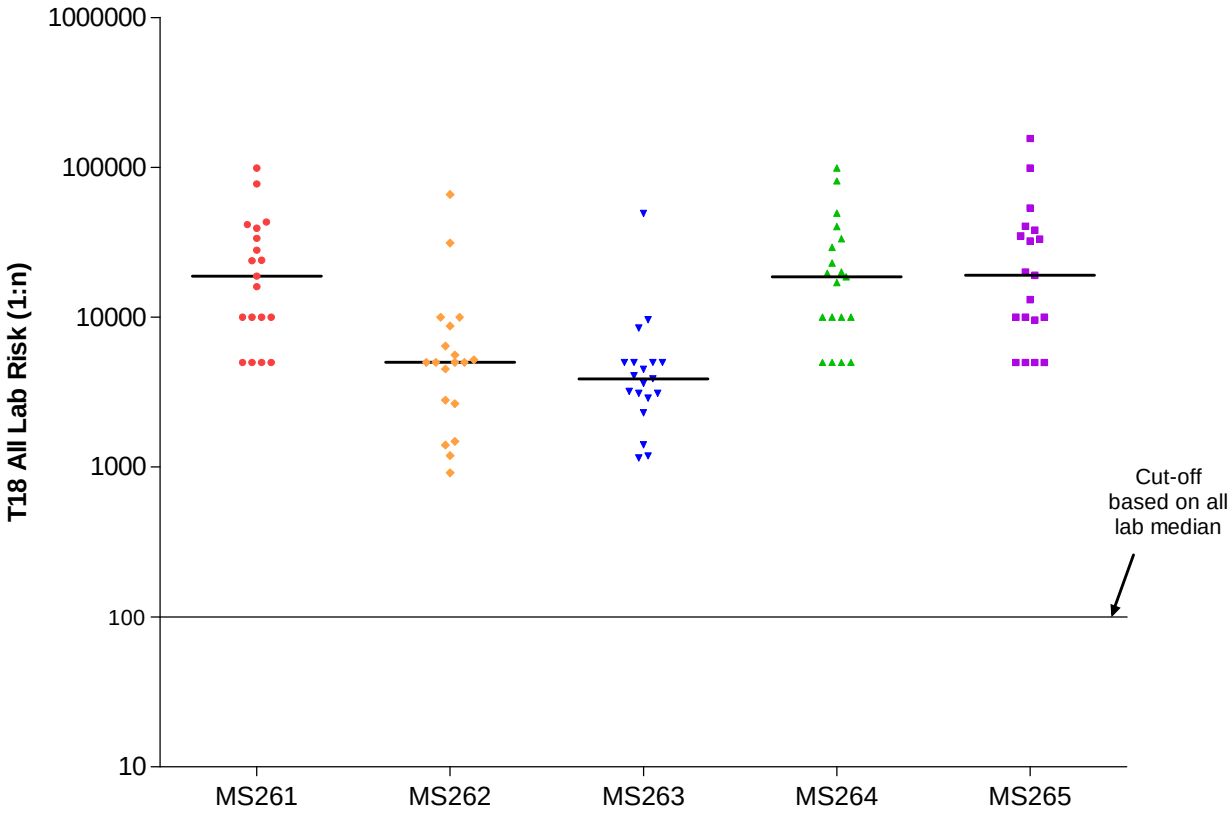


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

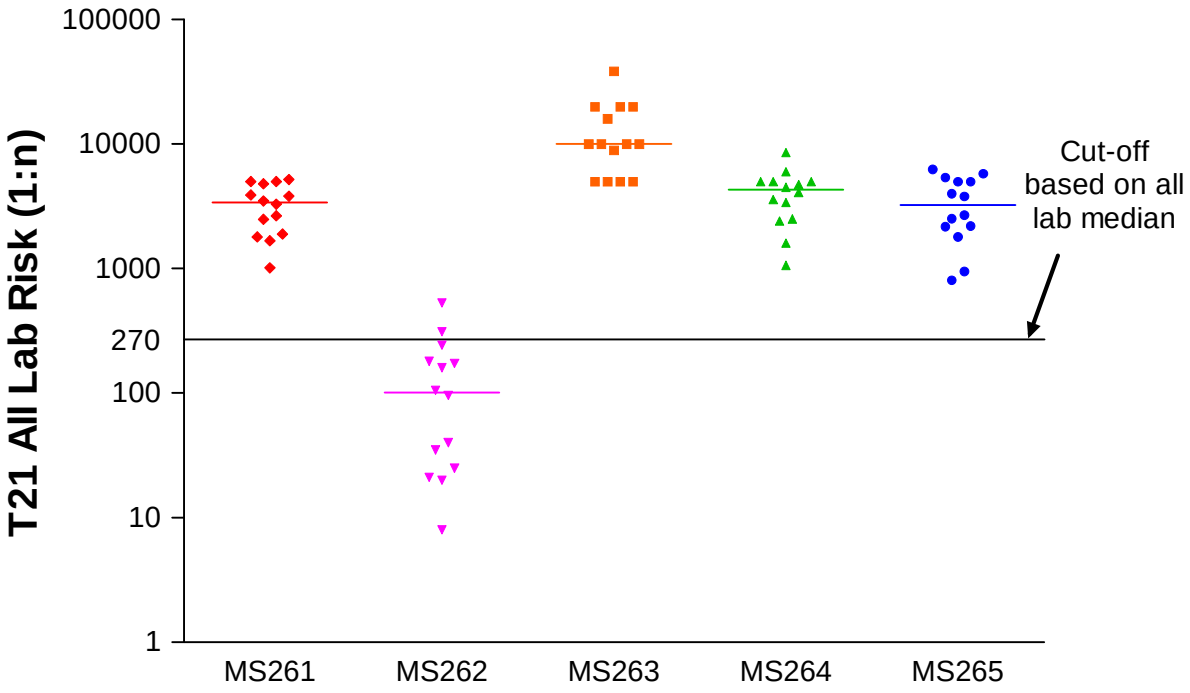


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

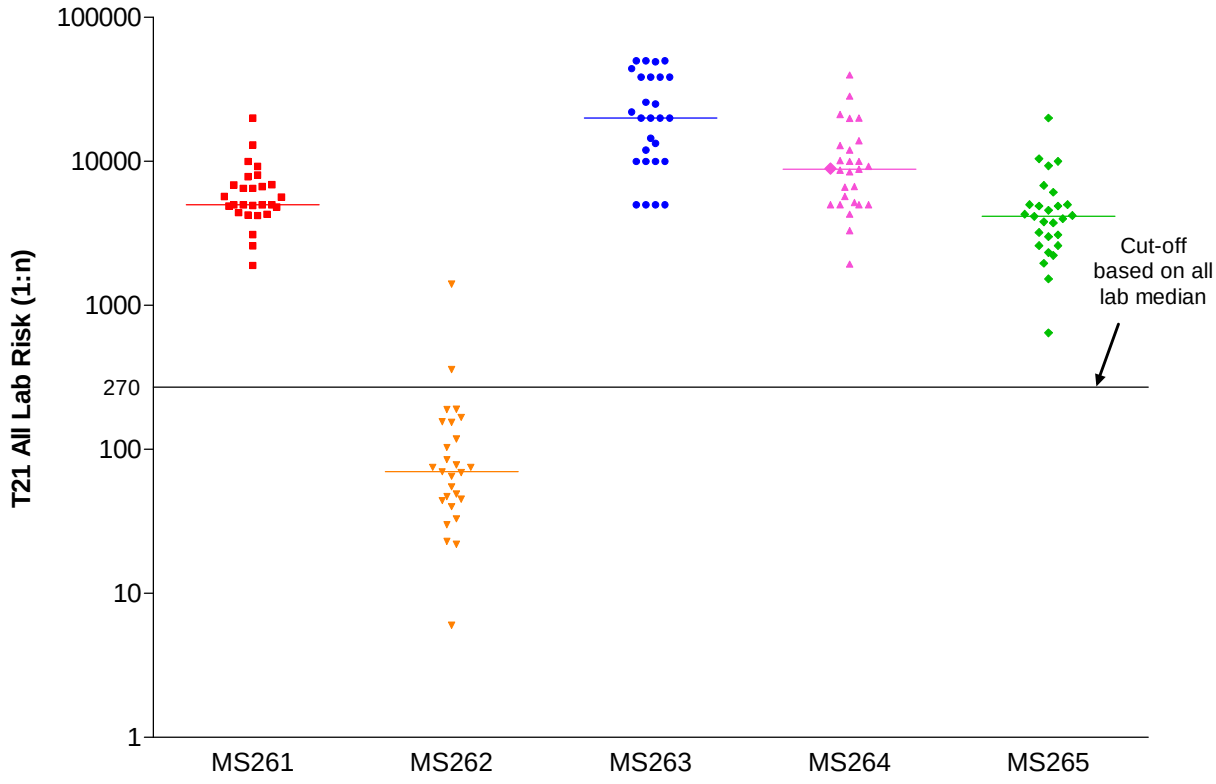


Figure 7A

MS AFP FEDM PT 1/11 Method Comparison

