NEW YORK
state department of
HEALTH

Howard A. Zucker, M.D., J.D.
Acting Commissioner of Health

Sue Kelly
Executive Deputy Commissioner

Fetal Defect Marker Proficiency Test Mailout[1] - September 2014

Dear Laboratory Director,
Below you will find a summary and critique of the Proficiency Testing mailout from September 9, 2014, 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 *No = 27	**Sample #**	**MS 316**	**MS 317**	**MS 318**	**MS 319**	**MS 320**
	Gestational Age (weeks)	20.0	17.0	16.0	15.0	18.0
Maternal Race	Ethnic Group	White	Hispanic	Black	White	White
Maternal Weight	Pounds (lbs)	150	140	145	160	155
Maternal Age	Years	25	20	28	30	32
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	198.3 ± 16.0	47.0 ± 3.3	39.7 ± 3.1	23.7 ± 1.6	32.3 ± 2.0
	MOM ± Std. Dev.	3.37 ± 0.34	1.19 ± 0.10	1.05 ± 0.09	0.85 ± 0.08	0.74 ± 0.06
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	1.13 ± 0.10	0.78 ± 0.08	0.62 ± 0.06	0.41 ± 0.05	0.49 ± 0.05
	MOM ± Std. Dev.	0.60 ± 0.10	0.78 ± 0.22	0.80 ± 0.22	0.68 ± 0.18	0.41 ± 0.10
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	20.0 ± 2.9	27.1 ± 3.4	31.6 ± 4.5	61.1 ± 8.5	49.0 ± 6.9
	MOM ± Std. Dev.	1.06 ± 0.13	1.00 ± 0.08	0.88 ± 0.07	1.50 ± 0.25	2.20 ± 0.23
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	166.7 ± 11.1	95.2 ± 6.0	83.9 ± 4.5	116.6 ± 7.4	209.8 ± 15.1
	MOM ± Std. Dev.	0.87 ± 0.08	0.55 ± 0.03	0.48 ± 0.03	0.64 ± 0.05	1.25 ± 0.09
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	G = 88% U = 92% A = 92%	NFA	NFA	NFA	NFA
	NTD Risk 1 in	24	5,445	10,000	6,525	11,000
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (B) (58%)	(+) (100%)
	Recommended Action**	NFA	NFA	NFA	G = 58% U = 25% A = 58% N = 8%	G = 92% U = 58% A = 83% N = 8%
	Risk Est. 1 in	4,704	2,705	3,200	218	27
2. Quad Test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (96%)
	Recommended Action **	NFA	NFA	NFA	NFA	G = 96% U = 65% A = 85% N = 8%
	Risk Est. 1 in	16,050	15,750	15,000	11,400	59
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	10,000	10,000	10,000	10,000	1,050

*No = 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 NTD/Down positive or negative/borderline screen). NFA = no further action; FA = further action; G = genetic counseling; U = ultrasound, A = amniocentesis, and N = Noninvasive prenatal testing. **This percentage is normalized to labs requesting further action. ‡ Insulin Dependent Diabetic pregnancy.

[1]The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 27 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 316 Wk 20.0	This specimen was obtained from a 25 year old White woman (Gravida = 3, parity = 1) in her 20th week gestation with a body weight of 150 lbs. She had a personal history of pregnancy loss. Her sample was a positive screen for NTD (100% consensus; MOM = 3.37). Her screen was negative for both Trisomies with all labs in agreement. Recommendations of further action from labs performing the NTD screen were: genetic counseling, 88%; ultrasound, 92%; and amniocentesis, 92% and repeat sample was 0%. The MS316 specimen had an amniotic fluid counterpart (AF316) which was also elevated (MOM = 2.24).
MS 317 Wk 17.0	This specimen was obtained from a 20 year old Hispanic woman (Gravida = 1, Parity = 0) in her 17th week of gestation with a body weight of 140 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight or ethnic correction indicated. The labs agreed that both Trisomy screens were negative. Specimen MS317 was not paired with an amniotic fluid specimen.
MS 318 Wk 16.0	This specimen was obtained from a 28 year old Black woman (Gravida = 1, Parity = 0) in her 16th week of gestation with a body weight of 145 lbs. She had no family history of reproductive complications. Her sample screened negative for NTD, and her aneuploidy screens were also negative for both Trisomy-18 and Trisomy-21. The MS318 sample was not paired to an amniotic fluid specimen.
MS 319 Wk 15.0	This specimen was obtained from a 30 year old White woman (Gravida = 3, Parity = 2) in her 15th week of gestation with a body weight of 160 lbs. She had a pre-existing autoimmune disease. Her sample screened negative for NTD, and her aneuploidy screen was borderline positive for Down syndrome but only by triple test. Further actions were recommended as: Genetic counseling 58%; ultrasound 25%; amniocentesis 58%; non-invasive prenatal testing, 8%. In contrast, no lab determined an elevated risk for trisomy by Quad test (see critique). This sample was not paired to an amniotic fluid specimen.
MS 320 Wk 18.0	This specimen was obtained from a 32 year old White woman (Gravida = 4, Parity = 2) in her 18th week gestation with a body weight of 155 lbs. She had a family (sibling) history of pregnancy complications. Her specimen screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100%). Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 96%; ultrasound, 65%; amniocentesis, 85% and noninvasive prenatal testing, 8%; while labs performing the triple test recommended genetic counseling, 92%; ultrasound 58%; and amniocentesis, 83% and noninvasive prenatal testing, 8%. Specimen MS320 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 = 0.69).

Notice of Gravida/Parity Clarification for Present and Future Mailouts;

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 pregnancies including the present one and parity = m indicates the patient already has m children; 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=20; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 316 Wk 20.0	AFP = 13.9 ± 1.1 µg/ml MOM = 2.24 ± 0.33	The AF316 sample was targeted as an NTD positive specimen in the upper gestational age screening range. All labs categorized AF316 as a positive NTD screen. This specimen had a maternal serum counterpart, MS316, which showed elevated levels of AFP (MOM = 3.37).
AF 317 Wk 18.7	AFP = 6.2 ± 0.6 µg/ml MOM = 0.73 ± 0.09	The AF317 sample was targeted for normal AFAFP value in the routine gestational age range. All labs called AF317 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 318 Wk 19.0	AFP = 10.9 ± 1.3 µg/ml MOM = 1.43 ± 0.21	The AF318 sample was targeted for a screen negative AFAFP value in the routine gestational age screening range. All labs reported this specimen as a screen negative AFAFP value. The AF318 specimen was not paired with a maternal serum sample.
AF 319 Wk 19.7	AFP = 7.5 ± 0.8 µg/ml MOM = 1.06 ± 0.12	The AF319 sample was targeted for a screen negative AFAFP value in the lower gestational age range. All labs reported this specimen as a screen negative AFAFP value. The AF319 specimen was not paired with a maternal serum sample.
AF 320 Wk 18.0	AFP = 6.5 ± 0.6 µg/ml MOM = 0.69 ± 0.12	The AF320 sample was targeted for a non-elevated AFAFP value in the routine gestational age range. All labs called AF320 a negative screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen MS320 whose AFP level was also low (MOM = 0.74).

3) First Trimester Maternal Sera:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *No = 17	**Sample #**	**FT 316**	**FT 317**	**FT 318**	**FT 319**	**FT 320**
	Gestational Age (weeks)	13.0	12.4	11.4	11.1	13.0
Maternal Race	Ethnic Group	Black	Asian	White	White	Hispanic
Maternal Weight	Pounds (lbs)	130	140	145	130	155
Maternal Age	Years	21	28	25	35	23
Fetal Physical Measurements	Crown Rump Length (mm)	67	60	47	44	68
	NT Thickness (mm)	1.50	1.40	2.50	1.20	1.60
	NT – MOM ± Std. Dev.	0.92 ± 0.06	0.95 ± 0.06	2.10 ± 0.11	1.06 ± 0.06	0.97 ± 0.06
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	63.8 ± 11.7	68.9 ± 11.0	172.3 ± 33.2	86.8 ± 16.2	66.0 ± 14.4
	MOM ± Std. Dev.	0.78 ± 0.11	0.84 ± 0.09	1.87 ± 0.21	0.86 ± 0.11	0.91 ± 0.10
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	3826.4 ± 1654.5	3087.9 ± 1296.2	1343.0 ± 632.1	2241.4 ± 917.0	3755.4 ± 1607.8
	MOM ± Std. Dev.	2.58 ± 1.31	3.23 ± 1.34	2.09 ± 0.90	3.46 ± 1.34	3.41 ± 1.38
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (B) (50%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	G = 50% U = 19% A = 25% C = 25% N = 13%	NFA	NFA
	Risk Estimate 1 in	20,000	20,000	270	10,000	20,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	10,000	4,650	10,000	10,000

*No = 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; A = amniocentesis; C = chorionic villus sampling, and N = Noninvasive prenatal testing; No = 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 E below.

B. Narrative Evaluation of First Trimester Screening Results:
No = 17 all-lab Consensus Values.

Sample#	Summary Comments:
FT 316 Wk 13.0	This specimen was obtained from a 21 year old Black woman with a body weight of 130 lbs. Her gestational age at the time of screening was 13.0 weeks. She had no prior history of pregnancy complications or difficulties. This FT specimen was screen negative and all testing labs were in agreement. The FT316 risk estimate for Trisomy-21 was 1 in 20,000 and the Trisomy-18 risk was 1 in 10,000.
FT 317 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 with an all-lab consensus of 100%. The FT317 risk estimate for Trisomy-21 was 1 in 20,000 (all lab median), and the Trisomy-18 risk was 1 in 10,000.
FT 318 Wk 11.4	This specimen was procured from a 25 year old White woman of average body weight (145 lbs.). Her gestational age at the time of screening was 11.4 weeks. She had no prior history of any pregnancy complications. This FT specimen was borderline screen positive for Trisomy-21 with 50% of testing labs reporting an elevated risk. The FT318 risk estimate for Trisomy-21 was 1 in 270, and the Trisomy-18 risk was 1 in 4,650.
FT 319 Wk 11.1	This specimen was procured from a 35 year old White woman of average body weight (130 lbs.). Her gestational age at the time of screening was 11.1 weeks. She had no prior family history of pregnancy complications and adverse outcomes. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT319 risk estimate for Trisomy-21 was 1 in 10,000, while the Trisomy-18 risk was 1 in 10,000.
FT 320 Wk 13.0	This specimen came from a 23 year old Hispanic woman with a body weight of 155 lbs. Her gestational age at the time of screening was 13.0 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for both Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT320 was 1 in 20,000, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

II. 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. The Caucasian maternal serum sample **MS316** was targeted as a positive specimen for NTD (Figs. 2a and 3) and was matched to the elevated **AF316** sample (Fig. 2b). All labs (100%) agreed that specimen **MS316** was screen positive for NTD and negative for both Trisomy screens using both the triple and quad tests (Figs. 4-6). Since the **MS316** sample matched to **AF316** (MSMOM = 3.37 vs AFMOM = 2.24), which exhibited elevated AFP levels, a diagnostic follow-up for the presence of an NTD would be indicated. A polyacrylamide gel electrophoresis is warranted to show the absence or presence of a diagnostic ACHE band which would confirm the presence of an NTD.

Sample **MS320** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS320** (MSAFP-MOM = 0.74, MSuE3-MOM = 0.41, MShCG-MOM = 2.20, DIA-MOM = 1.25) presented the canonical quad test profile of low MSAFP and low MSuE3, together with elevated MShCG and MSDIA (Fig. 1) resulting in a T21 positive screen with which all labs agreed (100% by both triple and quad test). Furthermore, the matched **AF320** specimen was low (MOM value = 0.64). The T21 risk was 1 in 27 by triple test and 1 in 59 by quad test (Figs. 4, 5). The recommended further actions for the sample **MS320** were genetic counseling, 96%; ultrasound, 65%; amniocentesis, 85%; and noninvasive prenatal testing, 8%, from labs performing the quad screen and genetic counseling, 92%; ultrasound, 58%; amniocentesis, 83%, and noninvasive prenatal testing, 8%, from labs performing the triple screen.

Two other specimens, **MS317** and **MS319**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS319** specimen at 15 weeks presented an interesting case involving low levels of MSAFP (MOM = 0.85), MSuE3 (MOM = 0.68), and slightly elevated MShCG levels (MOM = 1.5), and low MSDIA (MOM = 0.64); this profile resulted in a negative screen for T21 (Risk = 1 in 1400) (Fig. 5) for the quad test. However, using the triple test that does not include MSDIA, the average T21 risk was increased to 1 in 218 with individual lab risk values residing around the 1:270 cutoff (Fig. 4). Thus, while the quad test produced a negative risk for T21, the triple test resulted in a borderline positive risk (1 in 218) for Down syndrome. The quad test screen required no further action; however, the triple test indicated 58% genetic counseling, 25% ultrasound and 58% amniocentesis in support of the borderline positive risk assessment. Sample **MS319** was modeled after several literature case studies of pregnant women with multiple sclerosis disease (MSD) which contained aberrant triple test biomarkers. Prior to their present pregnancy, most of the MSD case study women had not experienced complicated pregnancies and had delivered normal term infants. Although the women had been counseled on the effects of medications for MSD taken prior to and during early pregnancy, the women chose to continue gestation and underwent further testing which included ultrasound, 3-D scans, and MSD-related tests including serum autoantibody assays. Some of the patients in the studies of autoimmune MSD had been treated with prednisone and corticosteroids prior to their pregnancy. All women in these studies had pre-existing MSD upon presentation at the first obstetrician's visit, and all delivered normal term infants with no signs of anatomical abnormalities. However, all patients had experienced cycles of MSD remissions and relapses prior to, during pregnancy, and in the perinatal period.

The maternal serum biomarker levels of **MS319** were modeled after one of the case reports which revealed an MSAFP MOM of 0.70, MSuE3 MOM of 0.63, and MSHCG MOM of 1.72 (1). Presently these values resulted in a borderline positive screen for T21. Previous reports from the literature had demonstrated that both the triple and quad test biomarkers often predicted pregnancy complications and adverse outcomes in addition to Down syndrome (2, 3). These outcomes included miscarriage, low birth weight, stillbirth, and increased placental thrombotic events (4, 5). In the present screen, specimen **MS319** produced a borderline positive prenatal screen for T21 due to a triple test profile of low MSAFP, MSuE3, and elevated hCG; these biomarkers will be discussed in the context of anti-inflammatory and immunoprotective agents directed against MSD.

Multiple sclerosis disease (MSD) is a chronic autoimmune-mediated neurological disorder acquired during young adulthood (6). Most patients manifest their onset of disease between the ages of 20 and 30 years of age. At least 400,000 people in the United States and 1 to 2 million people worldwide have MSD (7). This autoimmune neuro-inflammatory disease is three times more prevalent in women than in men (8). The majority of patients with MSD are of child-bearing age; hence, its occurrence in pregnancy is common. MSD is the most prevalent de-myelinating disease of the central nervous system (CNS) and is both an unpredictable and potentially disabling condition (9). No single gene or gene cluster is known to produce multiple sclerosis. The disease is highly influenced by pregnancy, hormonal factors, and anti-inflammatory agents which appear to serve as neuroprotective factors in the immune induction and effector stages of this neurological disease (10). Thus, changes in circulating pregnancy hormones and soluble circulating factors (estrogens, progesterones, prolactin, hCG, AFP, etc.) are thought to have protective effects against the immunoneurological damage that underlie the pathology of MSD.