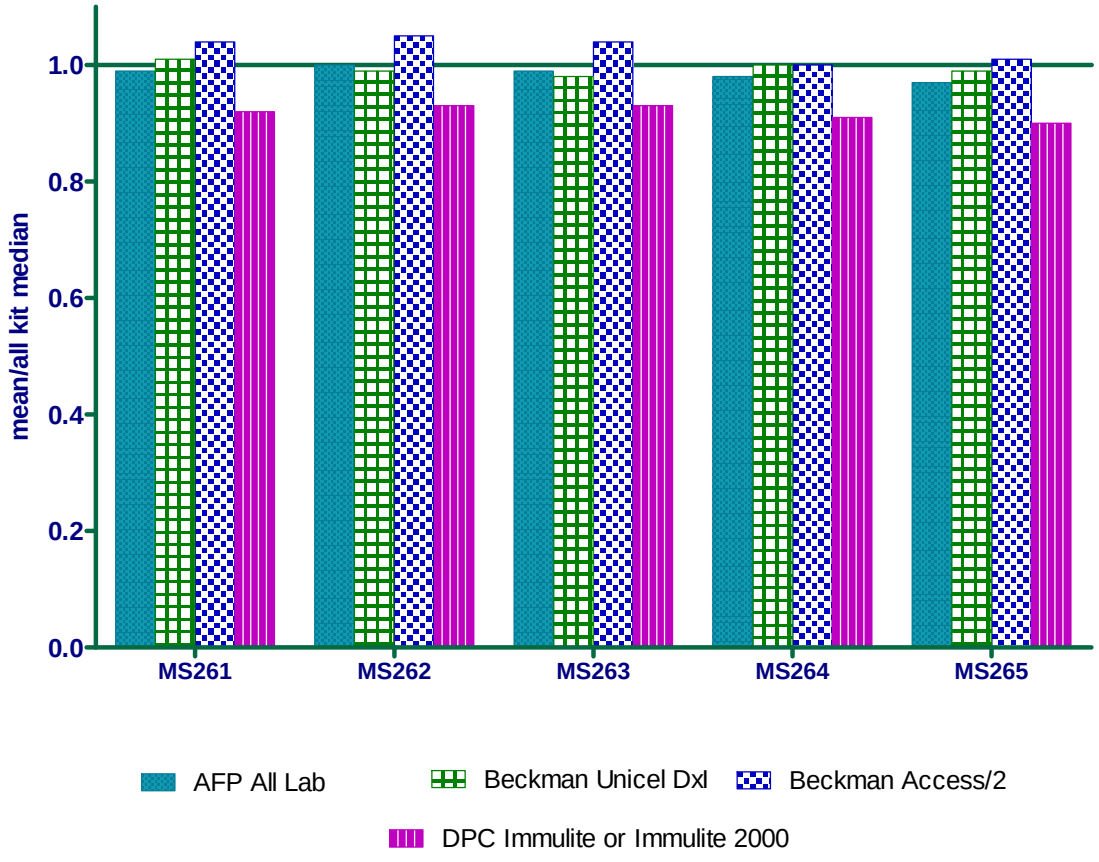


Figure 7B

AF AFP FEDM PT 1/11 Method Comparison

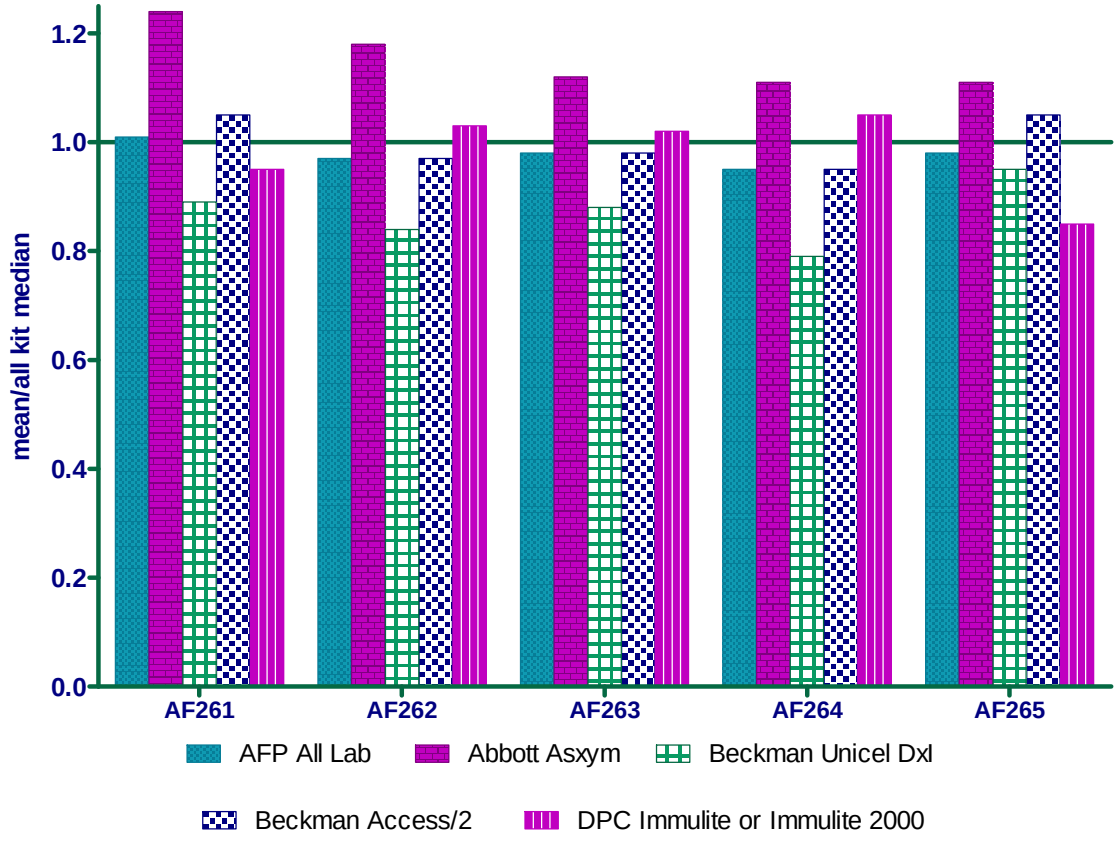


Figure 8A

MS uE3 FEDM PT 1/11 Method Comparison

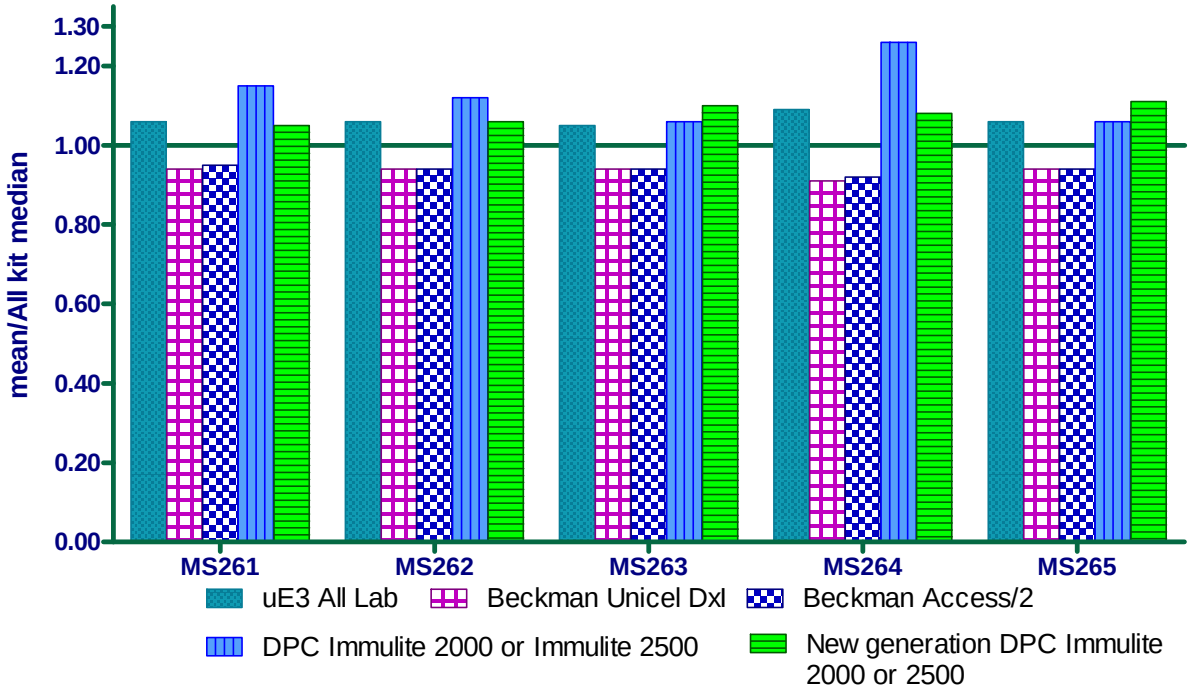


Figure 8 B

MS uE3 MOM FEDM PT 1/11 Method Comparison

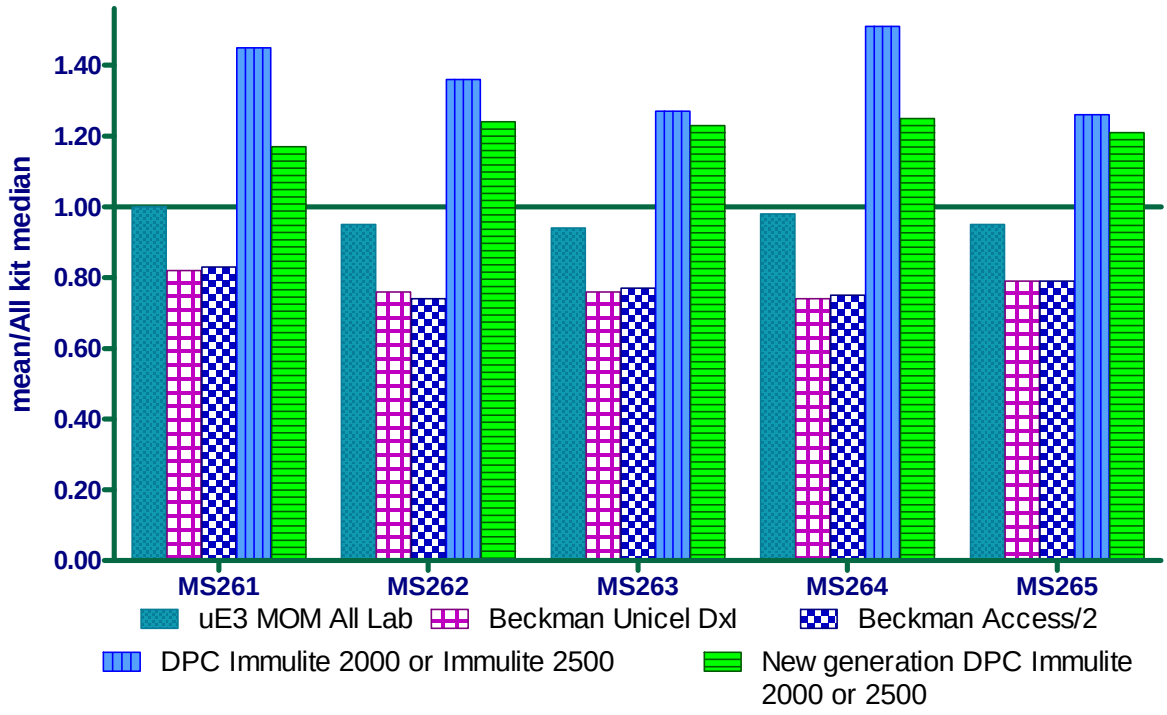


Figure 9

MS hCG FEDM PT 1/11 Method Comparison

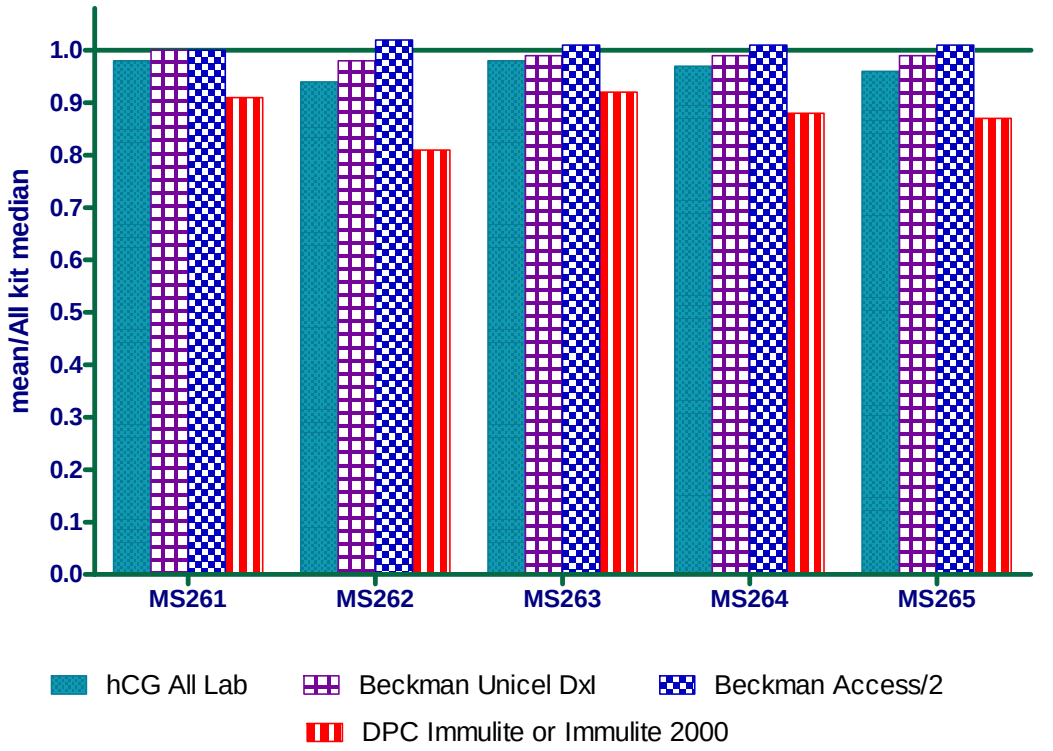


Figure 10

MS Inhibin A FEDM PT 1/11 Method Comparison

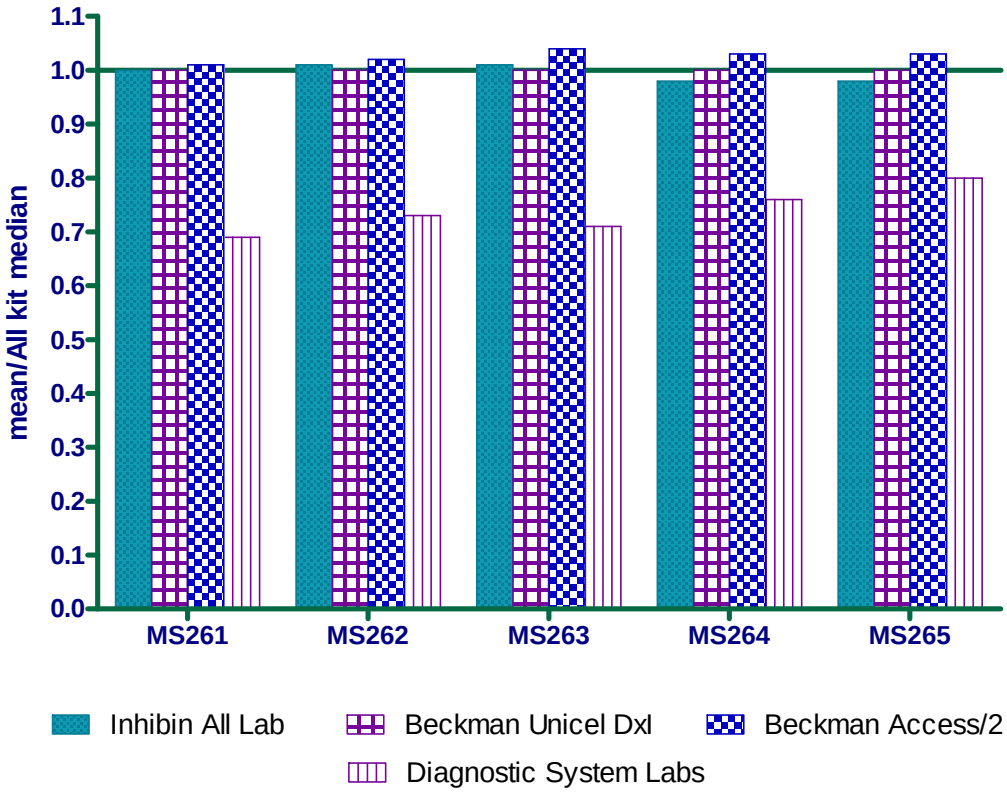


Figure 11