During pregnancy, MSD activity involves two major types, a period of profound reduction of MSD symptoms (remission) in the third trimester followed by an exacerbation of disease (relapses) in the postpartum period before returning to a pre-pregnancy disease state (7). Past and present data continue to support the conclusion that long-term MSD progression is not worse and may actually be lessened during pregnancy in patients with relapsing MSD. In a study of 935 pregnant women with MSD, relapses occurred 40-50% in the postpartum period, with only 10% relapses in the third trimester (9). Thus, investigators found a notable decrease in relapses in the third trimester of pregnancy, but a high increase in the first 3 months following delivery (11, 7). There appears to be soluble protective factors produced during pregnancy that causes the disease to be less active. Presumably, these are soluble factors that suppress the cell-mediated immune response. Several such factors have been proposed, including AFP, uE3, hCG, and more recently, an embryo-specific preimplantation factor (8).

The impact of pregnancy on MSD is only slight and no adverse effects have been reported. Furthermore, MSD patients are informed that their disease has no meaningful impact on the ability to conceive, pregnancy itself, ability to give birth, and fetal status and well-being (7). Furthermore, no convincing increase in spontaneous abortions, ectopic pregnancies, cesarean deliveries, or major obstetric complications have been demonstrated. Pregnancy also had no impact on the long-term progressive course of MSD or the likelihood of a secondary progression of MSD (10). Moreover, pregnancy and childbirth in MSD have been associated with less long-term disability and have no effect on fertility and family planning. However, there are risk factors for MSD during pregnancy which include: Vitamin-D deficiency, previous diethylstilbesterol (DES) exposure, late maternal initiation of prenatal care, maternal

overweight/obesity, diabetic pregnancy, maternal immune related medical conditions, cigarette smoking, excessive alcohol, and heavy caffeine use. Risks from a previous pregnancy may exist only if fetal loss, placental complications, and preterm delivery had been experienced (12).

Biomarkers of the triple test for Down syndrome have been implicated in the protection of pregnant women with MSD. The estrogens, especially estriol, have been shown to provide an anti-inflammatory and protective effect in experimental allergic encephalomyelitis (EAE), the animal model equivalent of multiple sclerosis (13). The anti-inflammatory effect appears to be mediated by estrogen nuclear receptors (ER alpha and ERbeta) expressed by T-regulatory cells bearing $CD4^+$ $CD25^+$ surface markers, by regulatory T-cells and dendritic cells (14). This anti-inflammatory mediation is eliminated in the absence of ER beta-receptors and programmed death-1 (PD-1) protein expressed on CD4+FOX3+T-reg cells (15). In non-pregnant MSD patients treated with pregnancy levels of uE3, amelioration of disease was evident by significant decreases in brain lesions shown by magnetic resonance imaging (16). MSD is a Th-1 lymphocyte mediated autoimmune disease; during pregnancy, a Th-1 to Th-2 expressing lymphocyte population shift occurs providing an immunoprotective effect for the mother. An immunomodulatory effect of estriol therapy on cytokine profiles demonstrated that significant increases in IL-5 and IL-10 occurred concomitant with decreased tumor necrosis factor (TNF) alpha levels. The increase in IL-5 was due to increased $CD4^+$ and $CD8^+$ expressing T-cells, while increased IL-10 levels were attributed to increased numbers of $CD64^+$ monocytes. The decreased TNF-alpha levels resulted from a decrease in the numbers of $CD8^+$ T-cells (16).

Alpha-fetoprotein (AFP) is a gestational-age-dependent biomarker present in both fetal and maternal serum during pregnancy. AFP has a long history as an immunomodulatory glycoprotein and is associated with normal fetal growth and development. Recombinant human AFP has been reported to reduce EAE-induced neuroinflammation, to increase apoptosis of activated immune cells by inhibiting BCL-2-related pathways, and to increase the presence of FAS-related (CD95) ligands (17). Furthermore, AFP increased both FOXp3 expression in lymph nodes and T-reg cell numbers in the CNS (18). AFP has been studied in animal models of MSD and was found to both treat and prevent the induction of EAE. AFP exerts significant immunosuppressive effects on T-cells in vitro at physiologic concentrations and has the capacity to induce suppressor T-cells (19). Overall, several investigators have shown a beneficial effect of AFP on the course of EAE in guinea pigs and other animal models (19-21). During human pregnancy, maternal serum AFP levels gradually increase until the 30-32 week period of the third trimester; this is the gestational period in which 50% of MSD patients experience disease remission. Thereafter, maternal serum AFP levels decrease to low nanogram/ml levels at postpartum when most relapses were found to occur.

Human chorionic gonadotrophin (hCG) constitutes a component of both the triple and quad tests for Down syndrome. HCG is a naturally occurring, immunomodulating agent that is highly expressed in pregnancy and contributes to clinical improvements in other autoimmune diseases such as rheumatoid arthritis during the gestation period. The precise mechanism of hCG immune modulation in MSD is not known. Studies in women who received hCG prior to in vitro fertilization-induced pregnancies demonstrated that hCG increased anti-inflammatory IL-27 expression, while reducing pro-inflammatory IL-17 expression (22). In addition, it was found that IL-10 levels increased as did elevated numbers of T-reg cells in the peripheral blood of hCG-treated women. In another study of MSD in non-pregnant women, 15 patients were treated with 10,000 I.U hCG daily for 12 weeks; results recorded on disease improvement and stabilization were obtained through patients' interview scores. Seven of 15 patients (47%) showed high overall disease improvement scores, with four patients (27%) showing improvement of neurological impairment scores, and four patients (27%) showing improved functional impairment scores (23). Finally, endocrine hormone impairments of the hypothalamic-pituitary-gonad axis were shown to exist in MSD patients that were given hCG and these were monitored by means of immunoassays (24).

The immune system in normal pregnancy does not produce an immunosuppressive state but rather an immunotolerant state with the mother adapting to the fetus as a uterine allograft. For MSD patients in pregnancy, four biological changes have been reported to occur (7). First, levels for a number of hormones increase markedly and then drastically decrease following birth; these include estrogens (especially estriol), progesterone, prolactin, and glucocorticoids. These hormones induce shifts in cytokine levels, a decrease in the presence of adhesion molecules and metalloproteinases, a decrease in antigen presentation to dendritic cells, and a boost in the numbers of regulatory T-cells (T-regs) (25) resulting in an overall decrease in inflammatory responses in the body. Second, significant immune cell numbers change during MSD pregnancy involving T-regs, T-helper cells, and natural killer cells. Third, fetal-derived antigens are released and interact with and modulate the maternal immune T-reg and dendritic cells (antigen-presenting cells). Overall, MSD pregnancy may result in positive benefits to the maternal CNS by promoting recovery mechanisms and enhancing the ability to respond to immune mediated injury (demyelination). Reports of patients with MSD have shown marked improvements in neurological symptoms during the course of pregnancy (9).

In regards to the patient case history represented by the MS319 specimen this patient and seven additional women with similar presentations were followed throughout pregnancy (1). Clinical conditions, T-cell subsets, and

levels of immunoreactive pregnancy-associated biomarkers were measured in these patients during the pregnancy and throughout the first postpartum year. None of the women's MSD became worse during pregnancy; however, six of the eight (75%) women experienced relapses within the first seven weeks following delivery. The number of CD8 suppressor T-cells were decreased, and the CD4 helper cell-to-CD8 suppressor T-cell ratios were increased in pregnant MSD patients compared to normal pregnant women. Overall, there was no evident relationship between the T-cell numbers/ratios and clinical disease activity even though differences were found in suppressor T-cell numbers. A reduction in the peripheral levels of CD4 helper T-cells have been reported during MSD pregnancy with a consequent lowering of the helper-suppressor T-cell ratio (26). Moreover, increased helper-suppressor T-cell ratios have been reported in association with MSD increased activity (27). AFP levels in these MSD patients were not elevated, but rather decreased, and levels of alpha-2- pregnancy associated glycoprotein and PAPP-A were not significantly different from controls.

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7-10). All participating labs used either a Beckman or Siemens Immulite method. As shown in Figs. 7A-7D, MS-AFP and AF-AFP mass measurements among the individual kits mostly agreed. The exception was Siemens Immulite in amniotic fluid, which returned values that were 10-20% higher than those from the Beckman methods. When the kit specific uE3 MOMs and mass values were compared, mass values from Siemens DPC Immulite 2000/2500 ranged 5-10% lower than those from the Beckman kits (Fig. 8A and 8B) whereas the corresponding MOMs were approximately 10% higher; however, preliminary studies in our lab suggest this may derive from a matrix effect in our samples. Regarding the hCG kits (Fig. 10A), results from the Beckman 5th generation kits (BCU/BC2; BCX BC2) were about 15% to 20% higher than those from the original Beckman kits (BCU/BC1;BCX/BC1), which were similar to the Siemens Immulite 2000 results. These differences were largely eliminated by the conversion to MOM values (Fig. 10B). Finally, the method comparison for Inhibin-A displayed in Fig. 9A shows that there was no difference between the results from the Beckman Access/2 and UNICEL instruments (Fig. 9B).

C) Second Trimester Screening Software Utilized:

The alpha and Benetech PRA software packages were each used by 33.3% and 25.9%, of the labs, respectively; Robert Maciel (RMA) software was employed by 25.9%; and in-house and "other" software comprised 14.8%. Programs classified as "other" are presumably proprietary software packages.

D) First Trimester Assay Kit Performance:

In order to compare the Beckman UNICEL assays (69% users) for PAPP-A with those of the older Siemens Immulite and AnshLabs assay platforms, a conversion factor given in the AnshLabs/Anshlite package insert of 0.00256 mIU/ml =1ng/ml was used.

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11, and 12 for the five FT samples. As shown in Fig 11A, FT hCG mass measurements by Beckman UNICEL 5th IS hCG kit were ~20% higher than those by the original Beckman kit, while the Siemens Immulite DPC instruments measured approximately 20% below the Beckman Access 2/UNICEL instruments. Overall, the hCG MoM values reflected the mass values but the differences were somewhat reduced (Fig. 11B). The results from the three PAPP-A kits, even when converted to the same mass units, were not consistent among one other (Fig. 12A) with Siemens Immulite nearly 2.5 times greater than Beckman, and Anshlite less than half of Beckman. Corresponding MOM values also reflected these differences.

E) First Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 25% and 19% of the labs, respectively; Robert Maciel (RMA) software was employed by 38%; and in-house software comprised 19%. None of the labs used programs classified as "other".

G.J. Mizejewski, Ph.D.

New and Related References (Suggested reading):

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Title: Alpha-fetoprotein – Derived Peptides as Epitopes for Hepatoma Immunotherapy: A Commentary

Therapeutic use of AFP in liver tumors

In order to assess AFP cytotoxicity against hepatoma cells, cytotoxic T lymphocytes (CTLs) have been induced by dendritic cells phagocytosing HLA-A2+ restricted epitope peptides encapsulated in polylactic acid (PLA) AFP-microspheres (PLA-AFP218-226). The studies utilized cell lines HepG2 and T2-hepatoma cells incubated with HLA-A2+ restricted epitope peptides derived from the second domain AFP-derived peptide epitope (HAFP #218–226, LLNQHACAV) (Table 2). Mature dendritic cells, obtained by inducing monocytes isolated from peripheral blood cells of HLA-A2+ healthy donors with GM-CSF and IL-4, were employed. On day 3 of the culture, PLA-AFP #218–226 was added to the culture medium and on day 6, LPS was added to induce the maturation of immature DCs. A high avidity was observed between HAFP #218–266 and the HLA-A2 cell surface proteins. The DCs phagocytosing PLA-AFP218-226 peptide highly expressed CD83, CD86, and CD40, while the CTLs induced by the DCs destroyed the HAFP #218–226-incubated HepG2 and T2 tumor cells. The strong cytotoxicity against HepG2 cell lines could be induced in vitro by DCs phagocytosing PLA-AFP #218– 226 microspheres, in hopes that such microspheres could serve as a new type of CTL epitope vaccine for the prophylaxis and treatment of hepatocellular carcinoma [40].

Alpha-fetoprotein-derived peptide epitopes are now being proposed as a potential resource for T cell-based immunotherapy for hepatocellular carcinoma (HCC); however, the number of its identified epitopes is still limited and the status of AFP-specific immunological responses in hepatoma patients has not been extensively characterized. To address this concept, investigators have studied the effects and consequences of inducing AFP-specific cytotoxic T cells (CTLs) employing HLA-A*2402-restricted T cell epitopes derived from AFP. The relationship between its frequency of occurrence and clinical features associated with patients having HCC was analyzed. Five newly-derived AFP-derived peptides, containing HLA-A*2402 binding motifs (Table 3), were studied; these showed high binding affinity to HLA-A*2402 antigens and induced CTLs to produce IFN-gamma that destroyed an AFP-secreting hepatoma cell line. The frequency of AFP-specific CTLs production in peripheral blood mononuclear cells was similar to other immunogenic cancer associated antigen-derived epitopes. Analyses of the relationships between AFP-specific CTL responses and clinical features of patients with hepatomas revealed that the AFP peptide epitopes were often recognized by CTLs in patients with advanced HCC and these data correlated with the stage of tumor progression. The analyses of CTL responses before and after therapy showed that the treatments changed the frequency of appearance of AFP-specific CTLs. Overall, the research group identified five new HLA-A*2402- restricted T cell epitopes (Table 2) derived from AFP which lent further credence to the concept of hepatoma AFP-based immunotherapy [33].

Other researchers have investigated the effects of pegylated (PEG)-interferon (IFN)-alpha2b on alpha-fetoprotein (AFP) expression as demonstrated by AFP protein and mRNA levels in several hepatoma cell lines [39]. The number of KIM-1 hepatoma cells in culture with PEG- IFN-alpha2b decreased between 24 and 240 h, whereas the levels of intracellular and secreted AFP increased, with levels 1.9-fold and 2.9-fold higher than control cells. The AFP mRNA levels increased between 72 and 192 h, achieving threefold higher levels than that of the control. In the 72-h culture utilizing PEG-IFN-alpha2b, dose-dependent increases occurred in both AFP protein and mRNA expression, while a dose-dependent decrease in cell number resulted from both apoptosis and blockage of the cell cycle at the S-phase. The rate of fucosylated AFP in the cell lysate decreased in a dose-dependent and time-dependent manner. In the PEG-IFN-alpha2b culture of other hepatoma cell lines, cell proliferation was suppressed while the expressions of AFP protein and mRNA increased in only two cell lines with suppression of cell proliferation unrelated to the increase in AFP expression. Overall, the findings showed that PEG-IFN-alpha2b induced an increase in AFP expression at both protein and mRNA levels [39].

The study of AFP-specific CD4(+) T cell responses have been applied in patients bearing hepatomas as shown above. Investigators have further shown that AFP specific CD4(+) T cell helper responses to three immunodominant epitopes in HCC patients were significantly expanded during and after embolization [4]. The development of higher

frequencies of AFP-specific CD4(+) T cells after treatment were significantly associated with the induction of >50% necrosis of tumor and an improved clinical outcome. Also, the authors identified two additional HLA-DR-restricted AFP-derived CD4(+) T cell epitopes HAFP #137–145 and HAFP #249–258 (Table 3) and showed that the CD4(+) T cells recognizing these epitopes produce Th-1 (IFN-gamma and TNF-alpha) but not Th-2 (IL-5)-type secreted cytokines. HAFP #137–145, HAFP #249–258, and HAFP #364–373-specific CD4(+) T cells were detected in HCC patients but not in the induction of tumor necrosis (Table 3). Thus, the conventional cancer treatment of embolization was found to unmask tumor rejection antigen cell-mediated immunity providing the rationale for combining embolization with immunotherapy in hepatoma patients [4].