FT hCG FEDM PT 1/11 Method Comparison

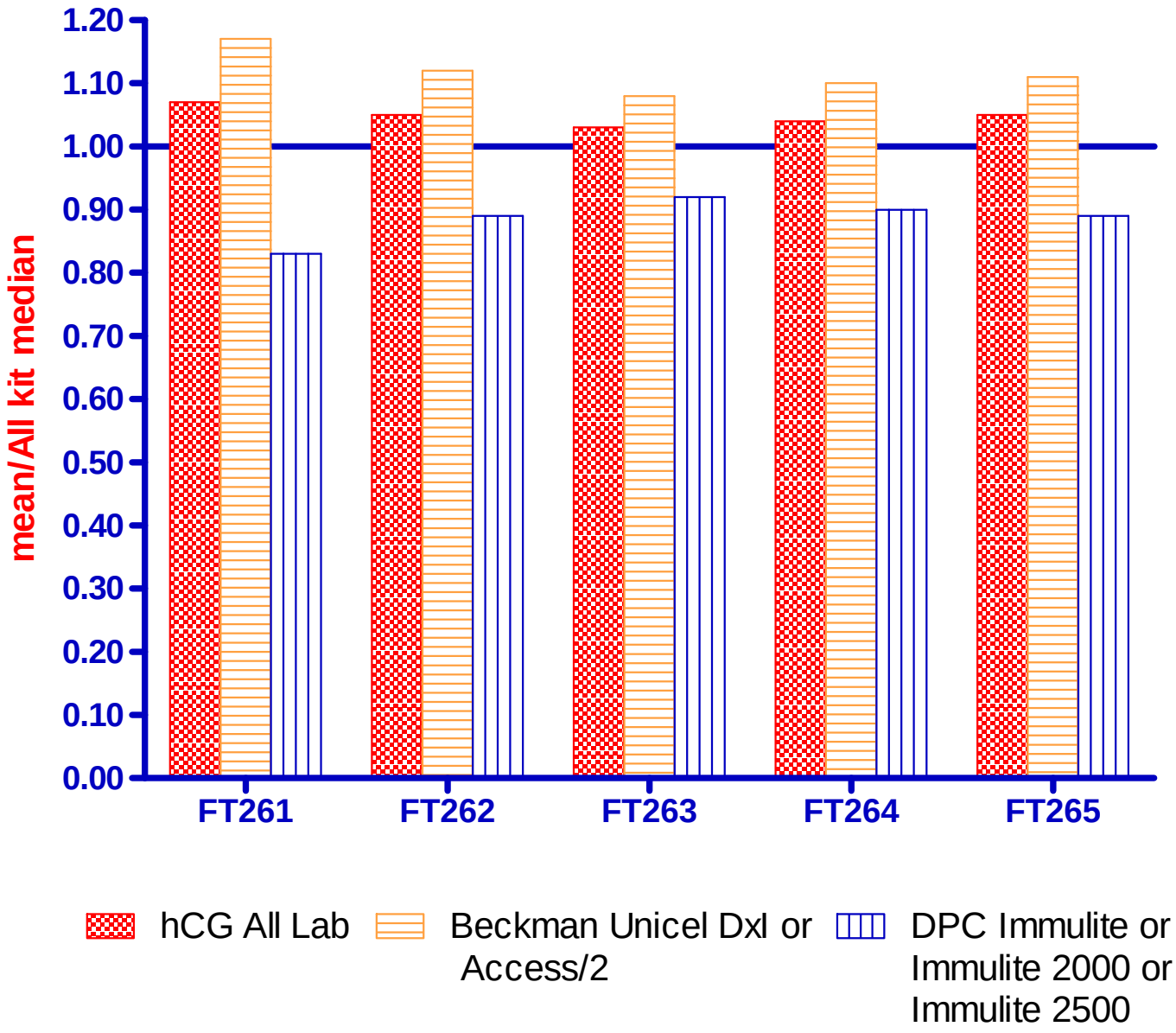
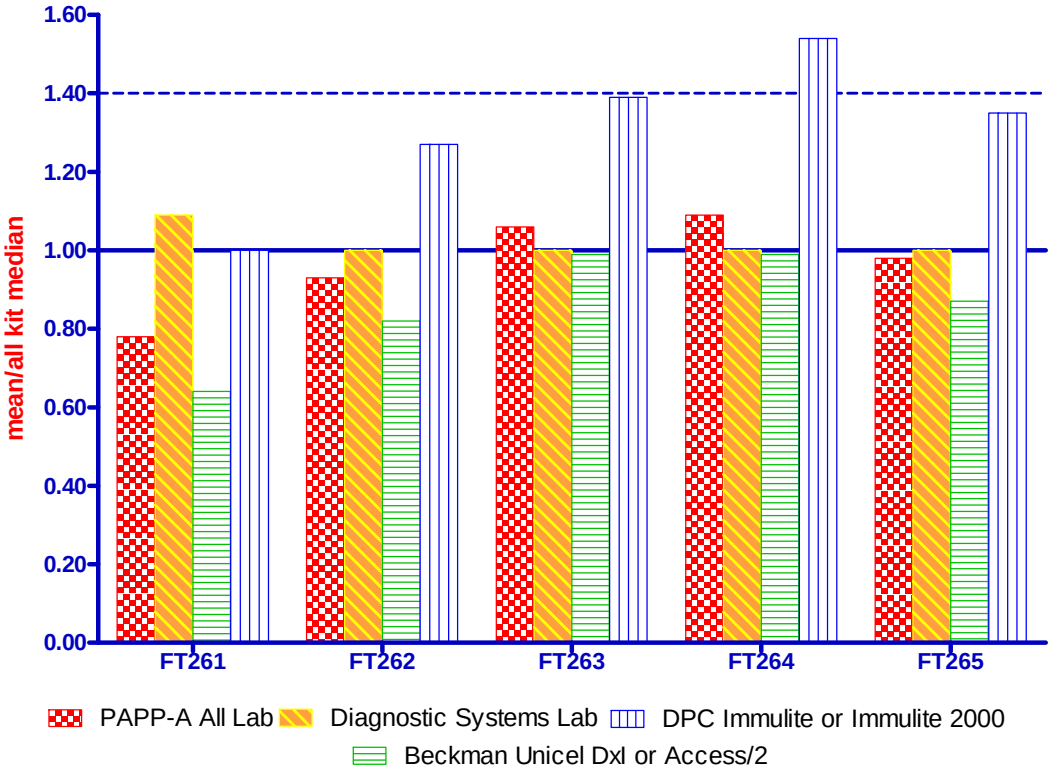


Figure 12A

FT PAPP- A FEDM PT 1/11 Method Comparison



*Please note: this graph is derived from converted values due to the difference in mass units used (mIU/ml -> ng/ml)