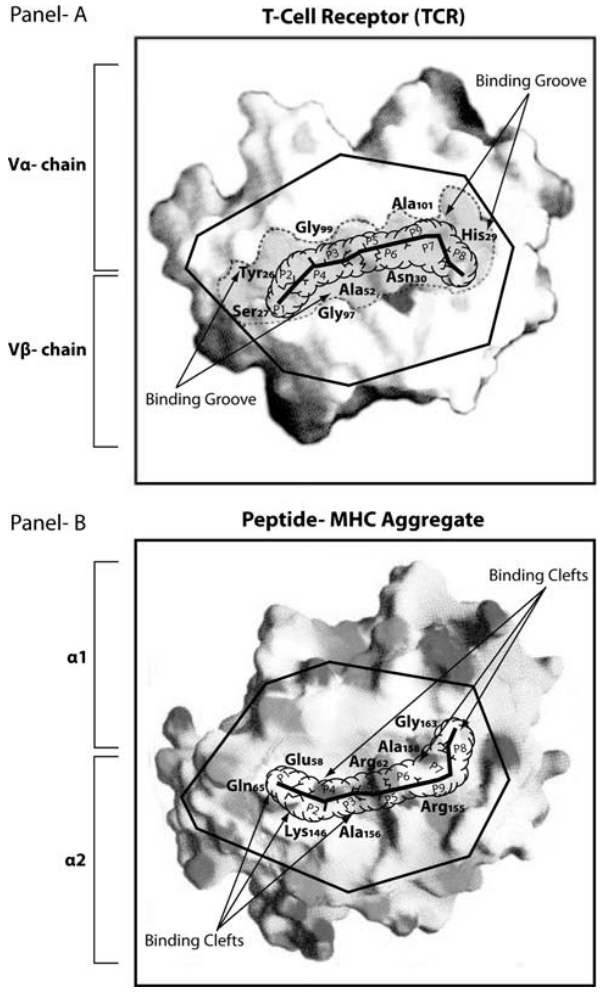

Fig. 2 *Panels A* and *B* an artist's steroscopic depiction of the surface molecular topology of the T cell receptor heterodimers (*top*, *Panel-A*) versus the HLA-A class-1 heterodimer (*bottom*, *Panel-B*). In this mock rendition of the electrostatic molecular surface, the two opposing TCR and MHC heterodimers are shown. The T cell to peptide-MHC contact surfaces are displayed by the rectangular (*solid-line*) boxed areas in both panels. The 8-mer peptide bound to the clefts of the MHC molecule is represented by the scalloped cloud rod-shaped peptide image in the center of the *rectangular boxes* in *Panel-B*. Similarly, the cloud-shaped peptide mirror image is also displayed on the opposing surface of the T cell receptor (*Panel-A*); a shaded binding groove runs lengthwise across the T cell molecular surface. The floor of the TCR binding groove is composed of eight beta strands, while the walls of the groove are comprised of two anti-parallel alpha helices composed of amino acids of the TCR which pair to the eight amino acids of the peptide epitope [15, 24]. The dark line perimeter on the molecular surface in *Panel-A* is presented as a mirror image of the molecular surface perimeter in *Panel-B*

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

(1) Source: Scand J Clin Lab Invest Suppl 74:41-47, 2014.

Title: Screening for Down syndrome.

Authors: Spencer, K.

Abstract: Abstract Screening for Down Syndrome was initially only related to maternal age and has successively developed by introducing biochemical markers and algorithms to estimate the risk for particularly trisomy 21 and 18. We now have a long experience of screening with four biochemical markers, alpha-fetoprotein, total hCG, unconjugated estriol and free beta-hCG during the second trimester. Screening is now moving towards screening in the first trimester using a combination of ultrasound (Nuchal Translucency) and the maternal serum biochemical markers free beta-hCG and Pregnancy Associated Plasma Protein-A (PAPP-A). This has become known as the combined test. Several maternal and pregnancy factors which can influence the concentrations of biochemical markers are discussed. The possibilities of screening for other aneuploidies in the first trimester and an outline of recent methods to improve overall screening performance are highlighted and the review will suggest some possible options for the future in which Cell Free DNA techniques may become part of an improved overall screening strategy. In conclusion it is emphasized that the time has come to invert the Pyramid of Antenatal Care to focus on the 11-13 week assessment.

(2) Source: Fetal Diagn Ther, 2014.

Title: Is MSAFP Still a Useful Test for Detecting Open Neural Tube Defects and Ventral Wall Defects in the Era of First-Trimester and Early Second-Trimester Fetal Anatomical Ultrasounds?

Authors: Roman AS, Gupta S, Fox NS, Saltzman D, Klauser CK, Rebarber A.

Abstract: Introduction: To evaluate whether maternal serum alpha-fetoprotein (MSAFP) improves the detection rate for open neural tube defects (ONTDs) and ventral wall defects (VWD) in patients undergoing first-trimester and early second-trimester fetal anatomical survey. Material and Methods: A cohort of women undergoing screening between 2005 and 2012 was identified. All patients were offered an ultrasound at between 11 weeks and 13 weeks and 6 days of gestational age for nuchal translucency/fetal anatomy followed by an early second-trimester ultrasound at between 15 weeks and 17 weeks and 6 days of gestational age for fetal anatomy and MSAFP screening. All cases of ONTD and VWD were identified via query of billing and reporting software. Sensitivity and specificity for detection of ONTD/VWD were calculated, and groups were compared using the Fisher exact test, with $p < 0.05$ as significance. Results: A total of 23,790 women met the criteria for inclusion. Overall, 15 cases of ONTD and 17 cases of VWD were identified; 100% of cases were diagnosed by ultrasound prior to 18 weeks' gestation; none were diagnosed via MSAFP screening ($p < 0.001$). First-trimester and early second-trimester ultrasound had 100% sensitivity and 100% specificity for diagnosing ONTD/VWD. Discussion: Ultrasound for fetal anatomy during the first and early second trimester detected 100% of ONTD/VWD in our population. MSAFP is not useful as a screening tool for ONTD and VWD in the setting of this ultrasound screening protocol. (c) 2014 S. Karger AG, Basel.

(3) Source: Obstet Gynecol 123 Suppl 1:40S-41S, 2014.

Title: Is there an association between placenta previa and serum analytes?

Authors: Boulis TS, Meirowitz N, Krantz D, Fleischer A, Sison C.

Abstract: INTRODUCTION: The objective of this study was to evaluate the association between maternal serum analytes and placenta previa. METHODS: Chart review of deliveries from 2004 to 2012 with two comparison groups: placenta previa without accreta and a control group. Patients in the control group were randomly selected from deliveries without placenta previa. Patients with previa were confirmed by third-trimester ultrasonography. Exclusion criteria were placenta previa with pathology-confirmed

accreta, multiple gestations, fetal anomalies, or growth restriction. Primary outcomes were maternal serum analytes in the first trimester: pregnancy-associated plasma protein A and free beta-human chorionic gonadotropin (beta-hCG), and second trimester: alpha-fetoprotein), free beta-hCG, unconjugated estriol, and inhibin. RESULTS: Twenty-six women with previa met inclusion criteria and 43 women in the control group. The groups did not differ with respect to maternal age, gravidity, or parity. Alpha-fetoprotein multiples of the median was significantly higher in women with previa cases than women in the control group (P<.005). Pregnancy-associated plasma protein A multiples of the median was higher (but was not statistically significant) in women with previa than in the women in the control group (P<.064). There were no differences between groups with respect to first-trimester free beta-hCG, second-trimester free beta-hCG, unconjugated estriol, or inhibin (). The following analytes were not available on all specimens: second-trimester free beta-hCG, unconjugated estriol, and inhibin. (Table is included in full-text article.) CONCLUSIONS: In our women with placenta previa, maternal serum alpha-fetoprotein was significantly higher than in women in the control group. We also observed higher levels of pregnancy-associated plasma protein A in women with previa but this did not reach statistical significance. Prospective studies are needed to confirm these findings and determine the relationship with pregnancy outcome.

B) Case History Screening "Picks-of-the-Month":

(1) Source: Turk Patoloji Derg:1-5, 2014.

Title: Pancreatoblastoma, a Rare Childhood Tumor: A Case Report.

Authors: Argon A, Celik A, Oniz H, Ozok G, Barbet FY.

Abstract: Pancreatoblastoma, rarely encountered in the literature, is a malignant exocrine tumor seen in the pancreas. A 5-year-old boy suffering from abdominal pain was sent to our institute for further examination and treatment. Clinical examination was normal but for a palpable abdominal tumor mass. Abdominal Doppler ultrasonography showed a mass with well-defined margins within the body of the pancreas. Laboratory tests, including lactic dehydrogenase, alpha-fetoprotein and cancer antigen 125 were abnormal. The tumor invading the splenic vein and transverse colon was removed totally. We observed a hypercellular tumor in histopathological examination. The tumor had epithelial acinar cells and squamoid morules (corpuscles) separated by stromal bands. Adjuvant chemotherapy was used after surgery. However, the patient died 14 months later. All data about pancreatoblastoma have to be collected in order to choose the treatment to elucidate the molecular pathogenesis of the tumor, to diagnose it early and to develop target-specific treatments.

(2) Source: Obstet Gynecol Sci 57:223-227, 2014.

Title: A meningomyelocele with normal intracranial signs on ultrasound and false-negative amniotic fluid alpha-fetoprotein and acetylcholinesterase.

Authors: Yoon CH, Kang SK, Jin CH, Park MS, Rho JH.

Abstract: Neural tube defects are the major targets of prenatal diagnoses, along with Down syndrome. Prenatal diagnosis of spina bifida is possible at second trimester of gestation through alpha-fetoprotein and acetylcholinesterase biochemistry assays and ultrasound. In particular, the discovery of characteristic intracranial signs on ultrasound leads to a very high diagnosis rate. However, it is rare for spina bifida to present without intracranial signs while also showing normal values of maternal serum alpha-fetoprotein, amniotic fluid alpha-fetoprotein, and acetylcholinesterase. In our hospital, a fetus with spina bifida was delivered at 37+5 weeks' gestation by cesarean section, and was continually followed up over 2 years to date.

(3) Source: Neurology, 2014.

Title: The pleiotropic movement disorders phenotype of adult ataxia-telangiectasia.

Authors: Meneret A, Ahmar-Beaugendre Y, Rieunier G, Mahlaoui N, Gaymard B, Apartis E, Tranchant C, Rivaud-Pechoux S, Degos B, Benyahia B, Suarez F, Maisonobe T, Koenig M, Durr A, Stern MH, Dubois d'Enghien C, Fischer A, Vidailhet M, Stoppa-Lyonnet D, Grabli D, Anheim M.

Abstract: OBJECTIVE: To assess the clinical spectrum of ataxia-telangiectasia (A-T) in adults, with a focus on movement disorders. METHODS: A total of 14 consecutive adults with A-T were included at 2 tertiary adult movement disorders centers and compared to 53 typical patients with A-T. Clinical evaluation, neurophysiologic and video-oculographic recording, imaging, laboratory investigations, and ATM analysis were performed. RESULTS: In comparison with typical A-T cases, our patients demonstrated later mean age at onset (6.1 vs 2.5 years, $p < 0.0001$), later loss of walking ability ($p = 0.003$), and longer survival ($p = 0.0039$). The presenting feature was ataxia in 71% and dysarthria and dystonia in 14% each. All patients displayed movement disorders, among which dystonia and subcortical myoclonus were the most common (86%), followed by tremor (43%). Video-oculographic recordings revealed mostly dysmetric saccades and 46% of patients had normal latencies (i.e., no oculomotor apraxia) and velocities. The alpha-fetoprotein (AFP) level was normal in 7%, chromosomal instability was found in 29% (vs 100% of typical patients, $p = 0.0006$), and immunoglobulin deficiency was found in 29% (vs 69%, $p = 0.057$). All patients exhibited 2 ATM mutations, including at least 1 missense mutation in 79% of them (vs 36%, $p = 0.0067$). CONCLUSION: There is great variability of phenotype and severity in A-T, including a wide spectrum of movement disorders. Karyotype and repeated AFP level assessments should be performed in adults with unexplained movement disorders as valuable clues towards the diagnosis. In case of a compatible phenotype, A-T should be considered even if age at onset is late and progression is slow.

C) News of Note: Abstracts of New Markers:

(1) Source: Prenat Diagn, 2014.

Title: Second trimester Maternal Serum Alpha-fetoprotein (MSAFP) as predictor of fetal hemoglobin Bart's disease.

Authors: Tongprasert F, Srisupundit K, Luewan S, Tongsong T.

Abstract: OBJECTIVE: To evaluate the efficacy of MSAFP levels in predicting hemoglobin (Hb) Bart's fetus. MATERIALS AND METHODS: A total of 199 pregnancies at risk of fetal Hb Bart's disease at 18-22 weeks were enrolled. Before performing cordocentesis for the Hb typing analysis, the MSAFP levels were analyzed and sonographic markers (middle cerebral artery peak systolic velocity, cardiothoracic ratio and placental thickness) were measured. The detection rates of MSAFP and the sonographic markers in predicting fetuses affected by Hb Bart's disease were evaluated. RESULTS: The MSAFP levels were significantly higher in pregnant women carrying affected fetuses (47 cases) than in those with unaffected fetuses (2.09 MoM vs 1.18 MoM, $P < 0.001$). MSAFP as a single marker effectively predicted fetal Hb Bart's disease (AUC ROC 0.832, 95% CI 0.752-0.911), with 87.2% sensitivity and 74.5% specificity using a cut-off value greater than 1.50 MoM. A combination of MSAFP with sonographic markers gave detection rates in the range of 91.5-100%. CONCLUSION: Second trimester MSAFP levels are significantly higher in pregnancies with Hb Bart's fetus. MSAFP as a single marker is relatively effective in Hb Bart's prenatal screening. A high detection rate is achieved when MSAFP is used in combination with sonographic markers.

(2) Source: Placenta, 2014.

Title: Maternal plasma levels of cell-free beta-HCG mRNA as a prenatal diagnostic indicator of placenta accrete.

Authors: Zhou J, Li J, Yan P, Ye YH, Peng W, Wang S, Wang XT.

Abstract: OBJECTIVE: Several biomarkers, including maternal serum creatinine kinase and alpha-fetoprotein, have been described as potential tools for the diagnosis of placental abnormalities. This study aimed to determine whether maternal plasma mRNA levels of the beta subunit of human chorionic gonadotropin (beta-HCG) could predict placenta accreta prenatally. METHODS: Sixty-eight singleton pregnant women with prior cesarean deliveries (CDs) were classified into three groups: normal placentation (35 women, control group); placenta previa alone (21 women, placenta previa group); and both placenta previa and placenta accreta (12 women, placenta previa/accreta group). Maternal plasma concentrations of cell-free beta-HCG mRNA were measured by real-time reverse-transcription polymerase chain reaction and were expressed as multiples of the median (MoM). RESULTS: Cell-free beta-HCG mRNA concentrations (MoM, range) were significantly higher in women with placenta accreta (3.65, 2.78-7.19) than in women with placenta previa (0.94, 0.00-2.97) or normal placentation (1.00, 0.00-2.69) (Steel-Dwass test, $P < 0.01$ and $P < 0.01$, respectively). In the placenta previa/accreta group, the concentration of cell-free beta-HCG mRNA was significantly higher among women who underwent CDs with hysterectomy (4.41, 3.49-7.19) than among women whose CDs did not result in hysterectomy (3.20, 2.78-3.70) (Mann-Whitney U test, $P = 0.012$). DISCUSSION: An increased level of cell-free beta-HCG mRNA in the maternal plasma of women with placenta accreta may arise from direct uteroplacental transfer of cell-free placental mRNA molecules. CONCLUSIONS: The concentration of cell-free beta-HCG mRNA in maternal plasma may be applicable to the prenatal diagnosis of placenta accreta, especially to identify women with placenta accreta likely to require hysterectomy.

(3) Source: Iran J Reprod Med 11:127-132, 2013.

Title: Investigating association between second trimester maternal serum biomarkers and pre-term delivery.

Authors: Sehat Z, Goshetasbi A, Taheri Amin M.

Abstract: Background: Considering the effect of preterm delivery in morbidity and mortality of newborns, its precaution and prevention is so important. Objective: To investigate the association between second trimester maternal serum biomarkers (Human Chorionic Gonadotropin, Alpha-fetoprotein, Non-conjugated estrogen, Inhibin A) and pre-term delivery. Materials and Methods: This is a historical cohort study that has been performed for 700 pregnant women, clients of Nilou Lab in the second trimester of pregnancy to take the Quad Marker test between March to September 2008. The information of mothers having required conditions to enter to study has been registered and after delivery, they called again to be interviewed. These data sets using statistical tests: chi-square test and Roc Curve was analysis. Results: There is a direct relationship between preterm delivery and increase of Alpha-fetoprotein ($p=0.011$) and inhibin A ($p=0.03$) serum level and. Also, there is an inverse relationship between the non-conjugated estrogen ($p=0.002$) serum level and preterm delivery. Moreover, there is not any relationship between the increase human chorionic gonadotropin ($p=0.68$) serum level and preterm delivery. Conclusion: The increase in the Alpha-fetoprotein and Inhibin A and decrease in Non-conjugated estrogen serum levels in the second trimester of pregnancy lead to enhance the probability of preterm delivery. Moreover, if the current study is done with higher samples and different sampling environment, it may have different results.

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

(1) Source: J Matern Fetal Neonatal Med:1-5, 2014.

Title: The effects of isolated single umbilical artery on first and second trimester aneuploidy screening test parameters.

Authors: Tulek F, Kahraman A, Taskin S, Ozkavukcu E, Soylemez F.

Abstract: Abstract Objective: Reliability of first and second trimester screening tests largely depends on accurate estimation of maternal serum marker values. Reduced reliability could lead redundant invasive tests or misdiagnosis. Adjustments of serum marker values for confounding factors like insulin-dependent

diabetes, maternal weight or maternal rhesus status are essential. We aimed to investigate whether isolated single umbilical artery alters first and second trimester test parameters or not. Methods: Routine detailed obstetric ultrasonographies performed were retrospectively screened for this study. Among spontaneously conceived singleton pregnancies, women who were found to have single umbilical artery without any additional structural anomalies or aneuploidies were selected. First and second trimester screening test results were accessible for 98 and 102 of the cases with isolated single umbilical artery, respectively. Results: Among first trimester screening test parameters, PAPP-A (pregnancy-associated plasma protein A) MoMs were found significantly higher in isolated single umbilical artery group. AFP MoMs were found significantly elevated in isolated single umbilical artery group in second trimester quadruple tests. Conclusion: Existence of single umbilical artery could alter the estimation of MoM values of maternal serum markers. Reliability of prenatal screening tests could be improved by adjusting these parameters in accordance with isolated single umbilical artery.

(2) Source: BMC Pediatr 14:191, 2014.

Title: Association between altered placental human chorionic gonadotrophin (hCG) production and the occurrence of cryptorchidism: a retrospective study.

Authors: Chedane C, Puissant H, Weil D, Rouleau S, Coutant R.

Abstract: BACKGROUND: An increase in cryptorchidism has been reported in many countries. One mechanism could be low fetal testosterone production possibly secondary to altered placental human chorionic gonadotrophin (hCG) release. Our Objective was to compare hCG values from maternal blood between boys with cryptorchidism and normal boys. METHODS: Total hCG and alpha-fetoprotein (AFP) values [12-16 weeks of gestation; from the double test for Down syndrome screening) were compared between cases of cryptorchidism and normal control boys who were matched for maternal age, maternal smoking, gestational age at time of hCG measurement (+/-1 day), birth weight and birth term. Measurements were performed in a single laboratory; values were expressed as absolute values (KU/L) and multiples of the median (MoM). Boys whose mothers had had a complicated pregnancy were excluded. Groups were compared using the Student's t test. Log transformation was used to normalize hCG, MoM hCG, AFP and MoM AFP distribution, and values were expressed as geometric means (-1, + 1 tolerance factor). RESULTS: Total hCG and MoM hCG levels were significantly lower in the 51 boys with cryptorchidism compared to 306 controls (21.4 (12.3; 37) KU/L vs 27.7 (15.9; 47.9) KU/L and 0.8 (0.5; 1.2) MoM vs 1.0 (0.6; 1.6) MoM, respectively, $p < 0.01$). By contrast, AFP and MoM AFP levels were similar between groups. CONCLUSION: This study showed a link between low maternal serum hCG levels and cryptorchidism in boys from uncomplicated pregnancy, while normal AFP levels indicated a normal fetoplacental unit. Whether these abnormalities were due to endogenous or exogenous factors remains to be determined.

(3) Source: Am J Obstet Gynecol 211:144 e141-149, 2014.

Title: Association between maternal characteristics, abnormal serum aneuploidy analytes, and placental abruption.

Authors: Blumenfeld YJ, Baer RJ, Druzin ML, El-Sayed YY, Lyell DJ, Faucett AM, Shaw GM, Currier RJ, Jelliffe-Pawlowski LL.

Abstract: OBJECTIVE: To examine the association between placental abruption, maternal characteristics, and routine first and second trimester aneuploidy screening analytes. STUDY DESIGN: Analysis of 1,017 women with and 136,898 women without placental abruption who had first and second trimester prenatal screening results, linked birth certificate, and hospital discharge records for a live born singleton. Maternal characteristics and first and second trimester aneuploidy screening analytes were analyzed using logistic binomial regression. RESULTS: Placental abruption was more frequent among women of Asian race, age > 34 years, women with chronic and pregnancy-associated hypertension, preeclampsia, preexisting diabetes, previous preterm birth and inter-pregnancy interval < 6 months. First trimester pregnancy associated plasma protein-A (PAPP-A) </= 5th percentile, second trimester alpha fetoprotein (AFP) >/= 95th percentile, unconjugated estriol (uE3) </= 5th percentile, and dimeric

inhibin-A (INH) >/= 95th percentile were associated with placental abruption as well. When logistic models were stratified by the presence or absence of hypertensive disease, only maternal age > 34 years (OR 1.4, 1.0-2.0), PAPP-A </=5th percentile (OR 1.9, 1.2-3.1), and AFP >/= 95th percentile (OR 2.3, 1.4-3.8) remained statistically significantly associated for abruption. CONCLUSION: In this large, population based cohort study, abnormal maternal aneuploidy serum analyte levels were associated with placental abruption, regardless of the presence of hypertensive disease.

E) Abstracts of New Assay Methodologies:

(1) Source: Talanta 129:106-112, 2014.

Title: Rapid chemiluminescent sandwich enzyme immunoassay capable of consecutively quantifying multiple tumor markers in a sample.

Authors: Kim J, Rho TH, Lee JH.

Abstract: Using the role of p-iodophenol in enzyme assay, enhanced 1,1'-oxalyldiimidazole chemiluminescent enzyme immunoassays (ODI-CLEIAs) were developed to consecutively quantify trace levels of triple tumor markers, such as alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), and prostate specific antigen (PSA) in a sample. Due to the high sensitivity of enhanced ODI-CLEIAs, it was possible to fix the incubation times (1) to capture a tumor marker with two antibodies, which are primary antibody immobilized on the surface of polystyrene strip-well and detection antibody-conjugated horseradish peroxidase (HRP), and (2) to form resorufin with the addition of substrates (e.g., Amplex Red, H2O2) in order to quantify triple markers in human serum. Enhanced ODI-CLEIAs capable of consecutively and rapidly quantifying triple markers with the same incubation time were more sensitive than conventional enzyme-linked immunosorbent assay (ELISA) capable of separately and slowly quantifying them with different incubation times. In addition, accuracy, precision, and recovery of enhanced ODI CLEIAs in the presence of p-iodophenol were acceptable within statistical error range.

(2) Source: Talanta 129:411-416, 2014.

Title: Facile fabrication of an ultrasensitive sandwich-type electrochemical immunosensor for the quantitative detection of alpha fetoprotein using multifunctional mesoporous silica as platform and label for signal amplification.

Authors: Wang Y, Li X, Cao W, Li Y, Li H, Du B, Wei Q.

Abstract: A novel and ultrasensitive sandwich-type electrochemical immunosensor was designed for the quantitative detection of alpha fetoprotein (AFP) using multifunctional mesoporous silica (MCM-41) as platform and label for signal amplification. MCM-41 has high specific surface area, high pore volume, large density of surface silanol groups (SiOH) and good biocompatibility. MCM-41 functionalized with 3-aminopropyltriethoxysilane (APTES), gold nanoparticles (Au NPs) and toluidine blue (TB) could enhance electrochemical signals. Moreover, primary antibodies (Ab1) and secondary antibodies (Ab2) could be effectively immobilized onto the multifunctional MCM-41 by the interaction between Au NPs and amino groups (-NH2) on antibodies. Using multifunctional MCM-41 as a platform and label could greatly simplify the fabrication process and result in a high sensitivity of the designed immunosensor. Under optimal conditions, the designed immunosensor exhibited a wide liner range from 10(-4)ng/mL to 10(3)ng/mL with a low detection limit of 0.05pg/mL for AFP. The designed immunosensor showed acceptable selectivity, reproducibility and stability, which could provide potential applications in clinical monitoring of AFP.

(3) Source: Methods Mol Biol 1200:69-77, 2014.

Title: High-performance lectin affinity chromatography.

Authors: Kobayashi Y.

Abstract: Lectin high-performance liquid chromatography techniques have contributed to the growing interest in glycoproteomics. Affinity chromatography is a very effective method to separate and purify trace amount of biological substances. In this chapter, we describe a basic procedure for separation of glycoproteins using commercially available lectin-HPLC columns. As an example, alpha-fetoprotein, known as a biomarker of liver cancer, can be separated at the level of their glyco-isomers by using a Lens culinaris agglutinin (LCA) column.

F) Special Abstract Selection:

(1) Source: Obstet Gynecol 123 Suppl 1:77S-78S, 2014.

Title: Does antidepressant exposure in pregnancy affect maternal serum markers for aneuploidy?

Authors: Schroeder C, Wu D, Merchant M, Ferber J, Currier R, Li DK.

Abstract: INTRODUCTION: The objective of this study was to examine the effect of maternal antidepressant exposure on first- and second-trimester maternal serum markers for aneuploidy. METHODS: We conducted a 10-year retrospective cohort study within a large health care organization. Pregnant women diagnosed with depression who underwent serum screening for aneuploidy were identified. Antidepressant exposure was defined by a filled prescription. Levels of pregnancy-associated plasma protein-A, alpha-fetoprotein, estriol, inhibin, and second-trimester human chorionic gonadotropin (hCG) were obtained, expressed as multiples of the mean. We compared levels of serum analytes between women who were and were not exposed to antidepressants. Using recorded Patient Health Questionnaire scores, we assessed depression severity as a confounder. RESULTS: Antidepressant exposure occurred in 52% of 19,186 pregnancies. Mean inhibin levels were significantly higher in the unexposed group (1.124 multiples of the median compared with 1.084 multiples of the median, P=.003) as were mean hCG levels (1.188 multiples of the median compared with 1.165 multiples of the median, P=.007). Mean estriol levels were lower in the unexposed group (1.005 multiples of the median compared with 1.015 multiples of the median, P=.030). There were no statistically significant differences in the mean values of pregnancy-associated plasma protein-A or alpha-fetoprotein. In bivariate analyses, there were no interactions between analyte values and depression severity. There were no significant differences in the proportion of patients with one or more abnormal level of serum analytes between exposed and unexposed groups. CONCLUSION: Antidepressant exposure affects mean levels of inhibin, hCG, and estriol. As a result of our large number of participants, small differences could be detected. Further research is necessary to determine if such differences are of clinical significance.

(2) Source: Am J Obstet Gynecol, 2014.

Title: Population-based biomarker screening and the development of severe preeclampsia in California.

Authors: Tache V, Baer RJ, Currier RJ, Li CS, Towner D, Waetjen LE, Jelliffe-Pawlowski LL.

Abstract: OBJECTIVE: The purpose of this study was to examine the relationship between second-trimester maternal serum biomarkers and the development of early- and late-onset severe preeclampsia in euploid pregnancies. STUDY DESIGN: Included were 136,139 pregnancies in second-trimester prenatal screening through the California Prenatal Screening Program with live births in 2006-2008. We identified severe preeclampsia diagnoses from hospital discharge records. We used log binomial regression to examine the association between abnormal second-trimester maternal serum biomarkers and the development of severe preeclampsia. RESULTS: Approximately 0.9% of all women (n = 1208) in our sample experienced severe preeclampsia; 329 women at <34 weeks' gestation and 879

women >/=34 weeks' gestation. High levels of alpha fetoprotein (AFP), human chorionic gonadotropin, inhibin (multiple of the median, >/=95th percentile), and low unconjugated estriol (multiple of the median, </=5th percentile), were associated with severe preeclampsia (relative risk, 2.5-11.7). Biomarkers were more predictive of early-onset severe preeclampsia (relative risk, 3.8-11.7). One in 9.5 pregnancies with combined high AFP, inhibin, and low unconjugated estriol levels experienced severe early-onset preeclampsia compared with 1 in 680.5 pregnancies without any abnormal biomarkers. CONCLUSION: The risk of the development of severe preeclampsia increases for women with high second-trimester AFP, human chorionic gonadotropin, inhibin, and/or low unconjugated estriol; this is especially true for early-onset severe preeclampsia. When abnormal biomarkers co-occur, risk dramatically increases. Although the screening value of second-trimester biomarkers is low, abnormal biomarkers, especially when occurring in combination, appear to indicate placental dysfunction that is associated with the development of severe preeclampsia.

(3) Source: Fetal Diagn Ther 35:118-126, 2014.

Title: First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing.

Authors: Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH.