Figure 12B

FT PAPP- A MOM FEDM PT 1/11 Method Comparison

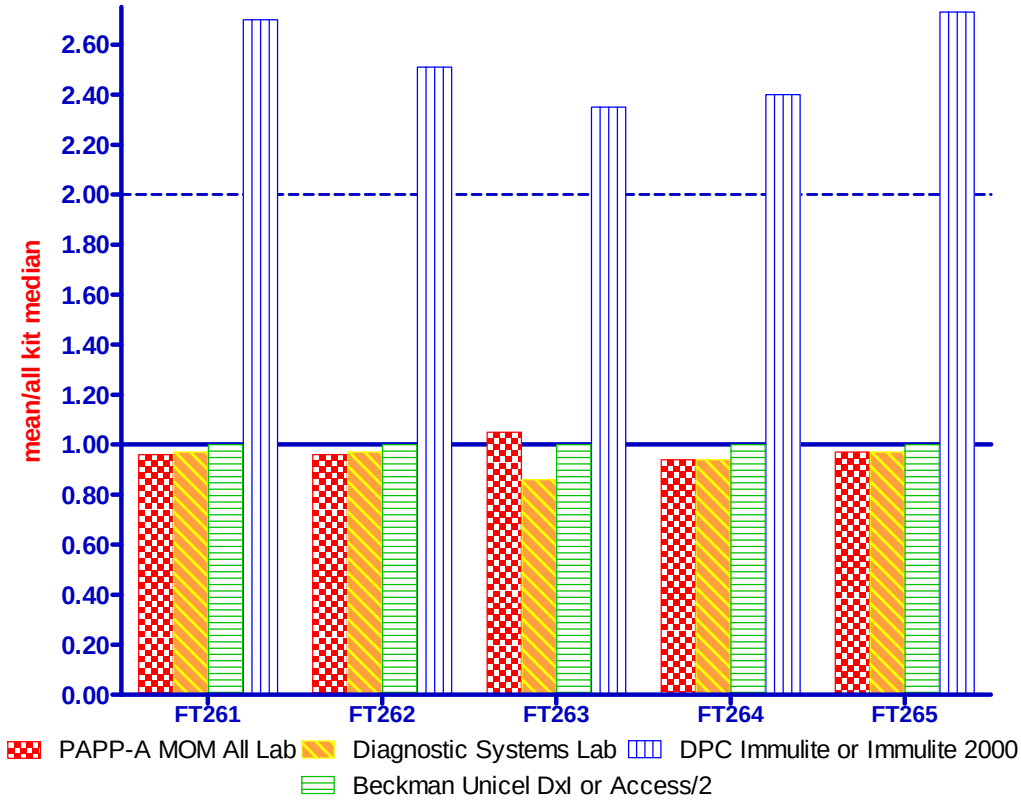


Figure 13

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

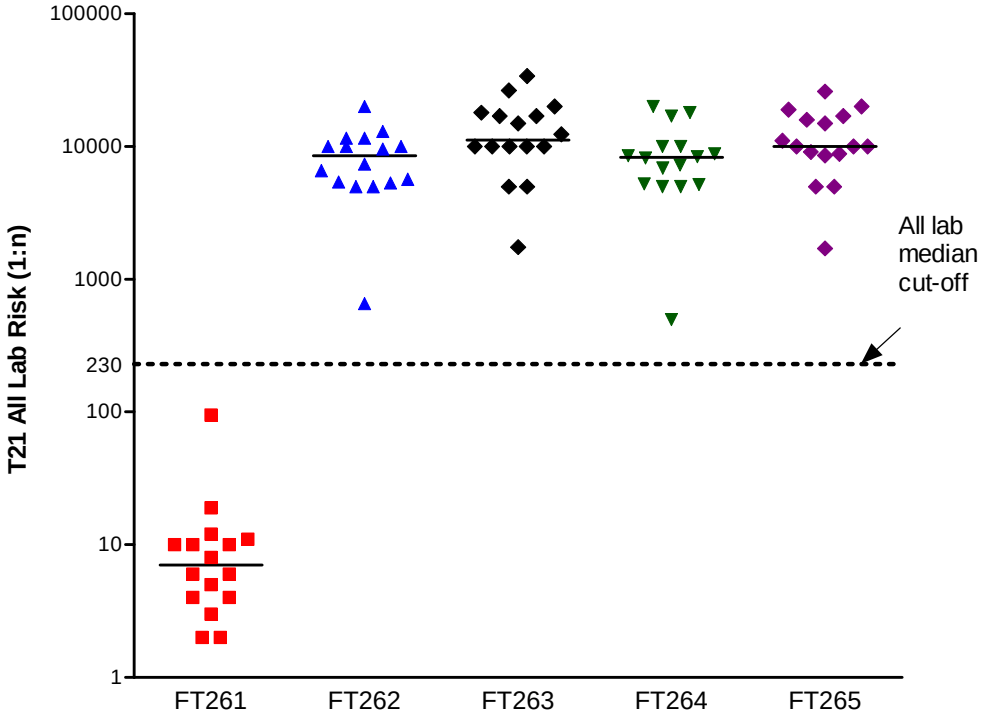
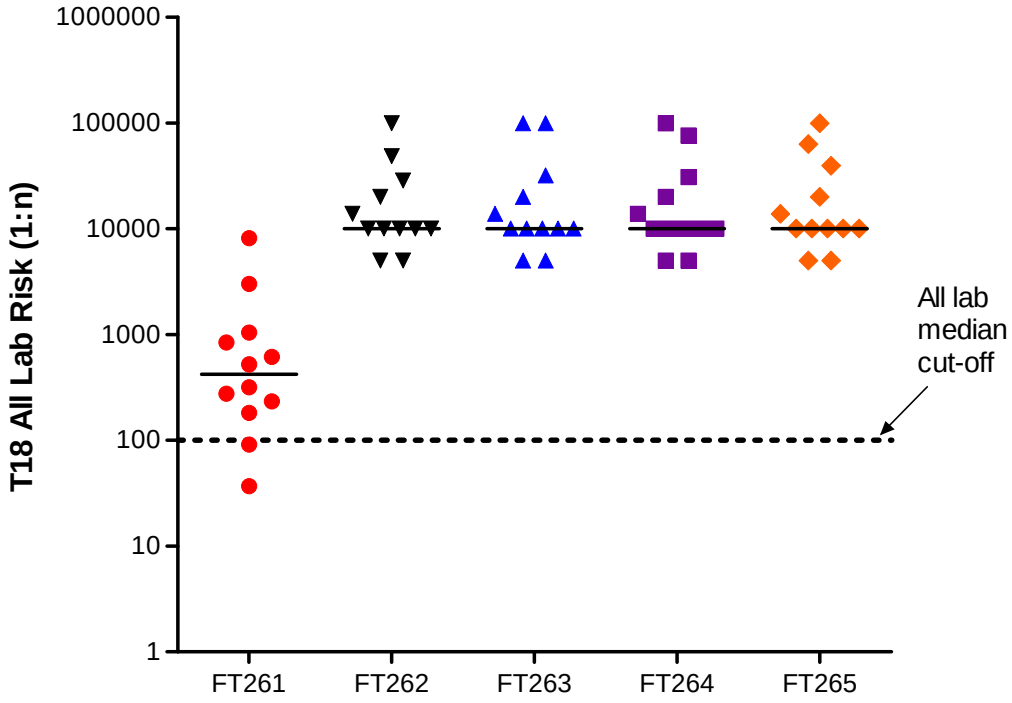


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates



New York State Fetal Defect Markers Proficiency Test,
January 2011
Summary of First Trimester Results

	FT261	FT262	FT263	FT264	FT265
FT Gestational Age All Lab Mean:					
Mean	11.9	11.2	12.4	13.0	11.4
SD	0.09	0.13	0.09	0.07	0.09
%CV	0.8%	1.2%	0.7%	0.5%	0.8%
X+3*SD	12.2	11.6	12.7	13.2	11.7
X-3*SD	11.6	10.8	12.1	12.8	11.2
N	18	18	18	18	18