Abstract: Objective: To examine the performance of screening for trisomies 21, 18 and 13 at 11-13 weeks' gestation using specific algorithms for these trisomies based on combinations of fetal nuchal translucency thickness (NT), fetal heart rate (FHR), ductus venosus pulsatility index for veins (DV PIV), and serum free beta-human chorionic gonadotropin (beta-hCG), pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PLGF) and alpha-fetoprotein (AFP). Methods: Model-based estimates of screening performance were produced for the distribution of maternal ages in England and Wales in 2011, and prospectively collected data on fetal NT, FHR, DV PIV, beta-hCG, PAPP-A, PLGF and AFP from singleton pregnancies undergoing aneuploidy screening. Results: In screening by NT, FHR, free beta-hCG and PAPP-A, using specific algorithms for trisomy 21 and trisomies 18 and 13 at the risk cutoff of 1:100, the estimated detection rate (DR) was 87.0% for trisomy 21 and 91.8% for trisomies 18 and 13, at a false-positive rate (FPR) of 2.2%. Addition of PLGF, AFP and DV PIV increased the DR to 93.3% for trisomy 21 and 95.4% for trisomies 18 and 13 and reduced the FPR to 1.3%. Conclusions: Effective screening for trisomies can be achieved using specific algorithms based on NT, FHR, DV PIV, beta-hCG, PAPP-A, PLGF and AFP.

(4) Source: Prenat Diagn 34:172-179, 2014.

Title: Prediction of obstetrical risk using maternal serum markers and clinical risk factors.

Authors: Metcalfe A, Langlois S, Macfarlane J, Vallance H, Joseph KS.

Abstract: OBJECTIVE: Abnormal maternal serum analytes (pregnancy associated plasma protein A, total human chorionic gonadotropin, alpha fetoprotein, Inhibin A, and unconjugated estriol) measured as part of aneuploidy screening programs have been associated with adverse obstetrical outcomes in euploid pregnancies. This study aimed to determine if their predictive ability could be enhanced with additional information on obstetrical history. METHOD: Forty-five thousand two hundred eighty-seven women participated in the screening program and delivered euploid singleton infants between 2010 and 2012 in British Columbia, Canada. A split-sample design was used to develop and validate prognostic models for serious perinatal events (stillbirth, preterm birth <32 weeks, or HELLP syndrome) and severe pre-eclampsia [pre-eclampsia with preterm birth <34 weeks or small for gestational age <10th percentile] using logistic regression. RESULTS: Three thousand five hundred four women (7.7%) had at least one abnormal marker using standard cut-off values. The combination of serum markers and clinical risk factors improved the ability of statistical models to predict a serious perinatal event [area under the curve (AUC) = 0.62] and severe pre-eclampsia (AUC = 0.78) compared with serum markers or clinical risk factors alone. CONCLUSIONS: While detection rates are low, the combination of maternal serum markers and obstetrical history helps to identify a small subset of women at higher risk for serious perinatal events and severe pre-eclampsia.

VI. Potentially helpful website connections/locations:

1) http://health.allrefer.com/health/alpha-fetoprotein-info.html

2) www.healthopedia.com/alpha-fetoprotein

3) http://pregnancy.about.com/cs/afp/a/afptesting.htm

4) http://www.webmd.com/baby/alpha-fetoprotein-afp-in-blood

5) http://pregnancy.about.com/od/afp/Alphafetoprotein_Testing.htm

6) http://www.americanpregnancy.org/prenataltesting/afpplus.html

New York State Fetal Defect Markers Proficiency Test,
September 2014
Summary of Second Trimester Results

	MS 316	MS 317	MS 318	MS 319	MS 320
Gestational Age All Lab Mean:					
Mean	20.0	17.0	16.0	15.0	18.0
SD	0.00	0.00	0.00	0.00	0.00
%CV	0.0%	0.0%	0.0%	0.0%	0.0%
mean+3*SD	20.0	17.0	16.0	15.0	18.0
mean-3*SD	20.0	17.0	16.0	15.0	18.0
N	27	27	27	27	27

	MS 316	MS 317	MS 318	MS 319	MS 320
MS AFP All Lab Mean:					
mean	198.3	47.0	39.7	23.7	32.3
SD	16.0	3.3	3.1	1.6	2.0
%CV	8.0%	7.0%	7.7%	6.6%	6.2%
mean+3SD	246.1	56.9	49.0	28.4	38.4
mean-3SD	150.4	37.1	30.5	19.0	26.3
N	27	27	27	27	27
median	198.4	47.0	39.7	23.6	31.8
mean/all kit median	1.00	0.99	1.01	1.00	0.99
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	197.4	46.6	39.4	23.5	32.0
SD	15.5	3.3	3.3	1.1	2.1
%CV	7.8%	7.1%	8.4%	4.6%	6.6%
mean + 3SD	243.8	56.5	49.3	26.7	38.3
mean - 3SD	151.0	36.7	29.5	20.2	25.7
N	18	18	18	18	18
Median	198.1	46.4	38.9	23.4	31.7
mean/All kit median	1.00	0.98	1.00	0.99	0.98
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	197.3	47.4	39.5	23.7	32.7
SD	21.5	4.2	2.8	2.8	2.1
%CV	10.9%	8.9%	7.0%	11.9%	6.5%
mean+3SD	261.7	60.0	47.7	32.2	39.1
mean-3SD	132.8	34.7	31.2	15.2	26.4
N	6	6	6	6	6
median	193.3	47.9	39.5	23.9	31.8
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	208.5	48.4	42.2	24.3	33.2
N	2	2	2	2	2
mean/all kit median	1.06	1.02	1.07	1.02	1.01
MS AFP kit average:					
mean	201.1	47.5	40.4	23.8	32.6
SD	6.5	0.9	1.6	0.4	0.6
all kit median	197.4	47.4	39.5	23.7	32.7

	MS 316	MS 317	MS 318	MS 319	MS 320
MS AFP MoM All Lab Mean:					
mean	3.37	1.19	1.05	0.85	0.74
SD	0.34	0.10	0.09	0.08	0.06
%CV	10.0%	8.5%	9.1%	9.7%	7.9%
mean+3SD	4.38	1.49	1.33	1.10	0.92
mean-3SD	2.36	0.88	0.76	0.61	0.57
N	27	27	27	27	27
All Median	3.37	1.18	1.06	0.84	0.73
mean/all kit median	0.99	1.01	1.01	1.01	1.01
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	3.35	1.18	1.03	0.84	0.74
SD	0.25	0.08	0.07	0.05	0.05
%CV	7.4%	6.6%	7.0%	6.4%	6.9%
mean + 3SD	4.09	1.41	1.25	1.00	0.89
mean - 3SD	2.61	0.94	0.81	0.68	0.58
N	18	18	18	18	18
Median	3.37	1.18	1.04	0.83	0.72
mean/all kit median	0.98	1.00	1.00	1.00	0.99
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	3.41	1.23	1.10	0.90	0.78
SD	0.60	0.17	0.15	0.15	0.09
%CV	17.7%	13.7%	14.1%	16.3%	11.3%
mean + 3SD	5.22	1.73	1.56	1.34	1.04
mean - 3SD	1.59	0.73	0.64	0.46	0.51
N	6	6	6	6	6
Median	3.44	1.25	1.08	0.93	0.77
mean/all kit median	1.00	1.05	1.06	1.07	1.05
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	3.53	1.15	1.04	0.81	0.74
N	2	2	2	2	2
mean/all kit median	1.04	0.98	1.00	0.96	1.00
MS AFP MoM kit average:					
mean	3.43	1.19	1.06	0.85	0.75
SD	0.09	0.04	0.04	0.05	0.02
all kit median	3.41	1.18	1.04	0.84	0.74

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	MS 316	MS 317	MS 318	MS 319	MS 320
MS uE3 All Lab Mean:					
mean	1.13	0.78	0.62	0.41	0.49
SD	0.10	0.08	0.06	0.05	0.05
%CV	8.5%	9.8%	9.8%	11.4%	10.0%
mean+3SD	1.42	1.01	0.80	0.55	0.64
mean-3SD	0.84	0.55	0.44	0.27	0.34
N	25	25	25	25	25
mean/all kit median	1.02	1.02	1.02	1.03	1.02
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	1.11	0.77	0.61	0.40	0.48
SD	0.09	0.08	0.05	0.04	0.04
%CV	8.5%	10.1%	8.7%	10.6%	8.2%
mean+3SD	1.39	1.00	0.77	0.53	0.60
mean-3SD	0.83	0.54	0.45	0.27	0.36
N	17	17	17	17	17
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS uE3 Beckman Access/2 (BCX/BC1) mean:					
mean	1.22	0.84	0.68	0.46	0.54
SD	0.06	0.05	0.02	0.03	0.02
%CV	4.8%	6.4%	2.8%	6.1%	3.8%
mean+3SD	1.39	1.00	0.73	0.54	0.61
mean-3SD	1.04	0.68	0.62	0.38	0.48
N	6	6	6	6	6
mean/all kit median	1.10	1.09	1.11	1.16	1.13
MS uE3 Siemens Immulite/2000 (DPD/DP5 or 6) mean:					
Mean	1.07	0.74	0.53	0.38	0.43
N	2	2	2	2	2
mean/all Kit Median	0.97	0.96	0.86	0.94	0.89
MS uE3 kit average:					
mean	1.13	0.78	0.60	0.41	0.48
SD	0.08	0.05	0.08	0.04	0.06
all kit median	1.11	0.77	0.61	0.40	0.48

	MS 316	MS 317	MS 318	MS 319	MS 320
MS uE3 MoM All Lab Mean:					
Mean	0.60	0.78	0.80	0.68	0.41
SD	0.10	0.22	0.22	0.18	0.10
%CV	17.1%	27.5%	27.8%	26.0%	25.1%
mean+3SD	0.91	1.43	1.47	1.21	0.72
mean-3SD	0.29	0.14	0.13	0.15	0.10
N	25	26	26	25	26
mean/all kit Median	0.96	0.96	0.97	0.95	0.94
MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:					
Mean	0.57	0.70	0.72	0.63	0.37
SD	0.07	0.11	0.08	0.09	0.04
%CV	11.5%	15.2%	11.3%	14.6%	11.5%
mean+3SD	0.77	1.02	0.96	0.91	0.50
mean-3SD	0.37	0.38	0.48	0.35	0.24
N	17	17	17	17	17
mean/all kit Median	0.91	0.86	0.87	0.88	0.85
MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:					
Mean	0.63	0.82	0.83	0.72	0.44
SD	0.04	0.08	0.06	0.07	0.03
%CV	6.9%	9.5%	7.8%	9.9%	6.6%
mean+3SD	0.76	1.05	1.02	0.93	0.52
mean-3SD	0.50	0.58	0.63	0.51	0.35
N	6	6	6	6	6
mean/all kit Median	1.00	1.00	1.00	1.00	1.00
MS uE3 MoM Siemens Immulite/2000 (DPD/DP5 or 6) Mean:					
Mean	0.78	0.93	0.90	0.81	0.45
N	2	2	2	2	2
mean/all kit Median	1.24	1.14	1.09	1.12	1.02
MS uE3 MoM kit average:					
mean	0.66	0.82	0.82	0.72	0.42
SD	0.11	0.11	0.09	0.09	0.04
all kit median	0.63	0.82	0.83	0.72	0.44

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	MS 316	MS 317	MS 318	MS 319	MS 320
MS hCG All Lab mean:					
mean	20.0	27.1	31.6	61.1	49.0
SD	2.9	3.4	4.5	8.5	6.9
%CV	14.4%	12.6%	14.3%	13.8%	14.0%
mean+3SD	28.7	37.4	45.1	86.4	69.6
mean-3SD	11.4	16.8	18.1	35.7	28.4
N	25	25	25	25	25
mean/all kit median	1.04	1.03	1.02	1.01	0.98
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	18.0	25.0	28.6	56.3	44.9
SD	1.8	2.6	2.9	5.4	4.7
%CV	9.8%	10.6%	10.0%	9.7%	10.6%
mean+3SD	23.3	33.0	37.2	72.6	59.1
mean-3SD	12.7	17.1	20.0	40.0	30.7
N	11	11	11	11	11
median	17.40	24.80	28.80	54.50	44.40
mean/All kit median	0.93	0.95	0.93	0.93	0.90
MS hCG Beckman Unicel 5th IS (BCU/BC2) mean:					
mean	22.8	30.1	34.9	68.4	53.6
SD	1.5	1.0	2.3	5.8	3.1
%CV	6.5%	3.3%	6.7%	8.4%	5.7%
mean+3SD	27.2	33.1	41.9	85.7	62.9
mean-3SD	18.3	27.1	27.9	51.1	44.4
N	6	6	6	6	6
median	23.1	30.0	34.9	66.9	53.1
mean/all kit median	1.18	1.14	1.13	1.13	1.07
MS hCG Beckman Access/2 (BCX/BC1) mean:					
mean	19.3	26.4	30.8	60.5	50.0
SD	0.4	1.5	0.3	5.0	2.5
%CV	1.9%	5.8%	1.0%	8.3%	5.1%
X+3SD	27.2	33.1	41.9	85.7	62.9
X-3SD	18.3	27.1	27.9	51.1	44.4
N	3	3	3	3	3
median	19.4	26.8	30.9	59.7	50.5
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS hCG Beckman Access/2 5th IS (BCX/BC2) mean:					
mean	24.0	31.6	38.7	71.5	58.9
SD	2.2	2.7	3.8	5.5	6.8
%CV	9.3%	8.5%	9.9%	7.7%	11.6%
X+3SD	27.2	33.1	41.9	85.7	62.9
X-3SD	18.3	27.1	27.9	51.1	44.4
N	3	3	3	3	3
median	25.0	31.6	39.2	73.7	59.6
mean/All kit median	1.24	1.20	1.26	1.18	1.18
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	17.9	23.7	28.0	50.3	40.9
N	2	2	2	2	2
mean/all kit median	0.92	0.89	0.95	0.87	0.87
MS hCG kit average:					
mean	20.4	27.4	32.2	61.4	49.7
SD	2.8	3.4	4.5	8.7	7.1
all kit median	19.3	26.4	30.8	60.5	50.0

	MS 316	MS 317	MS 318	MS 319	MS 320
MS hCG MoM All Lab Mean:					
mean	1.06	1.00	0.88	1.50	2.20
SD	0.13	0.08	0.07	0.25	0.23
%CV	11.8%	8.4%	8.4%	16.4%	10.5%
mean+3SD	1.43	1.25	1.10	2.25	2.90
mean-3SD	0.68	0.75	0.66	0.76	1.51
N	25	25	25	25	25
mean/All Kit Median	1.01	0.98	0.97	0.94	1.00
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	1.04	0.97	0.87	1.48	2.18
SD	0.13	0.11	0.07	0.13	0.27
%CV	12.6%	11.4%	8.1%	8.7%	12.5%
mean+3SD	1.44	1.30	1.08	1.86	2.99
mean-3SD	0.65	0.64	0.66	1.09	1.36
N	12	12	12	12	12
median	1.04	0.95	0.88	1.52	2.15
mean/All kit median	1.00	0.96	0.96	0.93	0.98
MS hCG MoM Beckman Unicel 5th IS (BCU/BC2) mean:					
mean	1.14	1.04	0.81	1.66	2.21
SD	0.07	0.03	0.05	0.07	0.05
%CV	6.2%	2.6%	6.6%	4.4%	2.4%
X+3SD	1.35	1.12	0.98	1.88	2.37
X-3SD	0.93	0.95	0.65	1.44	2.06
N	5	5	5	5	5
median	1.13	1.02	0.81	1.65	2.22
mean/All kit median	1.09	1.02	0.90	1.04	1.00
MS hCG MoM Beckman Access/2 (BCX/BC1) mean:					
mean	1.03	1.01	0.97	1.60	2.34
SD	0.17	0.03	0.04	0.26	0.18
%CV	16.8%	3.0%	4.5%	16.1%	7.8%
mean+3SD	1.44	1.30	1.08	1.86	2.99
mean-3SD	0.65	0.64	0.66	1.09	1.36
N	3	3	3	3	3
median	1.13	1.02	0.99	1.64	2.42
mean/All kit median	0.99	1.00	1.07	1.00	1.06
MS hCG MoM Beckman Access/2 5th IS (BCX/BC2) mean:					
mean	1.05	1.04	0.90	1.15	2.36
SD	0.09	0.04	0.04	0.51	0.09
%CV	8.1%	3.4%	4.2%	44.3%	3.8%
X+3SD	1.35	1.12	0.98	1.88	2.37
X-3SD	0.93	0.95	0.65	1.44	2.06
N	3	3	3	3	3
median	1.05	1.04	0.92	0.91	2.33
mean/All kit median	1.00	1.03	1.00	0.72	1.07
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	0.98	0.97	0.92	1.66	1.92
N	2	2	2	2	2
mean/All kit median	1.07	0.97	1.07	1.11	0.96
MS hCG MoM kit average:					
mean	1.05	1.01	0.89	1.51	2.20
SD	0.06	0.04	0.06	0.22	0.18
all kit median	1.04	1.01	0.90	1.60	2.21

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	MS 316	MS 317	MS 318	MS 319	MS 320
MS Inhibin A all lab mean:					
Mean	166.7	95.2	83.9	116.6	209.8
SD	11.1	6.0	4.5	7.4	15.1
%CV	6.7%	6.3%	5.4%	6.4%	7.2%
mean + 3SD	200.1	113.2	97.5	138.9	255.2
mean- 3SD	133.2	77.2	70.3	94.3	164.5
N	26	26	26	26	26
All Lab Median	166.5	95.8	84.1	117.1	210.3
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	165.2	94.4	82.9	116.8	207.7
SD	11.7	6.2	4.9	8.0	15.4
%CV	7.1%	6.6%	5.9%	6.8%	7.4%
mean + 3SD	200.4	112.9	97.5	140.7	254.0
mean- 3SD	130.0	75.8	68.3	93.0	161.4
N	17	17	17	17	17
Kit median	165.8	92.0	83.0	116.0	207.5
mean/all kit median	0.99	0.99	0.98	1.00	0.99
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	169.4	96.7	85.6	116.1	213.9
SD	10.0	5.7	3.4	6.7	14.4
%CV	5.9%	5.9%	3.9%	5.8%	6.7%
mean + 3SD	199.3	113.6	95.7	136.3	257.2
mean- 3SD	139.4	79.7	75.5	95.9	170.6
N	9	9	9	9	9
Kit median	170.0	98.7	86.9	118.2	214.8
mean/All kit median	1.01	1.01	1.02	1.00	1.01
MS Inhibin A kit average:					
mean	167.3	95.5	84.3	116.5	210.8
SD	2.9	1.6	1.9	0.5	4.4
all kit median	167.3	95.5	84.3	116.5	210.8

	MS 316	MS 317	MS 318	MS 319	MS 320
MS Inhibin A MoM All Lab mean:					
mean	0.87	0.55	0.48	0.64	1.25
SD	0.08	0.03	0.03	0.05	0.09
%CV	9.5%	6.0%	5.9%	7.9%	7.1%
mean+3SD	1.12	0.65	0.56	0.79	1.52
mean-3SD	0.62	0.45	0.39	0.49	0.98
N	26	26	26	26	26
mean/all kit median	1.00	1.00	0.99	1.00	1.00
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.87	0.55	0.47	0.64	1.24
SD	0.10	0.03	0.03	0.05	0.10
%CV	11.3%	4.8%	6.2%	8.0%	7.7%
mean + 3SD	1.16	0.63	0.56	0.79	1.53
mean- 3SD	0.57	0.47	0.38	0.49	0.95
N	17	17	17	17	17
Kit Median	0.85	0.55	0.47	0.65	1.24
mean/all kit median	1.00	0.99	0.98	1.01	0.99
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	0.87	0.56	0.49	0.63	1.27
SD	0.04	0.04	0.02	0.05	0.07
%CV	5.0%	7.7%	4.7%	7.9%	5.7%
mean + 3SD	1.00	0.69	0.56	0.78	1.49
mean- 3SD	0.74	0.43	0.42	0.48	1.06
N	9	9	9	9	9
Kit Median	0.86	0.55	0.47	0.64	1.24
mean/all kit median	1.00	1.01	1.02	0.99	1.01
MS Inhibin A MoM kit average:					
mean	0.87	0.55	0.48	0.63	1.26
SD	0.00	0.01	0.01	0.01	0.03
all kit median	0.87	0.55	0.48	0.63	1.26

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	AF316	AF317	AF318	AF319	AF320
AF AFP All Lab mean :					
mean	13.9	6.2	10.9	7.5	6.5
SD	1.1	0.6	1.3	0.8	0.6
%CV	8.1%	10.0%	11.7%	11.1%	9.7%
mean+3SD	17.2	8.1	14.8	9.9	8.4
mean-3SD	10.5	4.4	7.1	5.0	4.6
N	20	20	20	20	20
All kit median	13.9	6.1	10.7	7.3	6.4
mean/all kit mean	1.00	1.02	1.03	1.03	1.02
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	13.6	6.1	10.7	7.3	6.4
SD	1.2	0.5	1.1	0.8	0.5
%CV	8.7%	8.7%	10.2%	10.9%	8.0%
X+3SD	17.2	7.6	13.9	9.7	7.9
X-3SD	10.1	4.5	7.4	4.9	4.8
N	14	14	14	14	14
median	13.9	6.1	10.8	7.4	6.5
mean/all kit median	0.98	0.99	1.00	1.00	1.00
AF AFP Beckman Access/2 (BCX/BC1) mean:					
Mean	13.9	6.1	10.6	7.2	6.1
SD	0.5	0.3	1.0	0.3	0.3
%CV	3.7%	5.0%	9.7%	4.2%	4.3%
X+3SD	17.21	7.63	13.92	9.66	7.86
X-3SD	10.1	4.5	7.4	4.9	4.8
N	3	3	3	3	3
median	14.2	6.2	10.3	7.2	6.2
mean/all kit median	1.00	1.00	0.99	0.99	0.96
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	15.3	7.4	13.2	8.6	7.7
N	2	2	2	2	2
mean/all kit median	1.10	1.20	1.24	1.17	1.20
AF AFP kit average:					
mean	10.7	4.9	8.6	5.8	5.0
SD	0.9	0.7	1.5	0.8	0.8
all kit median	13.9	6.1	10.7	7.3	6.4

	AF316	AF317	AF318	AF319	AF320
AF AFP MoM All Lab Mean:					
mean	2.24	0.73	1.43	1.06	0.69
SD	0.33	0.09	0.21	0.12	0.12
%CV	14.8%	12.1%	14.3%	11.0%	17.0%
mean+3SD	3.24	1.00	2.05	1.41	1.04
mean-3SD	1.25	0.47	0.82	0.71	0.34
N	20	20	20	20	20
All median	2.23	0.73	1.46	1.08	0.70
mean/all kit median	1.01	1.01	0.99	0.99	0.99
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	2.24	0.72	1.42	1.04	0.69
SD	0.36	0.09	0.18	0.10	0.12
%CV	16.2%	12.0%	12.8%	9.6%	17.9%
X+3SD	3.33	0.98	1.96	1.34	1.06
X-3SD	1.15	0.46	0.87	0.74	0.32
N	14	14	14	14	14
median	2.23	0.71	1.44	1.08	0.69
mean/all kit median	1.00	0.99	1.00	0.97	1.00
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	2.22	0.73	1.35	1.07	0.62
SD	0.39	0.14	0.33	0.20	0.11
%CV	17.8%	19.5%	24.7%	18.9%	18.0%
X+3SD	3.40	1.16	2.36	1.68	0.95
X-3SD	1.19	0.33	0.67	0.47	0.46
N	3	3	3	3	3
median	2.12	0.70	1.24	1.01	0.60
mean/all kit median	0.99	1.00	0.96	1.00	0.90
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	2.37	0.76	1.68	1.08	0.79
N	2	2	2	2	2
mean/all kit median	1.06	1.04	1.18	1.00	1.15
AF AFP MoM kit average:					
mean	2.27	0.74	1.48	1.06	0.70
SD	0.08	0.02	0.17	0.02	0.09
all kit median	2.24	0.73	1.42	1.07	0.69

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	FT316	FT317	FT318	FT319	FT320
FT Gestational Age All Lab Mean:					
Mean	13.0	12.4	11.4	11.1	13.0
SD	0.05	0.06	0.09	0.10	0.06
%CV	0.4%	0.5%	0.8%	0.9%	0.5%
mean+3*SD	13.1	12.6	11.7	11.4	13.2
mean-3*SD	12.8	12.2	11.2	10.8	12.8
N	17	17	17	17	17

	FT316	FT317	FT318	FT319	FT320
FT NT MoM All Lab Mean:					
Mean	0.92	0.95	2.10	1.06	0.97
SD	0.06	0.06	0.11	0.06	0.06
%CV	6.0%	6.0%	5.4%	5.8%	6.0%
mean+3*SD	1.09	1.12	2.44	1.25	1.14
mean- 3*SD	0.75	0.78	1.76	0.88	0.79
N	16	16	16	16	16
All Median	0.92	0.96	2.08	1.06	0.97

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	FT316	FT317	FT318	FT319	FT320
FT hCG All Lab Mean:					
mean	63.8	68.9	172.3	86.8	66.0
SD	11.7	11.0	33.2	16.2	14.4
%CV	18.4%	15.9%	19.3%	18.6%	21.8%
mean+3*SD	99.0	101.8	271.9	135.3	109.1
mean- 3*SD	28.6	36.1	72.8	38.2	22.9
N	17	17	17	17	17
All lab median	64.0	63.7	167.2	82.9	61.9
mean/All kit median	1.01	1.01	1.03	1.03	1.03
FT hCG Beckman Unicel (BCU/BC1) mean:					
mean	58.7	63.0	158.7	80.2	61.7
SD	7.9	5.9	21.3	9.5	6.5
%CV	13.4%	9.4%	13.5%	11.9%	10.5%
mean+3SD	82.4	80.6	222.7	108.7	81.2
mean- 3SD	35.1	45.3	94.6	51.6	42.2
N	9	9	9	9	9
median	60.0	63.0	160.0	77.5	58.8
mean/All kit median	0.93	0.92	0.95	0.95	0.97
FT hCG Beckman Unicel (BCU/BC2) mean:					
mean	71.4	80.2	206.3	100.5	72.3
SD	5.3	8.9	14.2	0.6	4.7
%CV	7.4%	11.1%	6.9%	0.6%	6.4%
mean+3SD	82.38	80.63	222.69	108.72	81.25
mean- 3SD	35.07	45.30	94.64	51.63	42.20
N	3	3	3	3	3
median	71.1	75.2	209.6	100.4	69.8
mean/All kit median	1.13	1.17	1.23	1.19	1.13
FT hCG Beckman Unicel (BCX/BC1) mean:					
mean	67.2	73.8	177.0	88.8	66.0
N	2	2	2	2	2
mean/All kit median	1.07	1.08	1.05	1.05	1.03
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	55.6	61.7	139.8	71.7	52.1
N	2	2	2	2	2
mean/All kit median	0.88	0.90	0.83	0.85	0.82
FT hCG kit average:					
mean	63.2	69.6	170.4	85.3	63.0
SD	7.3	8.9	28.3	12.3	8.5
all kit median	63.0	68.4	167.8	84.5	63.9

	FT316	FT317	FT318	FT319	FT320
FT hCG MoM All Lab Mean:					
Mean	0.78	0.84	1.87	0.86	0.91
SD	0.11	0.09	0.21	0.11	0.10
%CV	14.3%	10.6%	11.1%	12.5%	10.9%
mean+3*SD	1.12	1.11	2.49	1.18	1.21
mean - 3*SD	0.45	0.57	1.25	0.54	0.61
N	15	15	15	15	15
All lab Median	0.80	0.82	1.84	0.87	0.90
mean/All kit Median	0.95	0.94	0.96	0.98	1.01
FT hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	0.77	0.82	1.82	0.83	0.91
SD	0.12	0.06	0.24	0.11	0.13
%CV	15.5%	6.7%	13.1%	12.9%	13.8%
mean+3SD	1.12	0.99	2.53	1.15	1.29
mean-3SD	0.41	0.65	1.10	0.51	0.53
N	9	9	9	9	9
median	0.78	0.80	1.78	0.83	0.86
mean/All kit median	0.93	0.92	0.93	0.94	1.01
FT hCG MoM Beckman Unicel (BCU/BC2) mean:					
mean	0.73	0.78	1.89	0.84	0.90
SD	0.08	0.10	0.11	0.06	0.02
%CV	10.5%	12.6%	5.8%	7.2%	1.9%
mean+3SD	1.12	0.99	2.53	1.15	1.29
mean-3SD	0.41	0.65	1.10	0.51	0.53
N	3.00	3.00	3.00	3.00	3.00
median	0.71	0.72	1.84	0.87	0.91
mean/All kit median	0.89	0.87	0.97	0.95	0.99
FT hCG MoM Beckman Access (BCX/BC1) mean:					
mean	0.91	0.97	2.01	0.92	0.96
N	1	1	1	1	1
mean/All kit median	1.11	1.09	1.03	1.05	1.06
FT hCG MoM DPC Immulite2000 (DPD/DP5) mean:					
mean	0.88	0.97	2.01	0.98	0.90
N	2	2	2	2	2
mean/All kit median	1.07	1.08	1.03	1.11	0.99
FT hCG MoM kit average:					
mean	0.82	0.88	1.93	0.89	0.92
SD	0.09	0.10	0.09	0.07	0.03
all kit median	0.82	0.89	1.95	0.88	0.91

New York State Fetal Defect Markers Proficiency Test,
September 2014
Summary of First Trimester Results

	FT316	FT317	FT318	FT319	FT320
FT PAPP-A All Lab Mean:					
Mean	3826.4	3087.9	1343.0	2241.4	3755.4
SD	1654.5	1296.2	632.1	917.0	1607.8
%CV	43.2%	42.0%	47.1%	40.9%	42.8%
mean + 3SD	8789.9	6976.6	3239.3	4992.3	8578.7
mean- 3SD	-1137.1	-800.8	-553.3	-509.6	-1068.0
N	16	16	16	16	16
All Lab Median	3572.8	2895.5	1223.8	2077.0	3500.5
mean/All kit median	1.06	1.05	1.11	1.06	1.06
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	3605.1	2936.4	1207.9	2119.1	3534.3
SD	182.0	220.0	58.6	112.7	176.7
%CV	5.0%	7.5%	4.9%	5.3%	5.0%
mean + 3SD	4151.2	3596.5	1383.8	2457.3	4064.4
mean - 3SD	3059.0	2276.3	1032.0	1781.0	3004.1
N	11	11	11	11	11
Kit Median	3613.0	2920.0	1234.0	2114.0	3533.0
mean/All kit median	1.00	1.00	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	8632.8	6777.3	3298.8	4902.3	8437.5
N	2	2	2	2	2
mean/All kit median	2.39	2.31	2.73	2.31	2.39