	FT261	FT262	FT263	FT264	FT265
FT NT MoMs All Lab Mean:					
Mean	2.22	0.97	0.96	0.66	0.92
SD	0.24	0.09	0.10	0.06	0.09
%CV	11.0%	9.7%	10.0%	9.7%	10.3%
X+3SD	2.95	1.25	1.25	0.85	1.21
X- 3SD	1.49	0.69	0.67	0.47	0.64
N	17	17	17	17	17
All Median	2.13	0.97	0.93	0.65	0.91

New York State Fetal Defect Markers Proficiency Test,
January 2011
Summary of First Trimester Results

	FT261	FT262	FT263	FT264	FT265
FT hCG All Lab Mean:					
mean	148.08	87.00	68.80	64.62	77.33
SD	27.69	13.68	9.17	8.85	10.85
%CV	18.7%	15.7%	13.3%	13.7%	14.0%
X+3SD	231.2	128.0	96.3	91.2	109.9
X-3SD	65.0	46.0	41.3	38.1	44.8
N	17	17	17	16	17
mean/All kit median	1.07	1.05	1.03	1.04	1.05
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	115.2	73.2	61.4	55.9	65.8
SD	18.5	10.5	7.1	4.3	6.4
%CV	16.1%	14.3%	11.6%	7.7%	9.7%
X+3SD	170.8	104.5	82.8	68.8	84.9
X-3SD	59.6	41.8	40.1	43.0	46.7
N	5	5	5	5	5
median	103.0	69.0	59.0	56.9	62.6
mean/All kit median	0.83	0.88	0.92	0.90	0.89
FT hCG kit average:					
mean	138.5	83.0	66.6	62.2	74.0
SD	33.0	13.9	7.4	8.9	11.5
all kit median	138.5	83.0	66.6	62.2	74.0

	FT261	FT262	FT263	FT264	FT265
FT hCG Beckman Unicel or Access (BCU or BCX/BC1) mean:					
mean	161.8	92.8	71.9	68.6	82.1
SD	17.1	10.4	8.3	7.4	8.4
%CV	10.6%	11.3%	11.5%	10.8%	10.3%
X+3SD	213.2	124.1	96.7	90.8	107.4
X-3SD	110.4	61.4	47.0	46.3	56.9
N	12	12	12	11	12
median	163.7	91.0	69.7	68.3	82.8
mean/All kit median	1.17	1.12	1.08	1.10	1.11

	FT261	FT262	FT263	FT264	FT265
FT hCG MoMs All Lab Mean:					
Mean	2.01	1.06	0.96	1.03	0.91
SD	0.28	0.12	0.09	0.13	0.09
%CV	13.7%	11.5%	9.3%	12.8%	9.8%
mean+3*SD	2.83	1.42	1.23	1.43	1.18
mean- 3*SD	1.18	0.69	0.69	0.63	0.64
N	16	16	16	15	16
All Median	2.04	1.08	0.99	1.04	0.94

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	FT261	FT262	FT263	FT264	FT265
FT PAPP-A All Lab Mean:					
Mean	383.68	661.97	1502.66	1146.64	773.23
SD	102.34	130.95	246.34	246.82	153.03
%CV	26.7%	19.8%	16.4%	21.5%	19.8%
mean + 3SD	690.70	1054.81	2241.68	1887.09	1232.33
mean- 3SD	76.66	269.12	763.64	406.18	314.12
N	17	17	17	16	17
All Lab Median	343.00	603.12	1410.55	1075.85	725.92
mean/All kit median	0.78	0.93	1.06	1.09	0.98

	FT261	FT262	FT263	FT264	FT265
***FT PAPP-A DPC Immullite or 2000 or 2500 (DPB or D or F/DP5) Mean:**					
Mean	489.73	903.64	1974.40	1618.51	1067.66
SD	51.33	41.54	77.34	45.38	70.25
%CV	0.10	0.05	0.04	0.03	0.07
X + 3SD	643.71	1028.27	2206.42	1754.66	1278.40
X - 3SD	335.76	779.02	1742.38	1482.36	856.92
N	3	3	3	3	3
Kit Median	471.16	893.59	1986.78	1622.38	1090.87
mean/All kit median	1.00	1.27	1.39	1.54	1.35

FT PAPP-A Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	313.71	582.51	1397.10	1034.38	688.70
SD	23.79	36.48	81.09	81.30	44.63
%CV	7.6%	6.3%	5.8%	7.9%	6.5%
X + 3SD	385.06	691.95	1640.37	1278.27	822.59
X - 3SD	242.35	473.07	1153.84	790.49	554.81
N	11	11	11	10	11
Kit Median	310.0	585.0	1403.4	1039.1	681.0
mean/All kit median	0.64	0.82	0.99	0.99	0.87

***FT PAPP-A Diagnostic Systems Lab (DS1) Mean:**					
Mean	534.18	711.64	1417.94	1048.96	788.72
SD	23.42	52.58	201.88	117.36	52.64
%CV	4.4%	7.4%	14.2%	11.2%	6.7%
X + 3SD	0.87	1.53	3.40	2.51	1.68
X - 3SD	0.42	0.75	1.52	1.19	0.96
N	3	3	3	3	3
Kit Median	520.66	718.64	1505.66	1089.69	790.72
mean/All kit median	1.09	1.00	1.00	1.00	1.00

FT PAPP-A kit average:					
mean	445.87	732.60	1596.48	1233.95	848.36
SD	116.60	161.59	327.45	333.12	196.39
all kit median	489.73	711.64	1417.94	1048.96	788.72

***Note: The above 2 tables contain converted values (mIU/ml->ng/ml) from equations obtained based on in house correlation data.**
(see critique)

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FT PAPP-A MoM All Lab Mean:

Mean	0.42	0.95	1.55	0.86	0.91
SD	0.08	0.18	0.53	0.20	0.16
%CV	18.4%	19.1%	34.2%	23.8%	17.2%
mean + 3SD	0.65	1.50	3.15	1.47	1.38
mean- 3SD	0.19	0.41	-0.04	0.25	0.44
N	14	14	16	13	14
All Lab Median	0.42	0.96	1.46	0.88	0.89
mean/ All kit median	0.96	0.96	1.05	0.94	0.97

FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:

Mean	1.18	2.48	3.47	2.20	2.57
SD	0.38	0.57	1.23	0.60	0.88
%CV	32.7%	22.9%	35.5%	27.1%	34.2%
X + 3SD	2.33	4.18	7.18	3.98	5.21
X - 3SD	0.02	0.78	-0.23	0.41	-0.07
N	3	3	3	3	3
mean/All kit median	2.70	2.51	2.35	2.40	2.73

FT PAPP-A MoM Beckman Unicel or Access (BCU or BCX/BC1) Mean:

Mean	0.44	0.99	1.48	0.92	0.94
SD	0.05	0.14	0.17	0.14	0.13
%CV	12.4%	14.4%	11.6%	14.9%	13.9%
X + 3SD	0.60	1.42	1.99	1.32	1.33
X - 3SD	0.27	0.56	0.96	0.51	0.55
N	10	10	10	9	10
Kit Median	0.43	0.96	1.50	0.96	0.89
mean/All kit median	1.00	1.00	1.00	1.00	1.00

FT PAPP-A MoM Diagnostic System Labs (DS1) Mean:

Mean	0.42	0.96	1.27	0.86	0.92
SD	0.11	0.19	0.16	0.18	0.16
%CV	25.3%	19.5%	12.7%	21.0%	17.7%
X + 3SD	0.74	1.52	1.76	1.40	1.40
X - 3SD	0.10	0.40	0.79	0.32	0.43
N	3	3	3	3	3
Kit Median	0.40	0.99	1.19	0.84	0.95
mean/ All kit median	0.97	0.97	0.86	0.94	0.97

FT PAPP-A MoM kit average:

mean	0.68	1.48	2.07	1.32	1.48
SD	0.43	0.87	1.22	0.76	0.95
all kit median	0.44	0.99	1.48	0.92	0.94

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	MS 261	MS 262	MS 263	MS 264	MS 265
Gestational Age All Lab Mean:					
Mean	18.0	15.0	17.0	16.0	20.0
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	16.0	20.0
X-3*SD	18.0	15.0	17.0	16.0	20.0
N	28	28	28	28	28