***Note: The above table contains converted values (mIU/ml->ng/ml) from conversion factor from Anshlabs PAPP-A Elisa Package insert. (see critique)**

	FT316	FT317	FT318	FT319	FT320
FT PAPP-A AnshLite (SMR, MPR or APM/AN1) Mean:					
Mean	1433.5	1184.0	534.6	915.6	1444.5
SD	150.3	119.4	42.8	97.3	237.5
%CV	10.5%	10.1%	8.0%	10.6%	16.4%
mean + 3SD	1884.4	1542.2	663.1	1207.4	2157.1
mean - 3SD	982.7	825.8	406.0	623.8	731.9
N	3	3	3	3	3
Kit Median	1432.0	1141.0	515.0	884.0	1351.0
mean/All kit median	0.40	0.40	0.44	0.43	0.41
FT PAPP-A kit average:					
mean	4557.1	3632.6	1680.4	2645.7	4472.1
SD	3692.9	2860.9	1441.4	2044.9	3589.6
all kit median	3605.1	2936.4	1207.9	2119.1	3534.3

	FT316	FT317	FT318	FT319	FT320
FT PAPP-A MoM All Lab Mean:					
Mean	2.58	3.23	2.09	3.46	3.41
SD	1.31	1.34	0.90	1.34	1.38
%CV	50.9%	41.4%	43.0%	38.6%	40.5%
mean + 3SD	6.51	7.25	4.78	7.47	7.56
mean- 3SD	-1.36	-0.78	-0.60	-0.55	-0.73
N	16	16	16	16	16
All Lab Median	2.75	3.31	2.07	3.41	3.45
mean/ All kit median	0.99	0.96	0.98	0.95	0.97
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	2.61	3.36	2.12	3.66	3.51
SD	0.37	0.47	0.31	0.56	0.27
%CV	14.0%	14.0%	14.5%	15.2%	7.7%
mean + 3SD	3.71	4.77	3.05	5.33	4.32
mean - 3SD	1.51	1.95	1.20	1.99	2.70
N	11	11	11	11	11
Kit Median	2.81	3.32	2.20	3.45	3.46
mean/All kit median	1.00	1.00	1.00	1.00	1.00
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	5.20	5.63	3.84	5.62	6.06
N	2	2	2	2	2
mean/All kit median	1.99	1.67	1.81	1.53	1.73

	FT316	FT317	FT318	FT319	FT320
FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	0.77	1.23	0.82	1.35	1.39
N	2	2	2	2	2
mean/ All kit median	0.29	0.36	0.39	0.37	0.39
FT PAPP-A MoM kit average:					
mean	2.86	3.40	2.26	3.54	3.65
SD	2.22	2.20	1.51	2.14	2.34
all kit median	2.61	3.36	2.12	3.66	3.51

Figure 1

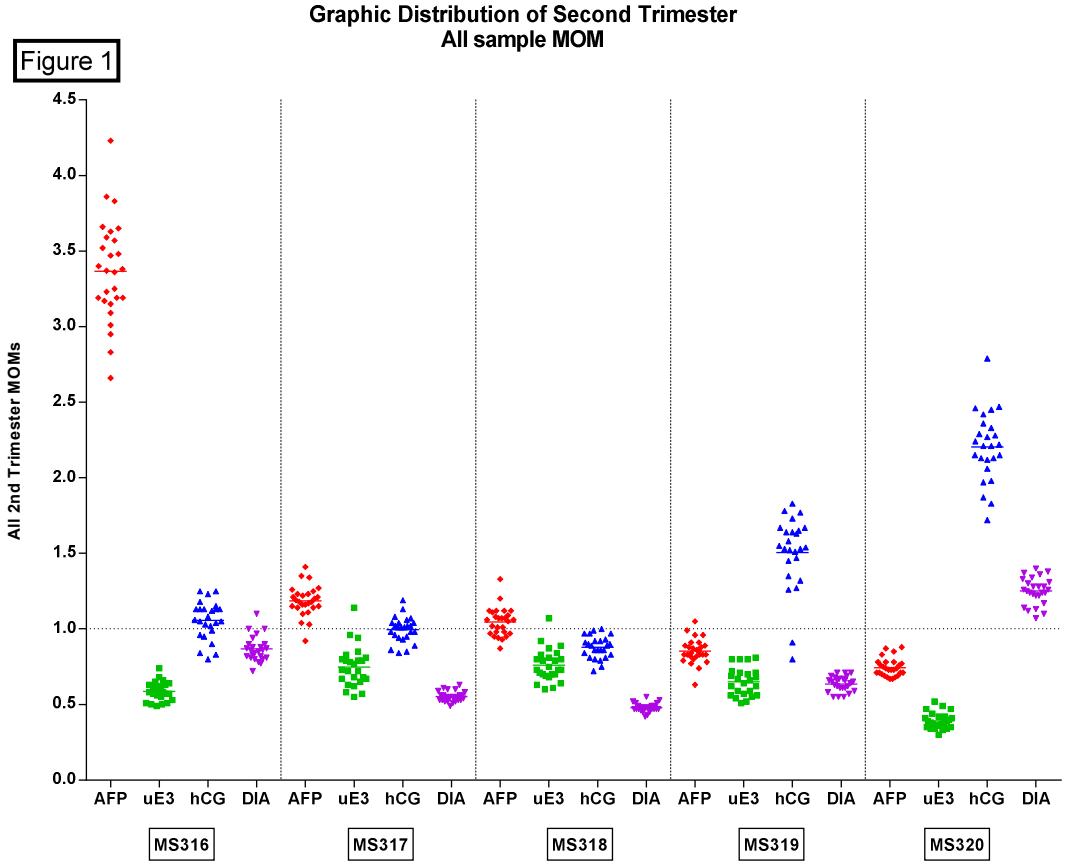


Figure 2a

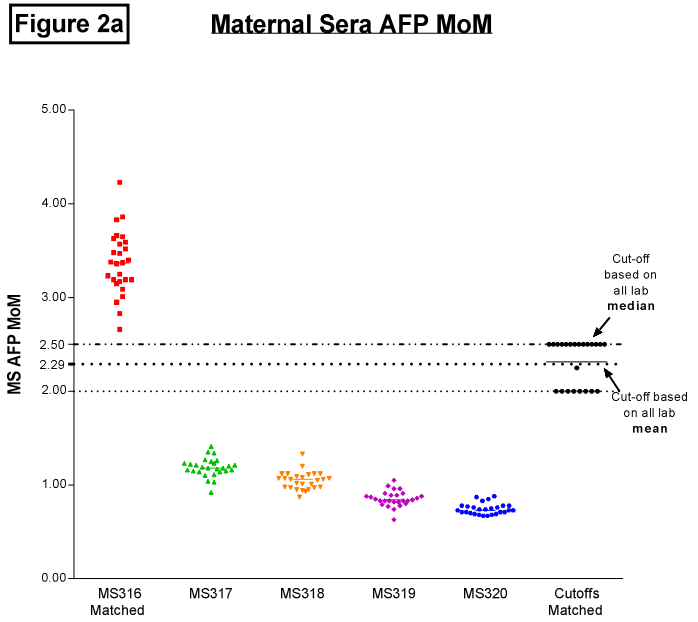


Figure 2b

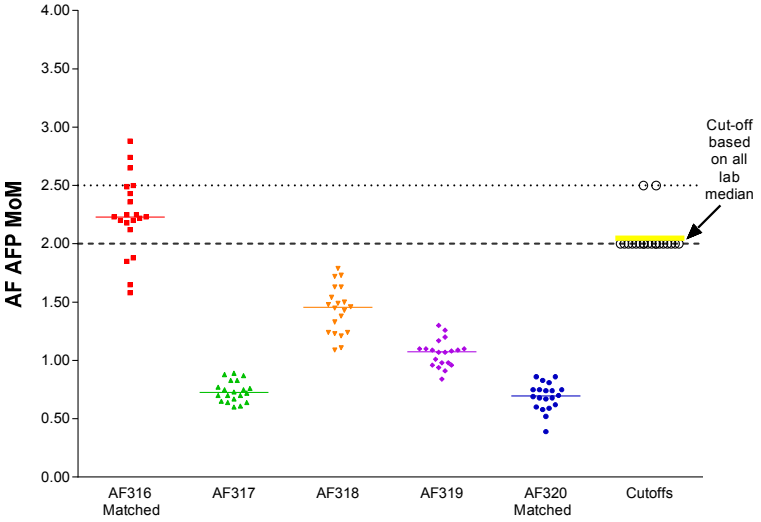


Figure 3

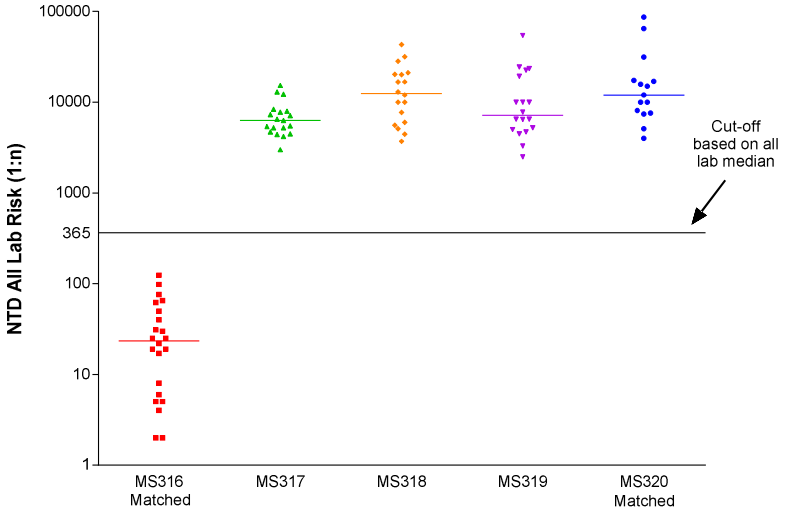


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

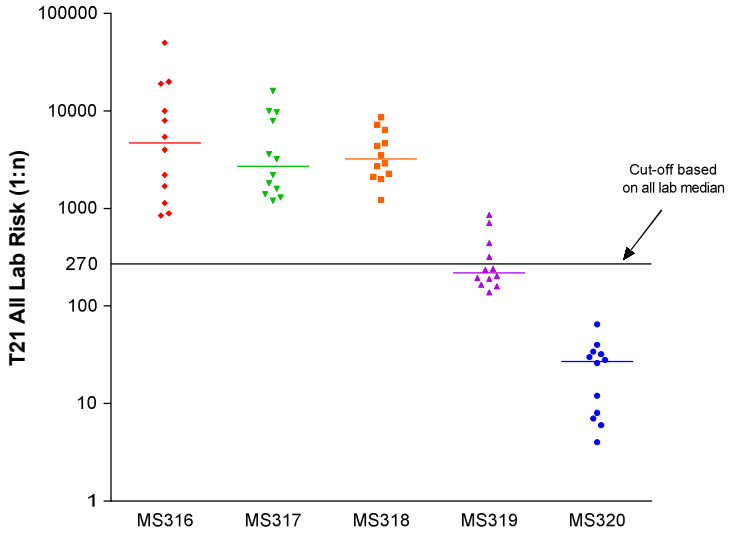


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

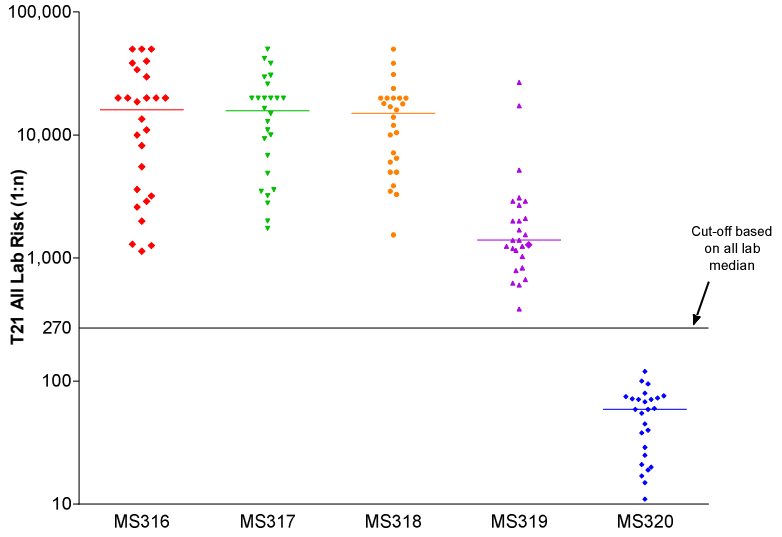
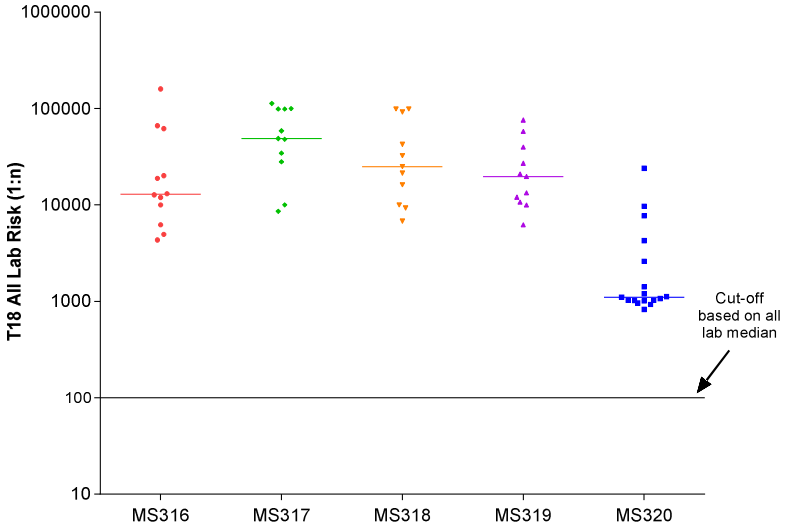


Figure 6

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates



NYS FEDM PT 5/14
Second Trimester

BCU/BC1 = Beckman Unicel Dxl
BCX/BC1 = Beckman Access/2
DPD/DP5 = Siemens Immulite 2000