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP All Lab Mean:					
mean	38.99	13.10	32.56	29.24	57.82
SD	2.61	0.90	2.43	1.77	3.69
%CV	6.7%	6.9%	7.5%	6.1%	6.4%
mean+3SD	46.8	15.8	39.9	34.6	68.9
mean-3SD	31.2	10.4	25.3	23.9	46.8
N	28	28	28	28	28
median	38.9	13.2	33.4	29.1	58.5
mean/all kit median	0.99	1.00	0.99	0.98	0.97

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	39.7	13.0	32.4	29.8	59.3
SD	1.9	0.5	2.7	1.3	3.6
%CV	4.7%	3.8%	8.4%	4.3%	6.1%
mean + 3SD	45.4	14.5	40.6	33.6	70.2
mean - 3SD	34.0	11.5	24.2	25.9	48.4
N	9	9	9	9	9
Median	39.7	13.0	33.7	29.2	59.2
mean/All kit median	1.01	0.99	0.98	1.00	0.99

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP MoMs All Lab Mean:					
mean	0.78	0.45	0.81	0.83	0.91
SD	0.08	0.04	0.08	0.07	0.08
%CV	10.2%	9.9%	9.9%	8.8%	8.4%
mean+3SD	1.02	0.58	1.05	1.05	1.14
mean-3SD	0.54	0.32	0.57	0.61	0.68
N	28	28	28	28	28

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP DPC Immulite or 2000 (DPB or DPD/DP5) mean:					
mean	36.3	12.2	30.6	27.2	53.7
SD	2.0	0.9	2.2	1.3	2.2
%CV	5.5%	7.6%	7.1%	4.7%	4.1%
mean+3SD	42.3	15.0	37.1	31.0	60.4
mean-3SD	30.3	9.4	24.0	23.4	47.0
N	8	8	8	8	8
median	36.4	12.3	30.6	27.3	54.2
mean/all kit median	0.92	0.93	0.93	0.91	0.90

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP Beckman Access (BCX/BC1) mean:					
mean	40.9	13.8	34.2	29.9	60.1
SD	2.1	0.4	1.0	1.1	1.7
%CV	5.2%	3.3%	2.8%	3.8%	2.8%
mean+3SD	47.4	15.1	37.0	33.3	65.0
mean-3SD	34.5	12.4	31.3	26.5	55.1
N	8	8	8	8	8
median	41.6	13.8	34.5	30.0	60.0
mean/all kit median	1.04	1.05	1.04	1.00	1.01

	MS 261	MS 262	MS 263	MS 264	MS 265
MS AFP kit average:					
mean	39.0	13.0	32.4	29.0	57.7
SD	2.4	0.8	1.8	1.5	3.5
all kit median	39.7	13.0	32.4	29.8	59.3

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 All Lab Mean:					
mean	1.16	0.32	0.93	0.75	1.48
SD	0.14	0.05	0.09	0.12	0.14
%CV	11.7%	14.6%	10.1%	16.3%	9.8%
mean+3SD	1.57	0.47	1.21	1.12	1.91
mean-3SD	0.76	0.18	0.65	0.38	1.04
N	27	27	27	27	27
mean/all kit median	1.06	1.06	1.05	1.09	1.06

MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	1.09	0.31	0.88	0.67	1.40
SD	0.11	0.05	0.08	0.07	0.09
%CV	10.1%	14.9%	9.7%	10.3%	6.3%
mean+3SD	1.42	0.44	1.13	0.88	1.66
mean-3SD	0.76	0.17	0.62	0.47	1.13
N	9	9	9	9	9
Median	1.08	0.29	0.87	0.64	1.39
mean/all kit median	0.94	0.94	0.94	0.91	0.94

MS uE3 BeckmanAccess (BCX/BC1) mean:					
mean	1.10	0.31	0.88	0.69	1.40
SD	0.08	0.04	0.07	0.07	0.12
%CV	6.9%	11.4%	8.2%	9.7%	8.9%
mean+3SD	1.33	0.41	1.10	0.89	1.77
mean-3SD	0.87	0.20	0.66	0.49	1.03
N	8	8	8	8	8
median	1.10	0.31	0.88	0.69	1.40
mean/all kit median	0.95	0.94	0.94	0.92	0.94

MS uE3 DPC Immulite 2000 or 2500(DPD or F/DP5) mean:					
Mean	1.34	0.36	0.98	0.94	1.57
SD	0.07	0.06	0.05	0.07	0.04
%CV	5.5%	16.6%	4.9%	7.6%	2.2%
mean+3SD	1.56	0.54	1.13	1.15	1.68
mean-3SD	1.12	0.18	0.84	0.72	1.47
N	5	5	5	5	5
Median	1.32	0.38	1.00	0.98	1.57
mean/all kit median	1.15	1.12	1.06	1.26	1.06

MS uE3 New generation DPC Immulite 2000 or 2500(DPD or F/DP6) mean:					
Mean	1.23	0.34	1.03	0.80	1.66
SD	0.13	0.03	0.06	0.08	0.11
%CV	10.5%	7.9%	5.6%	9.8%	6.9%
mean+3SD	1.56	0.54	1.13	1.15	1.68
mean-3SD	1.12	0.18	0.84	0.72	1.47
N	5	5	5	5	5
Median	1.25	0.36	1.02	0.77	1.71
mean/All Kit Median	1.05	1.06	1.10	1.08	1.11

MS UE3 kit average:					
mean	1.19	0.33	0.94	0.78	1.51
SD	0.12	0.03	0.08	0.12	0.13
all kit median	1.16	0.33	0.93	0.74	1.49

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs All Lab Mean:					
Mean	1.02	0.58	1.02	1.04	0.83
SD	0.28	0.19	0.29	0.36	0.21
%CV	27.1%	32.0%	28.8%	34.8%	25.0%
X+3SD	1.86	1.14	1.90	2.13	1.45
X-3SD	0.19	0.02	0.14	-0.05	0.21
N	27	27	27	27	27
mean/All Kit Median	1.00	0.95	0.94	0.98	0.95

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoM (DPD or F/DP5) Mean:					
Mean	1.48	0.83	1.38	1.61	1.09
SD	0.04	0.12	0.11	0.11	0.11
%CV	2.8%	14.8%	7.6%	6.9%	10.5%
X+3SD	1.60	1.19	1.69	1.94	1.44
X-3SD	1.35	0.46	1.06	1.28	0.75
N	5	5	5	5	5
mean/All Kit Median	1.45	1.36	1.27	1.51	1.26

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs (BCU/BC1) Mean:					
Mean	0.84	0.47	0.82	0.79	0.68
SD	0.10	0.07	0.09	0.08	0.06
%CV	11.4%	15.1%	11.4%	9.9%	9.4%
X+3SD	1.12	0.68	1.10	1.02	0.88
X-3SD	0.55	0.25	0.54	0.56	0.49
N	9	9	9	9	9
mean/All Kit Median	0.82	0.76	0.76	0.74	0.79

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoM (DPD or F/DP6) Mean:					
Mean	1.20	0.75	1.34	1.33	1.05
SD	0.18	0.13	0.28	0.24	0.12
%CV	15.3%	17.5%	21.2%	18.1%	11.3%
X+3SD	1.74	1.15	2.18	2.05	1.40
X-3SD	0.65	0.36	0.49	0.61	0.69
N	5	5	5	5	5
mean/All Kit Median	1.17	1.24	1.23	1.25	1.21

	MS 261	MS 262	MS 263	MS 264	MS 265
MS uE3 MoMs (BCX/BC1) Mean:					
Mean	0.84	0.45	0.83	0.80	0.69
SD	0.08	0.05	0.08	0.08	0.07
%CV	9.1%	11.3%	9.4%	9.6%	9.8%
X+3SD	1.07	0.60	1.07	1.03	0.89
X-3SD	0.61	0.30	0.60	0.57	0.49
N	8	8	8	8	8
mean/All Kit Median	0.83	0.74	0.77	0.75	0.79

	MS 261	MS 262	MS 263	MS 264	MS 265
MS UE3 MoM kit average:					
mean	1.09	0.62	1.09	1.13	0.88
SD	0.31	0.19	0.31	0.41	0.22
all kit median	1.02	0.61	1.08	1.06	0.87