Figure 7A

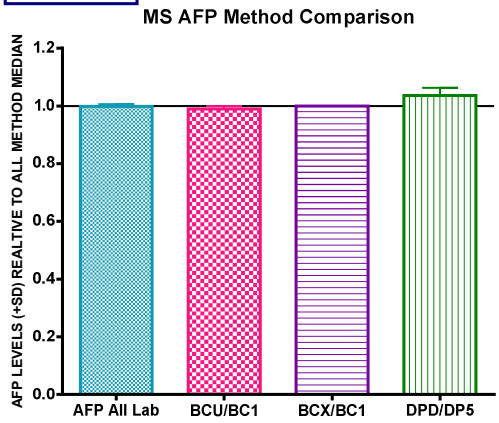


Figure 7B

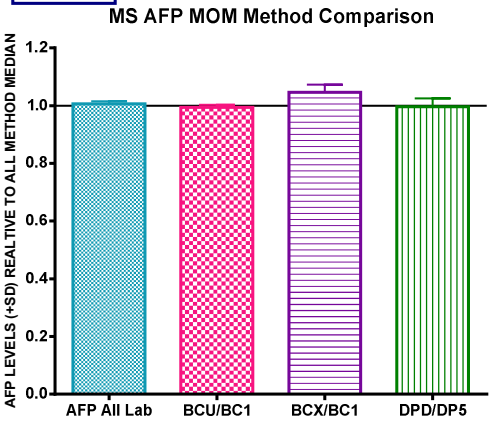


Figure 7C

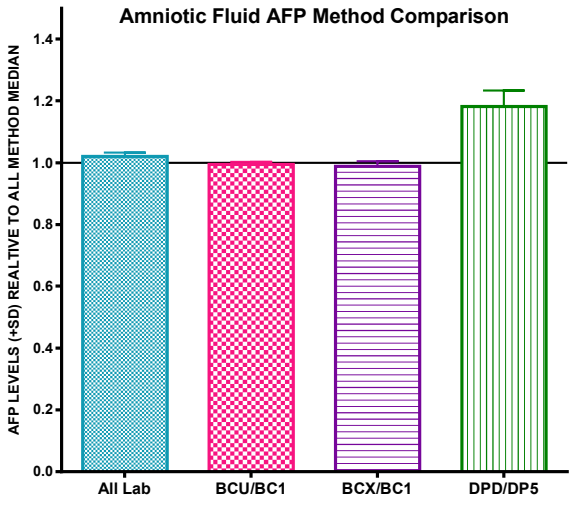
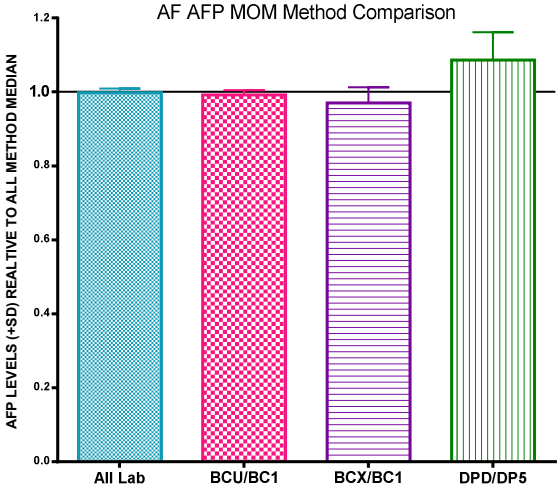


Figure 7D



NYS FEDM PT 9/14
Second Trimester

BCU/BC1 = Beckman Unicel Dxl
BCU/BC2 = Beckman Unicel Dxl 5th IS hCG
BCX/BC1 = Beckman Access/2
BCX/BC2 = Beckman Access/2 5th IS hCG
DPD/DP5 = Siemens Immulite 2000

Figure 8A

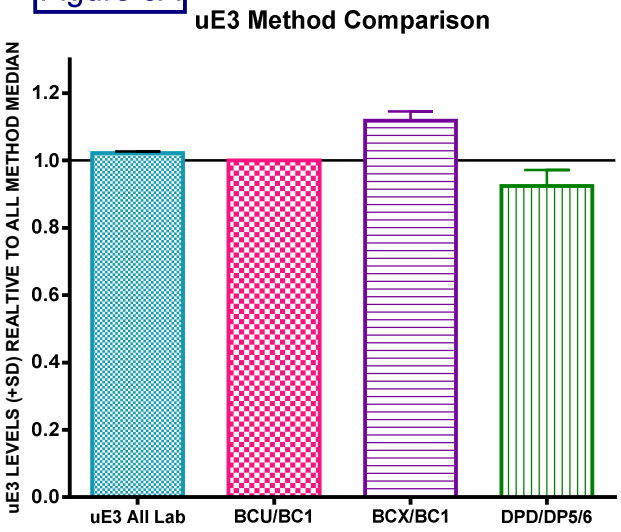


Figure 8B

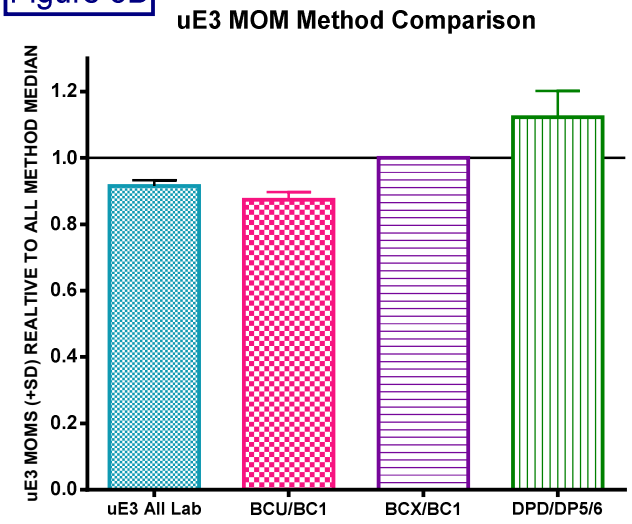


Figure 9A

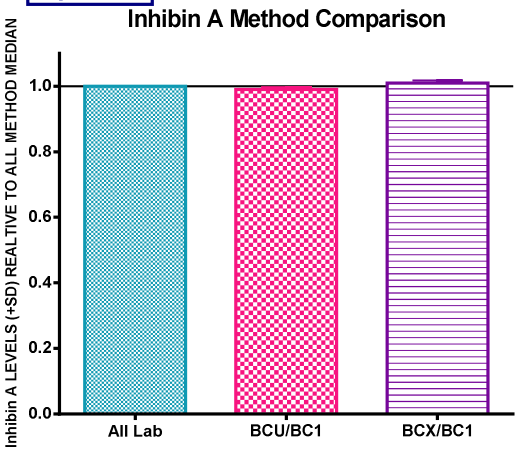


Figure 9B

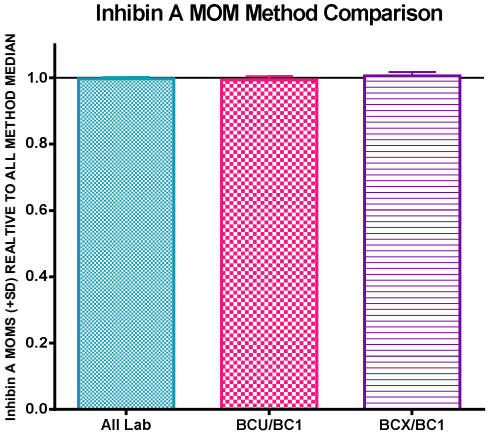


Figure 10A

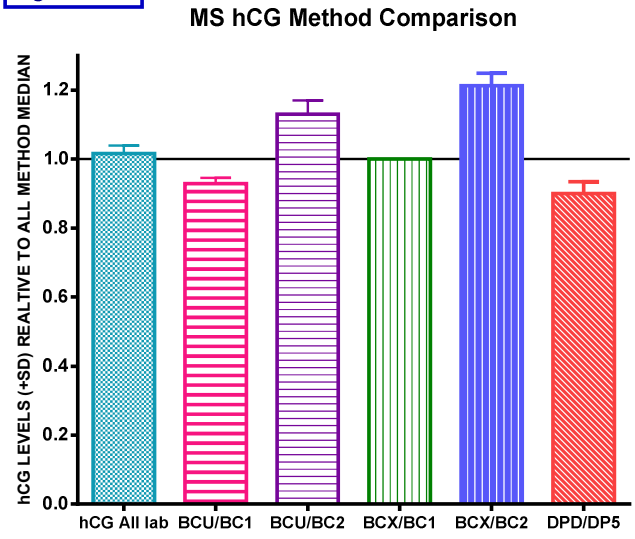
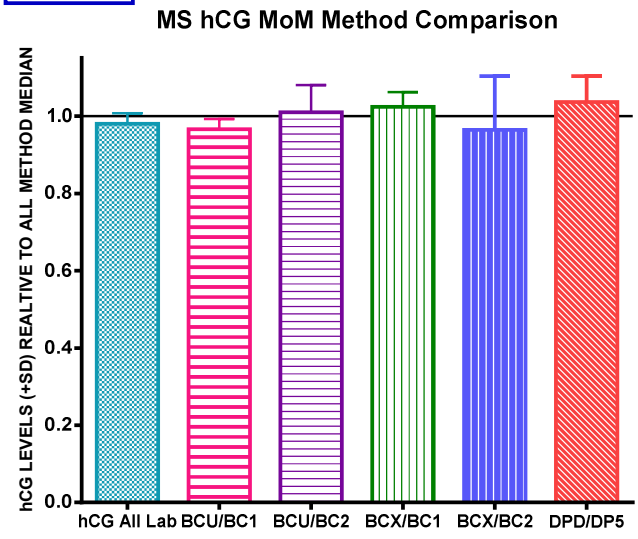


Figure 10B



NYS FEDM PT 9/14
First Trimester

Figure 11A

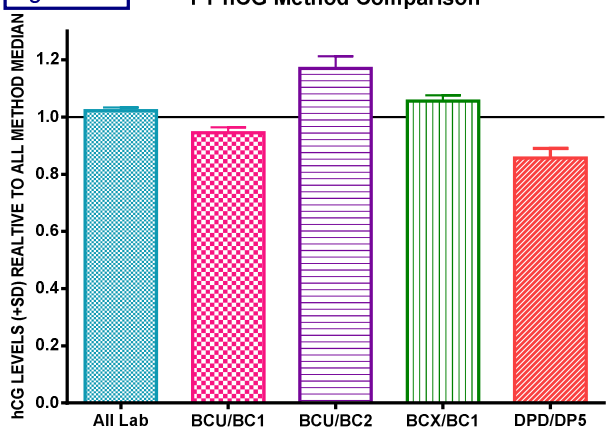


Figure 11B

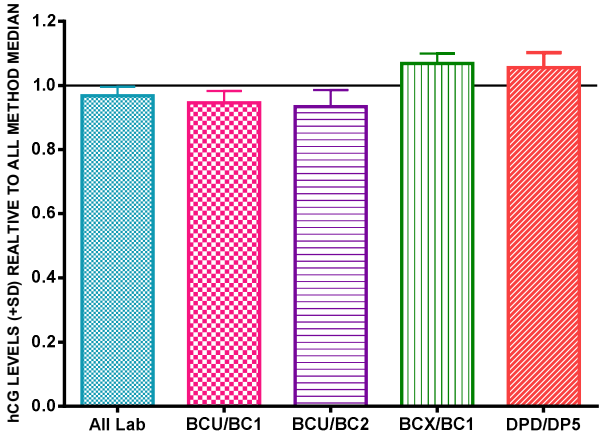


Figure 12A

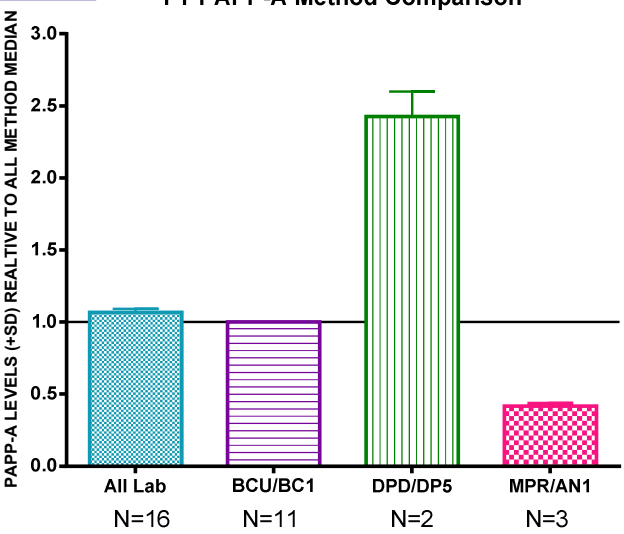
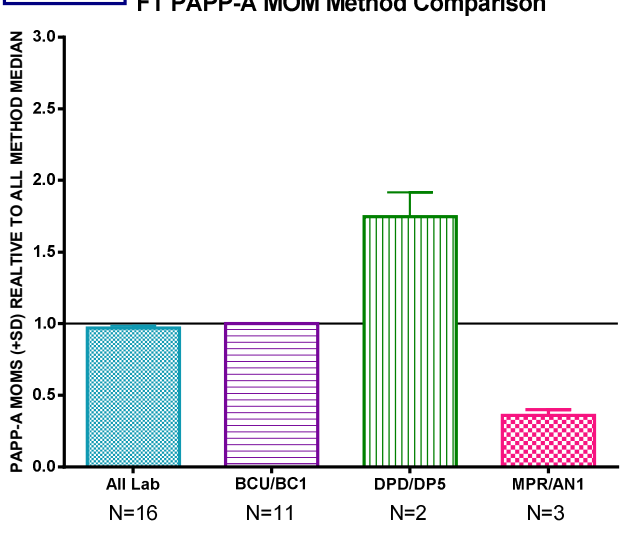


Figure 12B



BCU/BC1 = Beckman Unicel
BCU/BC2 = Beckman Unicel 5th IS hCG
BCX/BC1 = Beckman Access/2
DPD/DP5 = Siemens Immulite 2000
MPR/AN1 = AnshLite Reagents

Figure 13

Graphic Distribution of First Trimester Trisomy 21 Risk Estimates

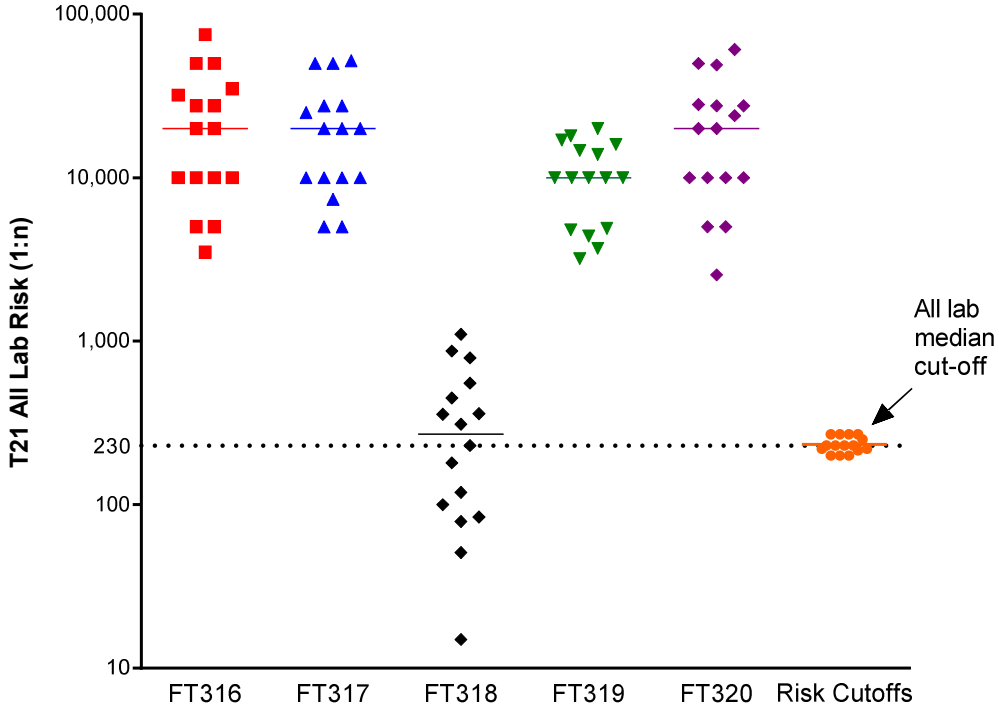


Figure 14

Graphic Distribution of First Trimester Trisomy 18 Risk Estimates