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG All Lab Mean:					
mean	18.23	57.98	7.49	25.07	16.91
SD	1.75	6.93	0.62	2.72	1.82
%CV	9.6%	12.0%	8.3%	10.8%	10.8%
mean+3SD	23.5	78.8	9.4	33.2	22.4
mean-3SD	13.0	37.2	5.6	16.9	11.4
N	28	28	28	28	28
mean/all kit median	0.98	0.94	0.98	0.97	0.96

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	18.69	60.36	7.59	25.52	17.48
SD	1.89	4.34	0.52	2.86	1.68
%CV	10.1%	7.2%	6.8%	11.2%	9.6%
mean+3SD	24.58	79.13	9.15	33.13	21.75
mean-3SD	12.79	46.97	6.30	19.09	13.75
N	9	9	9	9	9
median	18.50	59.90	7.70	25.80	17.60
mean/All kit median	1.00	0.98	0.99	0.99	0.99

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG Beckman Access (BCX/BC1) mean:					
mean	18.7	63.1	7.7	26.1	17.8
SD	2.0	5.4	0.5	2.3	1.3
%CV	10.5%	8.5%	6.1%	9.0%	7.5%
mean+3SD	24.6	79.1	9.1	33.1	21.7
mean-3SD	12.8	47.0	6.3	19.1	13.8
N	8	8	8	8	8
median	19.3	64.7	7.8	25.7	17.9
mean/all kit median	1.00	1.02	1.01	1.01	1.01

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	17.1	49.8	7.1	22.8	15.3
SD	1.2	2.8	0.6	1.7	1.2
%CV	6.8%	5.5%	9.1%	7.3%	7.9%
mean+3SD	20.6	58.0	9.0	27.7	18.9
mean-3SD	13.6	41.5	5.2	17.8	11.6
N	8	8	8	8	8
median	17.0	48.9	7.0	22.6	15.7
mean/all kit median	0.91	0.81	0.92	0.88	0.87

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG kit average:					
mean	18.2	57.7	7.5	24.8	16.8
SD	0.9	7.0	0.3	1.8	1.4
all kit median	18.7	60.4	7.6	25.5	17.5

	MS 261	MS 262	MS 263	MS 264	MS 265
MS hCG MoMs All Lab Mean:					
mean	0.85	1.57	0.31	0.83	0.95
SD	0.11	0.20	0.03	0.11	0.10
%CV	13.1%	13.0%	9.5%	12.7%	11.0%
mean+3SD	1.19	2.18	0.40	1.14	1.27
mean-3SD	0.52	0.96	0.22	0.51	0.64
N	27	27	27	27	27

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	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A all lab mean:					
Mean	137.33	262.97	131.10	115.09	229.79
SD	5.27	12.70	8.90	12.15	21.61
%CV	3.8%	4.8%	6.8%	10.6%	9.4%
mean + 3SD	153.1	301.1	157.8	151.5	294.6
mean- 3SD	121.5	224.9	104.4	78.6	165.0
N	24	24	25	27	27
All Lab Median	137.5	264.7	133.0	119.2	234.0
mean/all kit median	1.00	1.01	1.01	0.98	0.98

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Beckman Access (BCX/BC1) mean:					
Mean	138.6	266.8	135.7	121.4	240.6
SD	4.1	9.3	4.3	5.1	9.7
%CV	3.0%	3.5%	3.2%	4.2%	4.0%
mean + 3SD	151.0	294.6	148.6	136.6	269.7
mean- 3SD	126.2	238.9	122.7	106.2	211.4
N	12	12	12	12	12
median	139.9	265.2	134.8	122.8	239.9
mean/All kit median	1.01	1.02	1.04	1.03	1.03

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	137.1	261.6	130.4	117.5	233.7
SD	5.3	12.9	5.9	5.4	11.6
%CV	3.8%	4.9%	4.5%	4.6%	5.0%
mean + 3SD	152.9	300.2	148.0	133.8	268.6
mean- 3SD	121.3	223.0	112.7	101.2	198.8
N	11	11	11	11	11
median	136.6	262.6	131.5	119.0	231.2
mean/all kit median	1.00	1.00	1.00	1.00	1.00

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A Diagnostic System Labs (DS1) Mean:					
Mean	94.5	192.0	93.2	89.6	186.7
SD	26.3	33.3	19.2	6.4	16.0
%CV	27.8%	17.3%	20.6%	7.2%	8.6%
mean + 3SD	173.3	291.9	150.8	108.8	234.7
mean- 3SD	15.7	92.1	35.6	70.4	138.7
N	4	4	4	4	4
median	96.0	191.2	97.2	89.8	187.6
mean/all kit median	0.69	0.73	0.71	0.76	0.80

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A kit average:					
mean	123.4	240.1	119.7	109.5	220.3
SD	25.0	41.7	23.1	17.3	29.3
all kit median	137.1	261.6	130.4	117.5	233.7

	MS 261	MS 262	MS 263	MS 264	MS 265
MS Inhibin A MoM All Lab Mean:					
mean	0.85	1.38	0.76	0.65	1.16
SD	0.07	0.22	0.13	0.10	0.18
%CV	8.1%	16.2%	17.1%	14.9%	15.5%
mean+3SD	1.06	2.05	1.14	0.94	1.69
mean-3SD	0.65	0.71	0.37	0.36	0.62
N	24	27	27	27	27

New York State Fetal Defect Markers Proficiency Test,
January 2011
Summary of Results

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP All Lab Mean :					
mean	7.06	9.98	8.80	6.20	19.05
SD	1.01	1.32	1.02	0.84	2.37
%CV	14.3%	13.2%	11.6%	13.6%	12.4%
mean+3SD	10.1	13.9	11.9	8.7	26.2
mean-3SD	4.0	6.0	5.7	3.7	11.9
N	22	22	22	21	22
mean/all kit median	1.01	0.97	0.98	0.95	0.98

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP DPC Immulite or 2000 (DPB or D/DP5) mean:					
mean	6.7	10.7	9.2	6.9	16.5
SD	0.6	0.7	0.6	0.6	0.9
%CV	8.7%	6.4%	6.4%	8.3%	5.3%
mean+3SD	8.4	12.7	10.9	8.6	19.1
mean-3SD	4.9	8.6	7.4	5.2	13.9
N	5	5	5	5	5
median	6.5	10.6	9.1	6.7	16.3
mean/all kit median	0.95	1.03	1.02	1.05	0.85

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP MoMs All Lab Mean:					
mean	0.91	0.58	0.92	1.19	3.01
SD	0.09	0.06	0.10	0.15	0.38
%CV	10.1%	9.6%	10.4%	12.7%	12.7%
mean+3SD	1.18	0.75	1.21	1.65	4.17
mean-3SD	0.63	0.42	0.63	0.74	1.86
N	22	22	22	21	22

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	6.2	8.7	8.0	5.2	18.5
SD	0.7	0.8	1.3	0.3	1.8
%CV	10.5%	9.4%	15.8%	6.4%	9.9%
X+3SD	8.7	11.9	10.0	7.1	24.8
X-3SD	6.0	8.2	7.7	5.4	16.4
N	7	7	7	6	7
median	6.0	8.7	7.6	5.1	18.0
mean/All kit median	0.89	0.84	0.88	0.79	0.95

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Beckman Access (BCX/BC1) mean:					
mean	7.4	10.0	8.8	6.2	20.6
SD	0.4	0.6	0.4	0.3	1.4
%CV	6.0%	6.1%	4.3%	4.7%	6.8%
mean+3SD	8.7	11.9	10.0	7.1	24.8
mean-3SD	6.0	8.2	7.7	5.4	16.4
N	6	6	6	6	6
median	7.2	9.95	8.85	6.3	20.5
mean/all kit median	1.05	0.97	0.98	0.95	1.05

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP Abbott Axsym (ABB/AB2) mear:					
mean	8.7	12.2	10.1	7.3	21.7
N	2	2	2	2	2
mean/all kit median	1.24	1.18	1.12	1.11	1.11

	AF 261	AF 262	AF 263	AF 264	AF 265
AF AFP kit average:					
mean	7.2	10.4	9.0	6.4	19.3
SD	1.1	1.4	0.9	0.9	2.3
all kit median	7.0	10.3	9.0	6.6	19.5